



HAL
open science

Crossed effects of helminth infection and lead exposure on fitness: An experimental study in feral pigeons (*Columba livia*)

Aurélie Jeantet, Fabienne Audebert, Simon Agostini, Beatriz Decencière, Camille Lorang, Laura Jamet, Clarisse Giner, Emma Flandi, Nora Nardou, Baptiste Lemaire, et al.

► To cite this version:

Aurélie Jeantet, Fabienne Audebert, Simon Agostini, Beatriz Decencière, Camille Lorang, et al.. Crossed effects of helminth infection and lead exposure on fitness: An experimental study in feral pigeons (*Columba livia*). *Journal of Animal Ecology*, 2024, 93 (12), pp.1996-2009. <10.1111/1365-2656.14211>. <hal-04880752>

HAL Id: hal-04880752

<https://hal.science/hal-04880752v1>

Submitted on 17 Apr 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

RESEARCH ARTICLE

Crossed effects of helminth infection and lead exposure on fitness: An experimental study in feral pigeons (*Columba livia*)

Aurélie Jeantet^{1,2}  | Fabienne Audebert²  | Simon Agostini³ | Beatriz Decencière³  |
Camille Lorang^{1,4}  | Laura Jamet^{1,2} | Clarisse Giner¹ | Emma Flandi¹ | Nora Nardou¹ |
Baptiste Lemaire¹ | Pierre Federici¹  | David Rozen-Rechels¹  | Julien Gasparini¹ 

¹Sorbonne Université, UPEC, Paris 7, CNRS, INRA, IRD, UMR 7618, Institut d'Ecologie et des Sciences de l'Environnement de Paris, Paris, France

²Laboratoire de Biologie des Organismes et Écosystèmes Aquatiques, Sorbonne Université, MNHN, CNRS, IRD, UCA, UMR 8067, Paris, France

³Ecole Normale Supérieure, CNRS, UMS 3194, Centre de Recherche en Écologie Expérimentale et Prédicative (CEREEP-Ecotron IleDeFrance), PSL University, Saint-Pierre-lès-Nemours, France

⁴Laboratoire de Santé Animale, ANSES, INRAE, ENVA, UMR BIPAR, Maisons-Alfort, France

Correspondence

Aurélie Jeantet

Email: aurelie.jeantet@sorbonne-universite.fr

Funding information

EC2CO program (Ecosphère Continentale et Côtière), Grant/Award Number: 15690

Handling Editor: Isabella Cattadori

Abstract

1. Living organisms are exposed to multiple environmental factors that can affect their fitness. The negative effects of these simultaneous stressors can be additive or can interact in negative synergistic or antagonistic ways to affect the health of exposed individuals. Parasites can accumulate pollutants in their own tissues and have been shown to increase the tolerance of their hosts to different pollutants (antagonistic interaction between parasites and pollutants). Through an experimental approach, we tested the existence of combined antagonistic effects between intestinal parasites and lead exposure on urban feral pigeons (*Columba livia*) which are known to be exposed to trace metal pollution and harbour a wide variety of internal and external parasites.
2. We experimentally exposed wild feral pigeons in captivity to two treatments: an anthelmintic treatment to eliminate intestinal nematode parasites; an exposure to lead for a period of 6 months. We tested the effects of these crossed treatments on several components of fitness: immunocompetence, reproduction, and body mass.
3. Our findings suggest that the overall effects of lead exposure, either alone or in combination with the presence of intestinal parasites (without anthelmintic treatment) were negative, through either additive or synergistic means.
4. Our results reveal the existence of negative combined effects between pollutant exposure and intestinal parasites, highlighting the importance of accounting for multiple stress factors when studying the effects of exposure to pollutants and/or other environmental stressors on the fitness of organisms.

KEYWORDS

feral pigeon, helminths, parasitism, pollutants, stressors, urban ecology

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *Journal of Animal Ecology* published by John Wiley & Sons Ltd on behalf of British Ecological Society.

1 | INTRODUCTION

Growing anthropization in recent decades has led to substantial changes to the natural environment including the release of a variety of pollutants, many of which impart negative effects on living organisms. Among such pollutants are element trace metals (ETMs), whose concentration can be increased by some anthropic activities such as industry and agriculture (Cloquet et al., 2006). In addition to their exposure to pollutants, living organisms are also exposed to natural stressors, such as parasites, defined as any organism, whether micro or macroscopic, that negatively affects the fitness of its host by feeding on it or by using the host's resources for itself, without the host benefiting from this interaction itself (Combes et al., 2018). In addition to the direct costs due to the use of its resources, the host can suffer indirect costs, via a reallocation of its energy in antiparasitic strategies (immune or behavioural), to the detriment of its growth and reproduction for example. Thus, trade-offs may exist between antiparasitic strategies and other life history traits (Bize et al., 2004). Many studies have focused on the impacts of pollutants or parasites alone on various fitness components of exposed individuals, but few studies have investigated their combined effects on the hosts. However, parasites should be taken into account when studying the impact of pollutants on organisms because parasites have been demonstrated to modulate host tolerance to pollutants (interaction between pollutants and parasites; Bustnes et al., 2006; Chin et al., 2017; Goutte & Molbert, 2022; Jeantet et al., 2023;

Marcogliese & Pietrock, 2011; Molbert et al., 2020). Indeed, the combined impacts of exposure to parasites and to pollutants may be complex (Figure 1).

First, it may exist non-interactive cumulative effects of parasites and pollutants, where the independent effects of the two factors are additive (additive effects; Goutte & Molbert, 2022). Thus, these cumulative effects on the host are equivalent to the addition of the independent effects of pollutants and parasites. Second, parasites may be able to reduce host tolerance to pollutants via negative synergistic effects (Bustnes et al., 2006; Chin et al., 2017; Marcogliese & Pietrock, 2011), that is the combined effects of pollutants and parasites are greater than the addition of their independent effects. For example, Gheorgiu et al. (2005) found that the mortality of guppies (*Poecilia reticulata*) exposed to zinc was extremely low when guppies were not infected by ectoparasites (*Gyrodactylus turnbulli*) regardless of the zinc concentration. Whereas in ectoparasite-infected guppies, mortality increased linearly with zinc concentration, due to the guppies' inability to produce protective mucus when exposed to both ectoparasites and zinc. In this case, the cumulative effects of parasites and zinc exposure on hosts were greater than the addition of their independent effects (synergistic interaction). These combined negative effects may be due to direct or indirect costs of parasites and pollutants on the host. Resistance to parasites might for example be reduced with the impairment of other life history traits such as immunocompetence in response to pollutant contamination (Coors et al., 2008). Indeed, some pollutants are known for

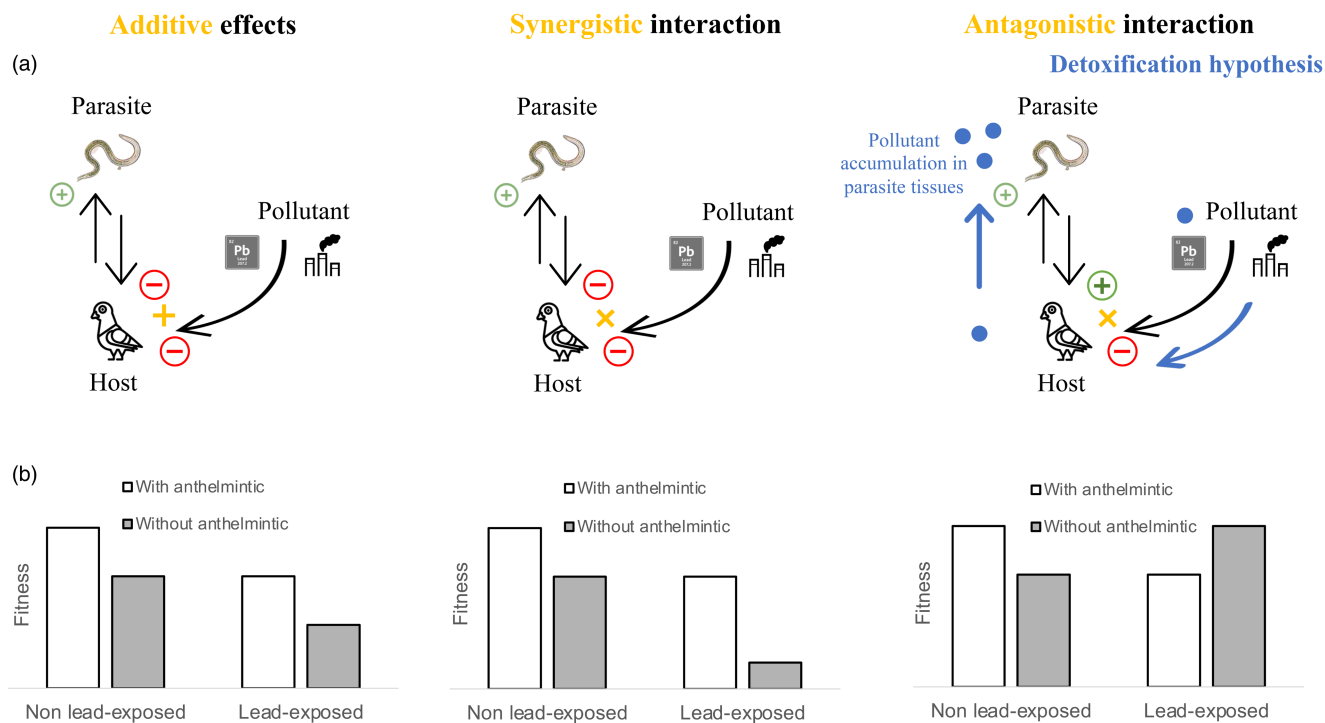


FIGURE 1 Theoretical description of the three different types of combined effects between lead exposure and helminth infestation (a) and the expected plots for each of the described effects (b). The three different types of combined effects are represented in yellow and the detoxification hypothesis on which our prediction is based is represented in blue.

their immunotoxic effects (Chatelain, Gasparini, & Frantz, 2016b), which could facilitate infestation by parasites (Marcogliese & Pietrock, 2011). In addition, parasites can modulate immune pathways, by stimulating them, or via suppressive effects (Larsen et al., 2002; Nunn et al., 2014). By inducing an increase in the energy allocated to the immune system, they would allow the host to fight more effectively the effects of pollutants, as mentioned by Sánchez et al. (2016). On the contrary, their suppressive effects on immune pathways could aggravate the effects of pollutants (Gheorgiu et al., 2005).

Finally, some parasites are able to increase host tolerance to pollutants via antagonistic effects (Brázová et al., 2021; Jeantet et al., 2023; Molbert et al., 2020; Morrill et al., 2019; Sánchez et al., 2016). For instance, Sánchez et al. (2016) have demonstrated that cestode parasites (*F. liguloides* and *C. podicipina*) of artemia (*Artemia parthenogenetica*) could increase host resistance to arsenic exposure (antagonistic interaction). This phenomenon might be explained by an increase in the host immune defences induced by the parasitic infection, which would allow infected organisms to more effectively fight the toxic effects of pollutants (Sánchez et al., 2016). Another hypothetical mechanism described in similar studies is based on the capacity of some parasites to accumulate pollutants in their own tissues and thus might constitute 'pollutant sinks'. Thus, the accumulation of pollutants in parasite tissues reduces the bio-availability of the pollutant in the host's body, thereby reducing the impact of the pollutants on the host (Jeantet et al., 2023; Molbert et al., 2020; Morrill et al., 2019; Sures et al., 2017). This accumulative capacity has been observed for a number of parasites, most of which are intestinal (Goutte & Molbert, 2022; Sures et al., 2017). Despite the complexity of their effects (Figure 1), there is currently no experimental study testing the combined effects of parasites and pollutants on their hosts.

In the present study, we attempt to address this lack of knowledge by experimentally tested the existence of combined effects of pollutants and helminths (parasitic worms) in feral pigeons (*Columba livia*) and whether they were additive, synergistic or antagonistic (Figure 1). This species represents a good model to test our hypotheses because this bird naturally harbours many identifiable species of parasites, especially helminths (Al-Barwari & Saeed, 2013; Tietz Marques et al., 2007). Moreover, pigeons mainly exploit the urban environment and are naturally exposed to trace metals (Chatelain et al., 2014). Feral pigeons have been previously shown to be impacted by lead exposure which reduce fitness parameters such as reproduction and immunocompetence (Chatelain, Gasparini, & Frantz, 2016a, 2016b; Jeantet et al., 2023). Finally, helminths are known for their ability to accumulate pollutants in their tissues (Molbert et al., 2020) and to modulate immune pathways (Larsen et al., 2002; Nunn et al., 2014), which makes them a good model to test our hypotheses and the mechanisms underlying the combined effects between pollutants and parasites. Thus, according to our results, if the effects of pollutants and helminths are additive, we predict that the parasites and lead exposure should negatively impact the fitness of individuals (negative effects of the two factors

without statistical interaction). Second, with a synergistic effect we would predict that the parasites and lead exposure should negatively interact to impact the host fitness, that is the negative effects of parasites and lead exposure would be greater than the addition of their effects taken independently (significant interaction of the two factors). Finally, we predict that an antagonistic effect would translate into a lower effect of lead on fitness in infected individuals (significant interaction of these two factors). In addition, we carried out immunocompetence measurements, in order to understand whether the combined effects of lead exposure and the presence of helminths could be explained, at least in part, by indirect negative effects on the immune system, as described by Gheorgiu et al. (2005) and Sánchez et al. (2016).

We challenged these predictions in 132 feral pigeons captured in Paris, France and housed in aviaries at the CEREEP (CEntre de Recherche en Ecologie Expérimentale et Prédicative) biological station. We experimentally exposed the pigeons to lead for a period of 6 months with or without anthelmintic treatment during the same period. We measured various fitness proxies: immunocompetence (cell-mediated and humoral-mediated immune responses), reproduction (egg mass, hatching success, chick body mass at hatching, chick survival rate, chick survival time, and number of fledglings produced) and bird body mass. In feral pigeons, based on previous results (Jeantet et al., 2023), we predicted antagonistic effects between lead exposure and helminths following the detoxification hypothesis.

2 | MATERIALS AND METHODS

2.1 | Bird sampling

A sample of 132 free-living pigeons (55 males and 77 females genetically sexed) was captured during January and February 2022 in Paris, France. Birds were individually identified with two plastic rings, one coloured and numbered on the left foot and the other only coloured on the right foot. Then, birds were placed in 12 outdoor aviaries (2.20×2.20m and 3m tall each) at the CEREEP field station (CEntre de Recherche en Ecologie Expérimentale et Prédicative-Ecotron Ile-de-France, UMS 3194, Ecole Normale Supérieure, Saint-Pierre-lès-Nemours, France). Birds were grouped to equilibrate mass (ANOVA, $F_{11,120}=0.20$, p -value=0.99), sex-ratio (GLM logistic, $\chi^2_{11}=7.95$, p -value=0.72), site of capture (Fisher's exact test, p -value=0.99) and melanin-based coloration plumage (Kruskall-Wallis, $\chi^2_{11}=1.81$, p -value=0.99) across groups, in order to avoid any correlation between birds' history (e.g. lead past exposure and parasite loads) and our crossed treatment.

This study was carried out in strict accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. All experiments and captures were approved by Charles Darwin Animal Experimentation Ethics Committee and French authorities (the 'Ministère de l'éducation nationale, de l'enseignement

supérieur et de la recherche', permit no. #17554 2018111610466351; and Ville de Paris).

2.2 | Experimental protocol

We adapted the experimental protocol described in Jeantet et al. (2023) for a period of 6 months. Birds were placed in aviaries 2 or 3 weeks before the start of the experiment for acclimation. Two crossed treatments were assigned to the pigeons in the 12 aviaries, with four experimental groups and three replicates per groups: pigeons exposed to lead during the experiment without anthelmintic treatment (group 1: $n=33$), pigeons exposed to lead with anthelmintic treatment (group 2: $n=33$), pigeons not exposed to lead during the experiment without anthelmintic treatment (group 3: $n=33$) and pigeons not exposed to lead with anthelmintic treatment (group 4: $n=33$). For groups 1 and 2 (pigeons exposed to lead during the experiment), the lead concentration was 10 ppm lead acetate, based on Parisian lead pollution, and placed into drinking troughs and baths, as described in (Chatelain, Gasparini, & Frantz, 2016a; Chatelain, Gasparini, Haussy, & Frantz, 2016). We chose this lead concentration based on both lead concentrations in blood measured in urban birds and the gastrointestinal absorption rate of lead in zebra finches (see Chatelain, Gasparini, & Frantz, 2016a; Chatelain, Gasparini, Haussy, & Frantz, 2016 for more details). For groups 3 and 4 (pigeons not exposed to lead during the experiment), drinking troughs and baths were filled with tap water only, which previous studies have shown does not contain significant amount of lead (shift from 24.34 to 0.71 ppm of lead in the feathers, after 1 year of drinking this tap water, Chatelain et al., 2014). Birds in groups 2 and 4 (pigeons with anthelmintic treatment), were dewormed with a levamisole solution (Capizol 15 mg/mL) dispensed directly into the bills of birds every month, according to Chege et al. (2017). Birds in groups 1 and 3 (pigeons without anthelmintic treatment), were administered tap water in the same way. Levamisole treatment significantly reduced the estimated number of helminth eggs counted at the end of the experiment by coproscopy in the faeces of pigeons with anthelmintic treatment (groups 2 and 4) as compared with faeces of pigeons without anthelmintic treatment (groups 1 and 3) (with anthelmintic: 5.07 helminth eggs/g of faeces ± 1.93 , without anthelmintic: 37.3 helminth eggs/g of faeces ± 5.99 ; $\chi^2_1 = 20.3$, $p < 0.01$). Coproscopies were realized using Macmaster flotation technique; faeces of each pigeon were collected then weighed and floated in a Falcon tube of 15 mL with a solution of saturated NaCl. After shaking the tubes and waiting for 15 min, the supernatant was spread on a Macmaster slide and helminth eggs were counted using an optical microscope (modified from Raynaud et al. (1970) method). This method allowed us to identify the presence of several nematode taxa: *Capillaria* sp., *Syngamus trachea*, *Ascaridia* sp. and *Trichostrongylus tenuis*. During the experiment, birds were fed ad libitum with a mix of maize, wheat and peas, and aviaries were enriched with baths and bamboo sticks as perches. At the end of the experiment, all birds were released into the wild near their site of capture.

2.3 | Immunocompetence

- *Cell-mediated immune response*: The intensity of the cell-mediated immune response was quantified at week 14, as described in Jacquin et al. (2011). All pigeons received phytohemagglutinin (PHA) by subcutaneous injection (0.1 mL concentrated to 5 mg/mL) into the patagium of the left wing. At the injection site, the thickness of the wing was measured three times just before (t_0) and three times 24 h after the PHA injection (t_{24}) using a pressure-sensitive spessimeter (Mitutoyo 0–1 mm, Tokyo, Japan). All measurements and injections were made by the same experimenter (AJ). The cell-mediated immune response was quantified by the increase in wing thickness at 24 h after the PHA injection, that is the average thickness at t_{24} minus the average thickness at t_0 . Note that the wing thickness measurements at 24 h were performed by a single operator who was blinded with respect to the treatments.
- *Humoral immune response*: The humoral immune response was assessed between week 16 and week 24 (9 weeks) as described in Chatelain, Gasparini, and Frantz (2016a). A solution containing 50 μ g of keyhole limpet hemocyanin (KLH, hemocyanin from *Megathura crenulata*, Sigma Aldrich) was injected subcutaneously in the neck at week 16 and week 20, and blood samples were collected every week for 9 weeks beginning with the week of the first injection. To quantify the humoral immune response, anti-KLH antibody levels in pigeon plasma were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (Jacquin et al., 2013). Thus, anti-KLH antibody levels at weeks 17, 18, 19, and 20 represented the primary humoral immune response (after the first injection of KLH) and the anti-KLH antibody levels at weeks 21, 22, 23, and 24 represented the secondary humoral immune response (after the second injection of KLH). Measurements of anti-KLH antibody levels were highly repeatable (intra-assay repeatability: $r = 0.77$, $F_{179,177} = 7.62$, $p < 0.001$ and inter-assay repeatability: $r = 0.92$, $F_{10,98} = 25.45$, $p < 0.001$).

2.4 | Reproduction

During our experiment, 36 pairs laid at least one egg, that is 71 pigeons invested in reproduction (one male fertilized two different females). Pigeons lay two eggs per clutch (Johnston & Janiga, 1995) and they can continue to produce eggs after removing a first clutch (Kennedy, 1991). Thus, breeding pigeons produced 1–6 eggs and up to 3 clutches. Offspring care is biparental, which allowed us to identify the social parents for each brood. Because there is no dimorphism between males and female chicks, we were not able to determine their sex. We measured different proxies of reproduction success:

- *Egg mass*: All eggs were weighed with the same precision scale. Newly laid eggs were removed daily from the nest and replaced

with false eggs during the weighing procedure, after which they were immediately replaced in their nests.

- *Hatching success*: Hatching was considered successful when a chick emerged from its shell and was alive.
- *Chick body mass at hatching*: On the day of hatching, chicks were weighed directly with a Pesola Newton scale to the nearest 2 g.
- *Chick survival rate and chick survival time*: After hatching, chicks were observed every day until 50 days old.
- *Number of fledglings produced*: Chick flight was considered successful when they were able to fly and feed alone.

2.5 | Bird body mass

During the entire experiment, birds were weighed every week with a Pesola Newton scale to the nearest 5 g.

2.6 | Statistics

Statistical analyses were performed using R (version 4.1.2). To test the effect of the crossed treatment on the measured variables, we systematically added anthelmintic treatment, lead exposure and their interaction in all models as explanatory factors. If we observed effects of lead exposure and anthelmintic treatment alone (i.e. without statistical interaction), this supported the hypothesis of additive effects. On the other hand, if we observed a significant statistical interaction between lead exposure and anthelmintic treatment, this was in favour of negative synergistic or antagonistic combined effects. In this case, post hoc tests have been performed to determine the direction of the effects. We also systematically added random effects in models: the aviary for the cell-mediated immune response, the number of hatched eggs and the number of fledglings produced; the pigeon ID nested in the aviary for the humoral immune response and the bird body mass; the mother's ID nested in the aviary for the egg mass, the chick body mass at hatching and the chick survival time; the chick ID nested in the mother's ID nested in the aviary for the chick survival rate. For each variable, different models were compared, with and without random effects, and cofactors, using likelihood ratio tests to determine which explained a higher proportion of the variance in the data. Furthermore, if the p -value associated with the statistical interaction between lead exposure and anthelmintic treatment was greater than 0.05, we removed this interaction from the model (which meant that there were no negative or synergistic effects) to test the existence of additive effects of these treatments on the measured variables by keeping lead exposure and anthelmintic treatment alone into the model. All initial models and final statistical models retained are summarized in the Table 1. For the egg mass (model 3), the number of hatched eggs (model 4), the chick body mass at hatching (model 5), the chick survival rate (model 6), the chick survival time (model 7), the number of fledging produced (model 8) and the bird body mass (model 9), the aviary random effects were finally not retained. For the cell-mediated immune response (model 1), the humoral immune response (model 2),

egg mass (model 3), chick body mass at hatching (model 5) and bird body mass (model 9), we used five linear mixed models (LMM). For the cell-mediated immune response, we added sex and the t_0 thickness of the wing measurement as cofactors and the aviary as a random effect. For the humoral immune response, we applied a log10 transformation to approximate normality and added the pigeon ID as a random effect and the week and the sex as cofactors. For egg mass and chick body mass at hatching, we added the mother's ID as a random effect. For bird body mass, we added the week as a cofactor, the centred initial mass as a covariate, and the pigeon ID as a random effect. For the number of hatched eggs (the number of hatched eggs produced by each female of the experiment, model 4) and the number of fledglings produced (the number of fledglings produced by each female of the experiment, model 8), we used two generalized linear models (GLM) for a Poisson distribution, as the aviary was not retained in the final model, with the total number of eggs laid by the pairs as a cofactor for the number of hatched eggs. For the chick survival time (model 7), we first ran a linear mixed model (LMM) with the mother's ID nested in the aviary as a random effect. The aviary was not retained in the final model; therefore, we finally performed a LMM with only the mother's ID as a random effect. For the chick survival rate (model 6), we used a mixed effects Cox model with the chick ID nested in the mother's ID as a random effect. Also, for the chick survival rate, we used the `survfit` function of the `survival` package to make the graph. In Section 3, when treatments (lead exposure and/or anthelmintic treatment) were not retained in the best model, the non-significant p -values of these treatments reported were extracted from likelihood ratio tests (LTR, see Tables 1 and 2). Finally, in order to illustrate our results by making the link with the hypothesis that there are combined effects between pollutants and parasites, we represented our measured variables among our two crossed treatments (lead exposure \times anthelmintic treatment) for all the figures, even when one of the two factors did not significantly affect the variable (Table 3).

3 | RESULTS

3.1 | Immunocompetence

We detected significant effects of lead exposure and sex on the wing swelling at the injection site (lead exposure: $F_{1,10.3}=7.48$, $p=0.02$, sex: $F_{1,122.5}=4.72$, $p=0.03$, Figure 2a). PHA-induced wing swelling was lower in birds exposed to lead as compared to birds not exposed to lead during the experiment (birds exposed to lead: $2.45 \text{ mm} \pm 0.09 \text{ SE}$ and birds not exposed to lead: $2.97 \text{ mm} \pm 0.12 \text{ SE}$) and greater in males than in females (males: $2.84 \text{ mm} \pm 0.12 \text{ SE}$ and females: $2.63 \text{ mm} \pm 0.10 \text{ SE}$). In contrast, we did not detect any effect of the anthelmintic treatment ($\text{LRT}=1.77$, $\text{df}=1$, $p=0.18$) or the interaction between the anthelmintic treatment and the lead exposure ($\text{LRT}=1.65$, $\text{df}=1$, $p=0.20$) on wing swelling.

We detected an interactive effect between anthelmintic treatment and lead exposure on the anti-KLH levels which differed over time (three-way interaction: $F_{8,987.4}=4.09$, $p<0.001$, Figure 2b).

TABLE 1 The initial models and final models retained to test the effects of the treatments (lead exposure × antiparasitic treatment) on the different variables measured.

Model ID	Measured variables	Model type	Fixed effects in the initial models	Random effects in the initial models	Fixed effects retained in the final models	Random effects retained in the final models
1	Cell-mediated immune response	LMM	Lead exposure × anthelmintic treatment + sex + t0 thickness of the wing measurement	Aviary	Lead exposure + sex + t0 thickness of the wing measurement	Aviary
2	Humoral immune response	LMM	Lead exposure × anthelmintic treatment × week + sex	Pigeon ID nested in the aviary	Lead exposure × anthelmintic treatment × week	Pigeon ID nested in the aviary
3	Egg mass	LMM	Lead exposure × anthelmintic treatment + laying date	Mother's ID nested in the aviary	Lead exposure	Mother's ID
4	Number of hatched eggs	GLM (Poisson)	Lead exposure × anthelmintic treatment + total number of eggs laid by the pairs	Aviary	Total number of eggs laid by the pairs	
5	Chick body mass at hatching	LMM	Lead exposure × anthelmintic treatment	Mother's ID nested in the aviary	Lead exposure + anthelmintic treatment	Mother's ID
6	Chick survival rate	Mixed effect Cox model	Lead exposure × anthelmintic treatment	Chick ID nested in the mother's ID nested in the aviary	1	Chick ID nested in the mother's ID
7	Chick survival time	GLMM (Negative binomial)	Lead exposure × anthelmintic treatment	Mother's ID nested in the aviary	1	Mother's ID
8	Number of fledging produced	GLM (Poisson)	Lead exposure × anthelmintic treatment + total number of eggs laid by the pairs	Aviary	1	
6	Bird body mass	LMM	Lead exposure × anthelmintic treatment × week + sex + centred initial mass	Pigeon ID nested in the aviary	Lead exposure: anthelmintic treatment + anthelmintic exposure: week + lead exposure: week + sex + week + anthelmintic treatment + lead exposure + centred initial mass	Pigeon ID

TABLE 2 Results of models used to test the effects of cross-treatment (lead exposure × anthelmintic treatment) and cofactors on immunocompetence (cell-mediated and humoral-mediated immune responses), reproduction (egg mass, hatching success, chick body mass at hatching, chick survival rate, chick survival time, and number of fledglings produced) and bird body mass in feral pigeons.

Variables	Fixed effects	<i>F</i> or χ^2	<i>p</i>
PHA-induced wing swelling	Lead exposure	$F_{1,10.3} = 7.48$	0.02
	Sex	$F_{1,122.5} = 4.72$	0.03
	Anthelmintic treatment	LRT = 1.77	0.18
	Lead-exposure × anthelmintic treatment	LRT = 1.65	0.20
Anti-KLH levels	Lead exposure × anthelmintic treatment × time	$F_{8,987.4} = 4.09$	<0.001
Egg mass	Lead exposure	$F_{1,46.4} = 4.02$	0.05
	Anthelmintic treatment	LRT = 1.74	0.19
	Lead-exposure × anthelmintic treatment	LRT = 0.20	0.66
Number of hatched eggs	Lead-exposure	LRT = 2.08	0.15
	Anthelmintic treatment	LRT = 0.08	0.78
	Lead-exposure × anthelmintic treatment	LRT = 0.49	0.48
Chick body mass at hatching	Lead-exposure	$F_{1,22.4} = 4.41$	0.047
	Anthelmintic treatment	$F_{1,21.5} = 5.0$	0.036
	Lead-exposure × anthelmintic treatment	LRT = 0.04	0.84
Chick survival rate	Lead-exposure	LRT = 0.35	0.55
	Anthelmintic treatment	LRT = 0.01	0.91
	Lead-exposure × anthelmintic treatment	LRT = 2.55	0.11
Chick survival time	Lead exposure	LRT = 0.18	0.68
	Anthelmintic treatment	LRT = 2.87	0.09
	Lead exposure and anthelmintic treatment	LRT = 1.62	0.20
Number of fledglings produced	Lead exposure	LRT = 2.95	0.086
	Anthelmintic treatment	LRT = 1.10	0.29
	Lead exposure and anthelmintic treatment	LRT = 0.88	0.35
Bird body mass	Lead-exposure × time	$F_{1,3281.3} = 29.90$	<0.001
	Anthelmintic treatment × time	$F_{1,3281.3} = 12.84$	<0.001
	Lead-exposure × anthelmintic treatment	$F_{1,126.1} = 3.77$	0.054
	Sex	$F_{1,126} = 25.3$	<0.001

Note: Fixed effects in bold in the table are those retained in the best final models, after comparison of several models by likelihood ratio test (see Section 2.6). When treatments (lead exposure and/or anthelmintic treatment) were not retained in the best model, the non-significant *p*-values of these treatments reported were extracted from likelihood ratio tests. Significant *p*-values (<0.05) are in bold in the table.

Indeed, at the peak of the secondary humoral immune response (week 21), we detected a significant interaction between lead exposure and anthelmintic treatment ($F_{1,121} = 6.72$, $p = 0.01$). In individuals not exposed to lead during the experiment, there was no difference in anti-KLH levels between birds with anthelmintic treatment and without anthelmintic treatment ($F_{1,62} = 1.89$, $p = 0.18$), while in lead-exposed environment, anti-KLH levels in birds with anthelmintic treatment were significantly higher than in birds without anthelmintic treatment ($F_{1,59} = 5.29$, $p = 0.03$, with anthelmintic treatment: 4.58 anti-KLH antibody levels ± 0.14 SE, without anthelmintic treatment: 4.01 anti-KLH antibody levels ± 0.20 SE). For the other weeks, including the peak of the primary immune response (week 16), we did not detect any interactive effect between lead exposure and anthelmintic treatment or any effect of lead-exposure alone or anthelmintic treatment alone (Figure 2b).

3.2 | Reproduction

We detected a significant effect of lead exposure on egg mass with eggs of birds exposed to lead being lighter than eggs of birds not exposed to lead during the experiment (birds exposed to lead: 15.12 g ± 0.22 SE and birds not exposed to lead: 16.14 g ± 0.15 SE, $F_{1,46.4} = 4.02$, $p = 0.05$; Figure 3a). However, egg mass was not significantly affected by the anthelmintic treatment alone (LRT = 1.74, $df = 1$, $p = 0.19$) or an interaction with lead exposure (LRT = 0.20, $df = 1$, $p = 0.66$).

We did not detect any effect of lead-exposure (LRT = 2.08, $df = -1$, $p = 0.15$), anthelmintic treatment (LRT = 0.08, $df = -1$, $p = 0.78$) or an interaction between lead-exposure and anthelmintic treatment (LRT = 0.49, $df = -1$, $p = 0.48$) on the number of hatched eggs.

We detected significant effects of the anthelmintic treatment and the lead exposure on chick body mass at hatching (anthelmintic treatment: $F_{1,21.5}=5.0$, $p=0.036$; lead-exposure: $F_{1,22.4}=4.41$, $p=0.047$; the interaction between anthelmintic treatment and lead-exposure was not significant: $LRT=0.04$, $df=1$, $p=0.84$). Birds

TABLE 3 The effects of the treatments on the variables measured, and whether the combined effects of lead exposure and helminth presence were additive, negative synergistic or antagonistic.

Effects of crossed treatments: Lead exposure × anthelmintic treatment	Variable to explained affected by the crossed treatments
No effects of exposure to lead or anthelmintic treatment	<ul style="list-style-type: none"> • Number of hatched eggs • Number of fledging produced • Chick survival rate
Negative effect of the anthelmintic treatment alone	<ul style="list-style-type: none"> • Chick survival time
Negative effect of exposure to lead alone	<ul style="list-style-type: none"> • PHA • Egg mass
Additive negative effects of exposure to lead and anthelmintic treatment	<ul style="list-style-type: none"> • Chick body mass at hatching • Bird body mass
Synergistic negative effects of exposure to lead and anthelmintic treatment	<ul style="list-style-type: none"> • KLH
Antagonistic effects of exposure to lead and anthelmintic treatment	

without anthelmintic treatment and birds exposed to lead produced lighter chicks at hatching than birds with anthelmintic treatment and birds not exposed to lead (birds exposed to lead without anthelmintic treatment: $10.77\text{g} \pm 0.49\text{ SE}$, birds exposed to lead with anthelmintic treatment: $12.21\text{g} \pm 0.38\text{ SE}$, birds not exposed to lead without anthelmintic treatment: $12.0\text{g} \pm 0.62\text{ SE}$ and birds not exposed to lead with anthelmintic treatment: $13.63\text{g} \pm 0.48\text{ SE}$, [Figure 3b](#)).

The chick survival rate was not significantly affected by the interaction between lead-exposure, anthelmintic treatment ($LRT=2.55$, $df=1$, $p=0.11$), or by anthelmintic treatment or lead-exposure alone (anthelmintic treatment: $LRT=0.01$, $df=1$, $p=0.91$; lead exposure: $LRT=0.35$, $df=1$, $p=0.55$).

We detected a non-significant trend of the anthelmintic treatment of the parents on the chick survival time ($LRT=2.87$, $df=1$, $p=0.09$), chicks of birds with anthelmintic treatment survived longer than those of birds without anthelmintic treatment (without anthelmintic treatment group: $6.60\text{days} \pm 1.35\text{ SE}$, with anthelmintic treatment group: $11.74\text{days} \pm 1.54\text{ SE}$, [Figure 3c](#)). We did not detect any effect of lead exposure or an interaction between lead exposure and anthelmintic treatment on chick survival time ($LRT=0.18$, $df=1$, $p=0.68$ and $LRT=1.62$, $df=1$, $p=0.20$, respectively).

We only detected a non-significant trend of lead-exposure on the number of fledglings produced ($LRT=2.95$, $df=-1$, $p=0.086$), with a lower number of fledglings produced by birds exposed to lead (0.29 fledglings produced $\pm 0.10\text{ SE}$) than by birds not exposed to lead during the experiment (0.66 fledglings produced $\pm 0.12\text{ SE}$). However, we did not detect any effect of anthelmintic treatment alone ($LRT=1.10$, $df=-1$, $p=0.29$) or an interaction with lead-exposure ($LRT=0.88$, $df=-1$, $p=0.35$).

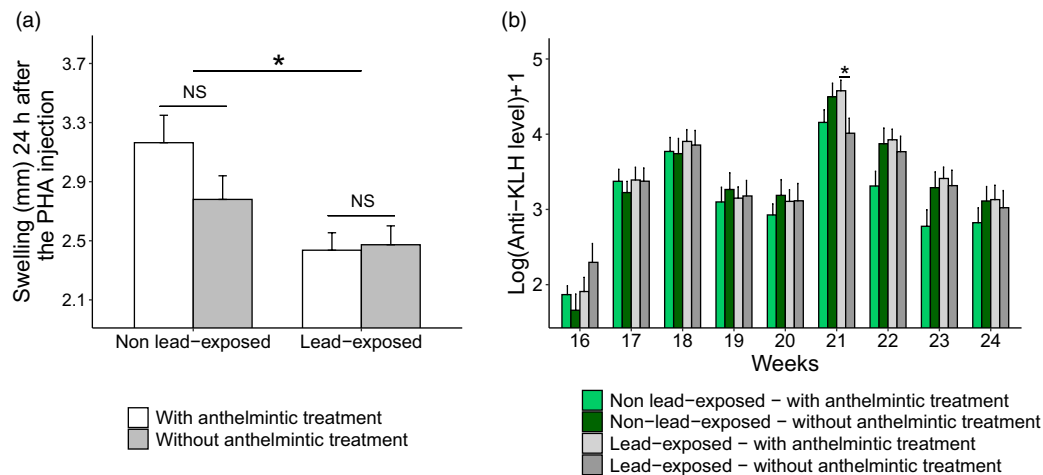


FIGURE 2 (a) Cell-mediated immune response (swelling response (mm) of the wing after PHA injection, mean \pm SE) of birds with anthelmintic treatment (white bars) or without anthelmintic treatment (grey bars) and exposed to lead or not at the 14th week of experiment. While the swelling response was significantly higher in males than in females ($F_{1,122.5}=4.72$, $p=0.03$), we also observed a significant negative effect of lead exposure on this swelling response ($F_{1,10.3}=7.48$, $p=0.02$) but no effect of anthelmintic treatment ($LRT=1.77$, $df=1$, $p=0.18$) or the interaction between anthelmintic treatment and lead exposure ($LRT=1.65$, $df=1$, $p=0.20$). (b) Humoral immune response (anti-KLH antibody levels in plasma, after two KLH injections, mean \pm SE) of lead-exposed birds (grey) and non-lead-exposed (green), with anthelmintic treatment (light) or without anthelmintic treatment (dark), across 9 weeks (week 16–24 of the 26 weeks of experiment). At the peak of the secondary humoral immune response (week 21), we detected a significant interaction between lead exposure and anthelmintic treatment ($F_{1,121}=6.72$, $p=0.01$). No difference between the treatments were detected in the other weeks (weeks 16–20 and 22–24 of the 26 weeks of experiment). Significant differences between groups are indicated by an asterisk (NS: $p>0.05$, *: $p<0.05$). SE, standard error.

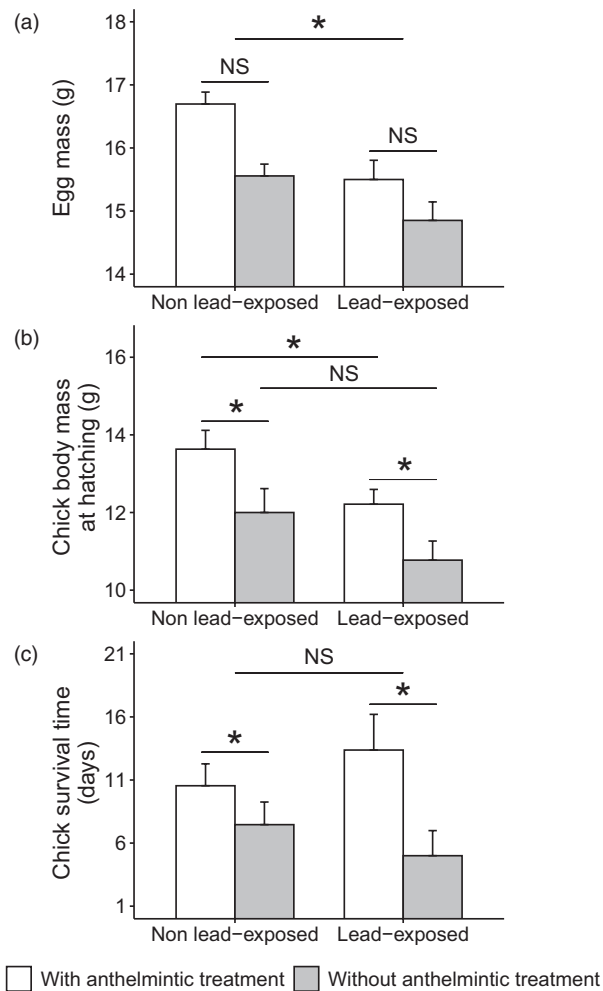


FIGURE 3 (a) Egg mass (g, mean + SE) laid by birds with anthelmintic treatment (white bars) or without anthelmintic treatment (grey bars) and exposed to lead or not. We detected a significant effect of lead exposure ($F_{1,46.4}=4.02$, $p=0.05$) but no effect of the anthelmintic treatment alone (LRT=1.74, $df=1$, $p=0.19$) or an interaction with lead exposure (LRT=0.20, $df=1$, $p=0.66$). (b) Chick body mass at hatching (g, mean + SE) produced by birds with anthelmintic treatment (white bars) or without anthelmintic treatment (grey bars) and exposed to lead or not. We detected effects of the anthelmintic treatment ($F_{1,21.5}=5.0$, $p=0.036$) and the lead exposure ($F_{1,22.4}=4.41$, $p=0.047$) on chick mass at hatching and the interaction between anthelmintic treatment and lead-exposure was not significant (LRT=0.04, $df=1$, $p=0.84$). (c) Chick survival time (days, mean + SE) for chicks produced by birds with anthelmintic treatment (white bars) or without anthelmintic treatment (grey bars) and exposed to lead or not. We detected a marginally non-significant trend of the anthelmintic treatment of the parents on the chick survival time (LRT=2.87, $df=1$, $p=0.09$), but we did not detect any effect of lead exposure (LRT=0.18, $df=1$, $p=0.68$) or an interaction between lead exposure and anthelmintic treatment (LRT=1.62, $df=1$, $p=0.20$). Significant difference between groups is indicated by an asterisk (NS: $p > 0.05$, *: $p < 0.05$). SE, standard error.

3.3 | Bird body mass

Bird body mass was significantly affected by the interaction between lead-exposure and time ($F_{1,3281.3}=29.90$, $p < 0.001$), with a more pronounced decrease in mass in pigeons not exposed to lead, compared to pigeons exposed (-0.33 ± 0.06), and by the interaction between the anthelmintic treatment and time ($F_{1,3281.3}=12.84$, $p < 0.001$), with a more pronounced decrease in mass in pigeons with anthelmintic treatment, compared to pigeons without anthelmintic treatment (-0.22 ± 0.06). Moreover, we detected a non-significant trend of the interaction between lead-exposure and anthelmintic treatment ($F_{1,126.1}=3.77$, $p=0.054$). Overall, we observed a decrease in mean body mass over time in all groups (Figure 4) compared to the mean body mass at the start of the experiment. However, the significant interactions found revealed that this body mass decline was less pronounced for individuals without anthelmintic treatment and exposed to lead during the experiment (Figure 4). In addition, males were significantly heavier than females ($F_{1,126}=25.3$, $p < 0.001$).

4 | DISCUSSION

Our results are consistent with the hypothesis that lead exposure and intestinal parasites have combined negative effects. Indeed, we observed negative additive effects of lead exposure and helminth presence (no anthelmintic treatment) on the mass of chicks at hatching and negative synergistic effects on the humoral immune response. However, we mainly observed a negative effect of lead exposure on the different variables measured rather than combined effects of lead exposure and helminth presence or an effect of helminth presence alone. Furthermore, in contrast to the predictions of the detoxication hypothesis (Jeantet et al., 2023; Figure 1), we did not observe antagonistic effects between lead exposure and the helminth presence on the different variables measured. However, we cannot exclude the existence of a detoxifying effect of intestinal parasites. Indeed, studies have shown that such effects of helminths on their hosts may only be observed under some conditions. Molbert et al. (2021) for example showed that the antagonistic effects between pollutants and helminths on the chub, *Squalius cephalus*, only appeared with a specific level of exposure to organic pollutants. Indeed, by exposing chubs to organic pollutants (at a concentration of 50 ng of polycyclic aromatic hydrocarbon (PAH) diluted in a gram of vegetable oil), they observed a higher reactive oxygen metabolites (ROM) concentration in infected chubs compared to uninfected chubs, whereas with a 100-fold greater exposure to PAH, they observed the opposite, that is a higher ROM concentration in uninfected chubs compared to infected chubs. These results suggest that at the lower concentration, the detoxifying benefit of the parasites was lower than the costs of the infestation, whereas

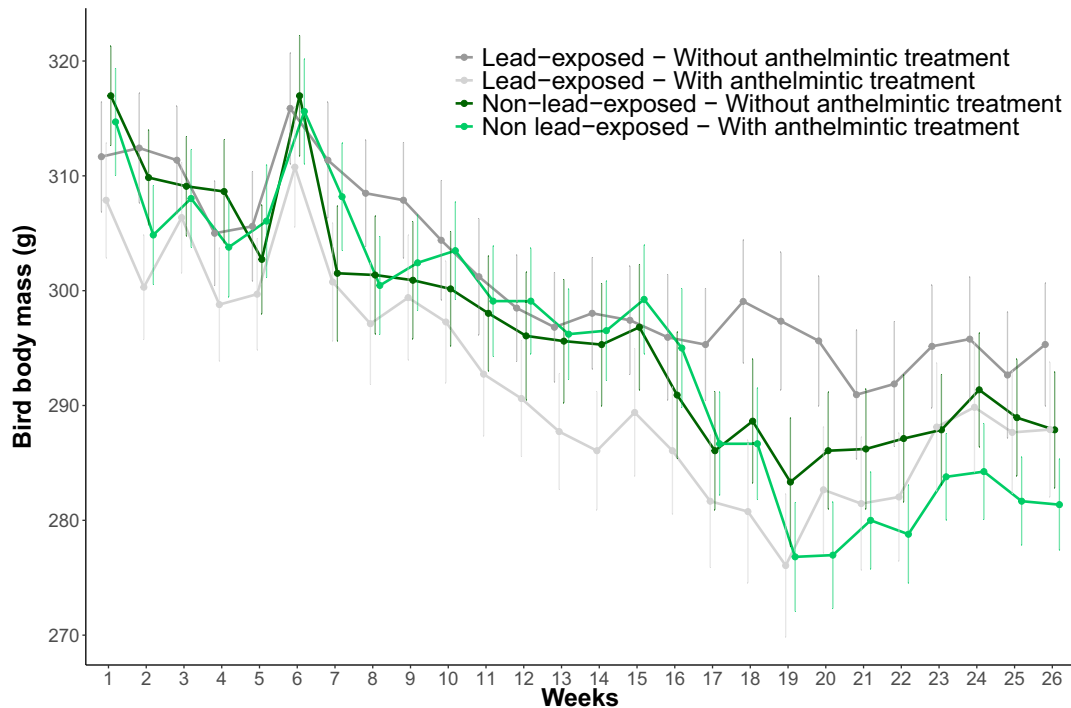


FIGURE 4 Bird body mass (g, mean \pm SE) across the 26 weeks of the experiment for non-lead-exposed birds without anthelmintic treatment (dark green line), non-lead-exposed birds with anthelmintic treatment (light green line), lead-exposed birds without anthelmintic treatment (dark grey line) and lead-exposed birds with anthelmintic treatment (light grey line). SE, standard error. We detected effects of the interactions between lead-exposure and time ($F_{1,3281.3} = 29.90$, $p < 0.001$), and between the anthelmintic treatment and time ($F_{1,3281.3} = 12.84$, $p < 0.001$) on the bird body mass.

at the 100-fold higher concentration, the benefit provided by the parasites was greater than their cost. Thus, in our study, we cannot fully reject the detoxifying capacity of helminths for pigeons, but if it exists, it seems that this benefit was lower than the costs of the infestation, at least in the range of lead pollution found in the city of Paris. Therefore, in urban environments, such as Paris, the levels of lead-pollution would not be high enough to detect the benefits of this detoxification process by parasites (but it may change especially with the increase of environmental lead after the Notre-Dame cathedral fire in 2019).

The main significant effects we found in this long-term experimental study concerned lead exposure that negatively impacted several fitness parameters alone, including the cell-mediated immune response and egg mass, or in a cumulative way with the helminth presence for chick body mass at hatching. These negative effects of lead exposure reinforce the idea that such trace metals are toxic, even at low levels, for organisms as previously reported for reproductive success (Chatelain, Gasparini, & Frantz, 2016a; Chatelain, Gasparini, Haussy, & Frantz, 2016; Eeva et al., 2009; Nam & Lee, 2006) and immunity (Chatelain, Gasparini, & Frantz, 2016b; Sanchez-Ramirez et al., 2007; Singh et al., 2003; Snoeijs et al., 2004). However, our treatment seems to have affected the quality of the offspring (egg mass and chick body mass at hatching) rather than the quantity, as we did not detect any effect of lead exposure or anthelmintic treatment on the number of hatched eggs and the number of fledglings produced. It would be interesting to test whether

these effects on quality may impact the long-term survival of the offspring. We predict that because of their lower quality at hatching, chicks whose parents had been exposed to lead and had not receive anthelmintic treatment will exhibit a reduced fitness if compared to chicks whose parents were not exposed to lead and had receive anthelmintic treatment with a lower survival and reproduction.

For the mass of chicks at hatching, we observed negative additive effects of lead and helminth presence (but without a statistical interaction): chicks whose parents had been exposed to lead and had not receive anthelmintic treatment were lighter than chicks whose parents were exposed only to lead or than chicks whose parents had not receive anthelmintic treatment. In contrast, we observed that lead exposure and helminth presence interacted to affect the humoral immune response. Our results indicate that 1 week after the second injection of KLH (peak of the secondary immune response, week 21, Figure 2b), in the absence of lead exposure, the secondary immune responses of pigeons were not affected by helminth presence, whereas, when exposed to lead, pigeons whose had not receive anthelmintic treatment had a significantly weaker humoral response than those had receive anthelmintic treatment. In this situation, it is possible that lead exposure reduced the tolerance of birds to helminth presence. This in line with the study of Marcogliese et al. (2005), who found that the levels of oxidative stress in yellow perch (*Perca flavescens*) were negatively affected by infestation by the nematode *Raphidascaris acus* but only in polluted environments. These results suggest that, in feral pigeons, negative combined

effects between pollutants exposure and parasites occur, and can have different effects depending on the variables considered: the negative effects can be cumulative (negative additive effects) or multiplied (negative synergistic effects) (Figure 1).

We observed a statistical interaction between lead exposure and helminth presence that influenced pigeon body mass over time. First, we found that pigeons in all groups lost significant body mass from the start of the experiment to around the 19th week of treatment (Figure 4). This could be partially explained by the investment in reproduction during this time (Freed, 1981; Rands et al., 2006). However, we cannot exclude an effect of stress related to captivity and handling (Angelier et al., 2016). Surprisingly, body mass loss was less pronounced in the pigeons exposed to lead and whose had not received anthelmintic treatment and was more pronounced in the pigeons not exposed to lead and whose had received anthelmintic treatment (Figure 4). We observed that chick body mass at hatching (which could result from greater investment in reproduction) were higher for pigeons not exposed to lead and that had received anthelmintic treatment, which was also the group that lost the most body mass. Thus, the cost of parental care may have contributed to the highest body mass losses in this group (Freed, 1981).

Overall, we observed very high chick mortality in all groups, whatever the treatment. To our knowledge, there are no reported estimates for pigeon chick survival rates in the natural environment but such low overall survival rates might be explained by infestation

by helminth presence through the faeces in the nest. The survival rate was nevertheless better for pigeons not exposed to lead in combination with anthelmintic treatment suggesting negative effects of pollutants in addition to the effect of helminth presence (Figure 5). We also observed that the chicks of parents that had received anthelmintic treatment survived longer on average than the chicks of parents without anthelmintic treatment. Before the death of the chicks, we observed that they no longer gained body mass or even lost mass. This effect on the mass of the chicks could be partially explained by lower energy availability of the parents that had not received anthelmintic treatment to feed their young, compared to the parents that had received anthelmintic treatment, due to the cost of the helminth infestation (Hicks, Burthe, Daunt, Newell, Butler, et al., 2018; Hicks, Burthe, Daunt, Newell, Chastel, et al., 2018).

Moreover, we observed that helminth presence had only limited impacts on the fitness variables. Our coproscopy results nevertheless indicated that the anthelmintic treatment efficiently reduced nematode load. We propose two hypotheses to explain this result. First, we only controlled the presence/absence of nematodes through the anthelmintic treatment; without controlling the abundance of intestinal parasites in the groups without anthelmintic treatment. It is possible that the natural nematode abundance in pigeons that had not received anthelmintic treatment was low and therefore limited our capacity to detect an effect of the anthelmintic treatment. In addition, a low natural infestation may have

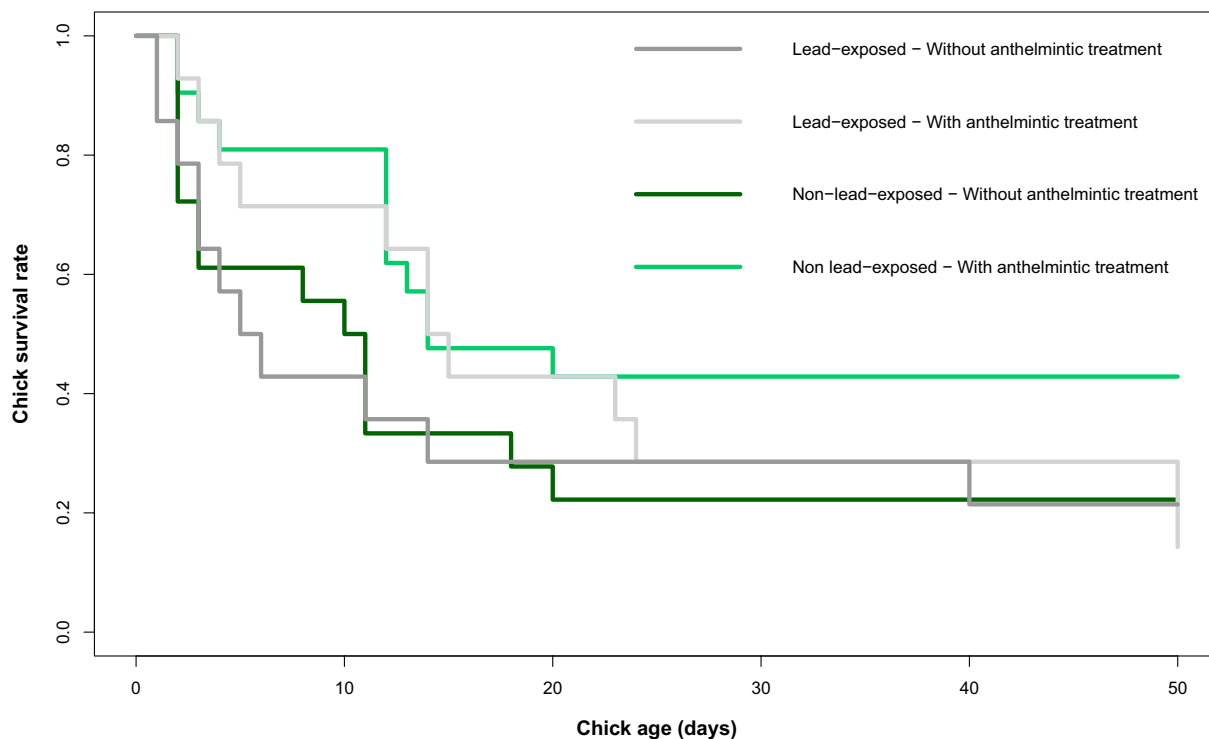


FIGURE 5 Chick survival rate over the chick age by experimental group (dark green line: Non-lead-exposed without deworming treatment, light green line: Non-lead-exposed with deworming treatment, dark grey line: Lead-exposed without anthelmintic treatment, light grey line: Lead-exposed with anthelmintic treatment), with a similar trend for each curve. The chick survival rate was not significantly affected by the interaction between lead-exposure and anthelmintic treatment (LRT = 2.55, $df = 1$, $p = 0.11$), or by anthelmintic treatment (LRT = 0.01, $df = 1$, $p = 0.91$) or lead-exposure (LRT = 0.35, $df = 1$, $p = 0.55$) alone.

been influenced by the living conditions in the aviaries, which were cleaned every week, and may have limited re-infestations via faeces (Lloyd, 2003). This hypothesis could be investigated by limiting the cleaning of infrastructures in future studies to more closely resemble the natural living conditions of pigeons in the wild and allowing nematode re-infestation in the group without anthelmintic treatment. Second, it is possible that the effects of anthelmintic treatment could have been masked by the presence of intestinal parasites other than the nematodes eliminated by the anthelmintic treatment. Indeed, birds typically host a variety of different parasites, including several species of intestinal parasites. These intestinal parasites live in interaction with each other in different part of the gut (Biard et al., 2015; Graham, 2008; Larsen et al., 2002; Nunn et al., 2014). Removing one group can therefore positively or negatively impact the other parasite species present and may have masked the effects of the anthelmintic treatment. Finally, the effects of pollutants and parasites could be exacerbated in low-resources conditions, because of a higher immune cost in low-resources conditions for example (Bize et al., 2010). As birds were fed ad libitum throughout the experiment, we hypothesized that it might have minimized the effects of helminth presence or lead exposure.

In conclusion, our results demonstrate that helminth presence and lead exposure may impair costs in pigeons either in an additive way or a synergistic negative way, but they did not confirm the presence of an antagonistic effect between pollutants and parasites (detoxication effect). Nevertheless, in general, our results reveal the existence of negative combined effects between pollutant exposure and intestinal parasites, highlighting the importance of accounting for multiple stress factors when studying the effects of exposure to pollutants and/or other environmental stressors on the fitness of organisms. Our study suggests that the combination of parasite and pollutant pressures in urban ecosystems might increase the constraints experienced by local organisms and in turn increase the probability of extinction of their population.

AUTHOR CONTRIBUTIONS

Aurélije Jeantet, Julien Gasparini and Fabienne Audebert conceived the ideas and designed the methodology; Aurélije Jeantet analysed the data and led the writing of the manuscript together with Julien Gasparini, David Rozen-Rechels and Fabienne Audebert. All the authors collected the data, contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

This work was supported by the French National program EC2CO (Ecosphère Continentale et Côtière). AJ's PhD grant was funded by the 'Biodiversity, Evolution, Ecology, Society' initiative of the Sorbonne University Alliance. The authors thank Dr. K. Liittschwager (USA) for revising the grammar of the text. This study was carried out in strict accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. All experiments and captures were approved by Charles Darwin Animal

Experimentation Ethics Committee and French authorities (the "Ministère de l'éducation nationale, de l'enseignement supérieur et de la recherche", permit no. #17554 2018111610466351; and Ville de Paris).

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data available from the Zenodo Repository: <https://doi.org/10.5281/zenodo.10621236> (Jeantet et al., 2024).

ORCID

Aurélije Jeantet  <https://orcid.org/0009-0000-7261-1642>

Fabienne Audebert  <https://orcid.org/0000-0002-6039-4254>

Beatriz Decencièrre  <https://orcid.org/0000-0002-2847-7631>

Camille Lorang  <https://orcid.org/0009-0005-4698-4695>

Pierre Federici  <https://orcid.org/0009-0009-5615-6104>

David Rozen-Rechels  <https://orcid.org/0000-0003-4351-8961>

Julien Gasparini  <https://orcid.org/0000-0003-2594-6567>

REFERENCES

- Al-Barwari, S., & Saeed, I. (2013). The parasitic communities of the rock pigeon *Columba livia* from Iraq: Component and importance. *Turkish Journal of Parasitology*, 36(4), 232–239. <https://doi.org/10.5152/tpd.2012.56>
- Angelier, F., Parenteau, C., Trouvé, C., & Angelier, N. (2016). Does the stress response predict the ability of wild birds to adjust to short-term captivity? A study of the rock pigeon (*Columbia livia*). *Royal Society Open Science*, 3(12), 160840. <https://doi.org/10.1098/rsos.160840>
- Biard, C., Monceau, K., Motreuil, S., & Moreau, J. (2015). Interpreting immunological indices: The importance of taking parasite community into account. An example in blackbirds *Turdus merula*. *Methods in Ecology and Evolution*, 6(8), 960–972. <https://doi.org/10.1111/2041-210X.12371>
- Bize, P., Piau, R., Gasparini, J., & Roulin, A. (2010). Indirect costs of parasitism are shaped by variation in the type of immune challenge and food availability. *Evolutionary Biology*, 37(4), 169–176. <https://doi.org/10.1007/s11692-010-9092-5>
- Bize, P., Roulin, A., Tella, J. L., Bersier, L., & Richner, H. (2004). Additive effects of ectoparasites over reproductive attempts in the long-lived alpine swift. *Journal of Animal Ecology*, 73(6), 1080–1088. <https://doi.org/10.1111/j.0021-8790.2004.00880.x>
- Brázová, T., Miklisová, D., Barčák, D., Uhrovič, D., Šalamún, P., Orosová, M., & Oros, M. (2021). Hazardous pollutants in the environment: Fish host-parasite interactions and bioaccumulation of polychlorinated biphenyls. *Environmental Pollution*, 291, 118175. <https://doi.org/10.1016/j.envpol.2021.118175>
- Bustnes, J. O., Erikstad, K. E., Hanssen, S. A., Tveraa, T., Folstad, I., & Skaare, J. U. (2006). Anti-parasite treatment removes negative effects of environmental pollutants on reproduction in an Arctic seabird. *Proceedings of the Royal Society B: Biological Sciences*, 273(1605), 3117–3122. <https://doi.org/10.1098/rspb.2006.3687>
- Chatelain, M., Gasparini, J., & Frantz, A. (2016a). Do trace metals select for darker birds in urban areas? An experimental exposure to lead and zinc. *Global Change Biology*, 22(7), 2380–2391. <https://doi.org/10.1111/gcb.13170>
- Chatelain, M., Gasparini, J., & Frantz, A. (2016b). Trace metals, melanin-based pigmentation and their interaction influence immune

- parameters in feral pigeons (*Columba livia*). *Ecotoxicology*, 25(3), 521–529. <https://doi.org/10.1007/s10646-016-1610-5>
- Chatelain, M., Gasparini, J., Haussy, C., & Frantz, A. (2016). Trace metals affect early maternal transfer of immune components in the feral pigeon. *Physiological and Biochemical Zoology*, 89(3), 206–212. <https://doi.org/10.1086/685511>
- Chatelain, M., Gasparini, J., Jacquin, L., & Frantz, A. (2014). The adaptive function of melanin-based plumage coloration to trace metals. *Biology Letters*, 10(3), 20140164. <https://doi.org/10.1098/rsbl.2014.0164>
- Chege, H. W., Kemboi, D. C., Bebora, L. C., Maingi, N., Mbutia, P. G., Nyaga, P. N., & Njagi, L. W. (2017). Efficacy of Piperazine citrate, levamisole hydrochloride and Albendazole in the treatment of chicken naturally infected with gastrointestinal helminths. *Livestock Research for Rural Development*, 29(5).
- Chin, H. M.-H., Luong, L. T., & Shostak, A. W. (2017). Longitudinal study of parasite-induced mortality of a long-lived host: The importance of exposure to non-parasitic stressors. *Parasitology*, 144(14), 1943–1955. <https://doi.org/10.1017/S0031182017001251>
- Cloquet, C., Carignan, J., Libourel, G., Sterckeman, T., & Perdrix, E. (2006). Tracing source pollution in soils using cadmium and lead isotopes. *Environmental Science & Technology*, 40(8), 2525–2530. <https://doi.org/10.1021/es052232+>
- Combes, C., Gavotte, L., Moulia, C., & Sicard, M. (2018). *Parasitisme: Écologie et évolution des interactions durables*. Dunod.
- Coors, A., Decaestecker, E., Jansen, M., & De Meester, L. (2008). Pesticide exposure strongly enhances parasite virulence in an invertebrate host model. *Oikos*, 117(12), 1840–1846. <https://doi.org/10.1111/j.1600-0706.2008.17028.x>
- Eeva, T., Ahola, M., & Lehtikoinen, E. (2009). Breeding performance of blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*) in a heavy metal polluted area. *Environmental Pollution*, 157(11), 3126–3131. <https://doi.org/10.1016/j.envpol.2009.05.040>
- Freed, L. A. (1981). Loss of mass in breeding wrens: Stress or adaptation? *Ecology*, 62(5), 1179–1186. <https://doi.org/10.2307/1937282>
- Gheorgiu, C., Marcogliese, D. J., & Scott, M. (2005). Concentration-dependent effects of waterborne zinc on population dynamics of *Gyrodactylus turnbulli* (Monogenea) on isolated guppies (*Poecilia reticulata*). *Parasitology*, 132(2), 225. <https://doi.org/10.1017/S003118200500898X>
- Goutte, A., & Molbert, N. (2022). Benefits of parasitism in polluted environments: A review and perspectives. *Frontiers in Ecology and Evolution*, 10, 847869. <https://doi.org/10.3389/fevo.2022.847869>
- Graham, A. L. (2008). Ecological rules governing helminth microparasite coinfection. *Proceedings of the National Academy of Sciences of the United States of America*, 105(2), 566–570. <https://doi.org/10.1073/pnas.0707221105>
- Hicks, O., Burthe, S. J., Daunt, F., Newell, M., Butler, A., Ito, M., Sato, K., & Green, J. A. (2018). The energetic cost of parasitism in a wild population. *Proceedings of the Royal Society B: Biological Sciences*, 285(1879), 20180489. <https://doi.org/10.1098/rspb.2018.0489>
- Hicks, O., Burthe, S. J., Daunt, F., Newell, M., Chastel, O., Parenteau, C., & Green, J. A. (2018). The role of parasitism in the energy management of a free-ranging bird. *Journal of Experimental Biology*, 221, jeb.190066. <https://doi.org/10.1242/jeb.190066>
- Jacquín, L., Haussy, C., Bertin, C., Laroucau, K., & Gasparini, J. (2013). Darker female pigeons transmit more specific antibodies to their eggs than do paler ones: Melanin and maternal antibodies. *Biological Journal of the Linnean Society*, 108(3), 647–657. <https://doi.org/10.1111/bij.12001>
- Jacquín, L., Lenouvel, P., Haussy, C., Ducatez, S., & Gasparini, J. (2011). Melanin-based coloration is related to parasite intensity and cellular immune response in an urban free living bird: The feral pigeon *Columba livia*. *Journal of Avian Biology*, 42(1), 11–15. <https://doi.org/10.1111/j.1600-048X.2010.05120.x>
- Jeantet, A., Audebert, F., Agostini, S., Decencièrre, B., Lorang, C., Jamet, L., Giner, C., Flandi, E., Nardou, N., Lemaire, B., Federici, P., Rozen-Rechels, D., & Gasparini, J. (2024). Data from: Crossed effects of helminth infection and lead exposure on fitness: An experimental study in feral pigeons (*Columba livia*). *Zenodo*, <https://doi.org/10.5281/ZENODO.10621236>
- Jeantet, A., Sandmeyer, L., Campech, C., Audebert, F., Agostini, S., Pellerin, A., & Gasparini, J. (2023). The “parasite detoxification hypothesis”: Lead exposure potentially changes the ecological interaction from parasitism to mutualism. *Ecotoxicology*, 32(5), 666–673. <https://doi.org/10.1007/s10646-023-02678-z>
- Johnston, R. F., & Janiga, M. (1995). *Feral pigeons*. Oxford University Press.
- Kennedy, E. D. (1991). Determinate and indeterminate egg-laying patterns: A review. *The Condor*, 93(1), 106–124. <https://doi.org/10.2307/1368612>
- Larsen, A. H., Bresciani, J., & Buchmann, K. (2002). Interactions between ecto- and endoparasites in trout *Salmo trutta*. *Veterinary Parasitology*, 103(1–2), 167–173. [https://doi.org/10.1016/S0304-4017\(01\)00577-5](https://doi.org/10.1016/S0304-4017(01)00577-5)
- Lloyd, C. (2003). Control of nematode infections in captive birds. *In Practice*, 25(4), 198–206. <https://doi.org/10.1136/inpract.25.4.198>
- Marcogliese, D., Brambilla, L., Gagné, F., & Gendron, A. (2005). Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*. *Diseases of Aquatic Organisms*, 63, 77–84. <https://doi.org/10.3354/dao063077>
- Marcogliese, D. J., & Pietrock, M. (2011). Combined effects of parasites and contaminants on animal health: Parasites do matter. *Trends in Parasitology*, 27(3), 123–130. <https://doi.org/10.1016/j.pt.2010.11.002>
- Molbert, N., Agostini, S., Alliot, F., Angelier, F., Biard, C., Decencièrre, B., Leroux-Coyau, M., Millot, A., Ribout, C., & Goutte, A. (2021). Parasitism reduces oxidative stress of fish host experimentally exposed to PAHs. *Ecotoxicology and Environmental Safety*, 219, 112322. <https://doi.org/10.1016/j.ecoenv.2021.112322>
- Molbert, N., Alliot, F., Leroux-Coyau, M., Médoc, V., Biard, C., Meylan, S., Jacquín, L., Santos, R., & Goutte, A. (2020). Potential benefits of acanthocephalan parasites for chub hosts in polluted environments. *Environmental Science & Technology*, 54(9), 5540–5549. <https://doi.org/10.1021/acs.est.0c00177>
- Morrill, A., Provencher, J. F., Gilchrist, H. G., Mallory, M. L., & Forbes, M. R. (2019). Anti-parasite treatment results in decreased estimated survival with increasing lead (Pb) levels in the common eider *Somateria mollissima*. *Proceedings of the Royal Society B: Biological Sciences*, 286(1910), 20191356. <https://doi.org/10.1098/rspb.2019.1356>
- Nam, D.-H., & Lee, D.-P. (2006). Reproductive effects of heavy metal accumulation on breeding feral pigeons (*Columba livia*). *Science of the Total Environment*, 366(2–3), 682–687. <https://doi.org/10.1016/j.scitotenv.2006.02.004>
- Nunn, C. L., Brezine, C., Jolles, A. E., & Ezenwa, V. O. (2014). Interactions between micro- and macroparasites predict microparasite species richness across primates. *The American Naturalist*, 183(4), 494–505. <https://doi.org/10.1086/675362>
- Rands, S. A., Cuthill, I. C., & Houston, A. I. (2006). Explaining individual variation in patterns of mass loss in breeding birds. *Theoretical Biology and Medical Modelling*, 3(1), 20. <https://doi.org/10.1186/1742-4682-3-20>
- Raynaud, J.-P., William, G., & Brunault, G. (1970). Etude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Annales de Parasitologie Humaine et Comparée*, 45(3), 321–342. <https://doi.org/10.1051/parasite/1970453321>
- Sánchez, M. I., Pons, I., Martínez-Haro, M., Taggart, M. A., Lenormand, T., & Green, A. J. (2016). When parasites are good for health: Cestode parasitism increases resistance to arsenic in brine shrimps. *PLoS Pathogens*, 12(3), e1005459. <https://doi.org/10.1371/journal.ppat.1005459>

- Sanchez-Ramirez, C., Vidal-Martinez, V. M., Aguirre-Macedo, M. L., Rodriguez-Canul, R. P., Gold-Bouchot, G., & Sures, B. (2007). *Cichlidogyrus sclerosus* (Monogenea: Ancyrocephalinae) and its host, the Nile tilapia (*Oreochromis niloticus*), as bioindicators of chemical pollution. *Journal of Parasitology*, 93(5), 1097–1106. <https://doi.org/10.1645/GE-1162R.1>
- Singh, V. K., Mishra, K. P., Rani, R., Yadav, V. S., Awasthi, S. K., & Garg, S. K. (2003). Immunomodulation by Lead. *Immunologic Research*, 28(2), 151–166. <https://doi.org/10.1385/IR:28:2:151>
- Snoeijs, T., Dauwe, T., Pinxten, R., Vandesande, F., & Eens, M. (2004). Heavy metal exposure affects the humoral immune response in a free-living small songbird, the great tit (*Parus major*). *Archives of Environmental Contamination and Toxicology*, 46(3), 399–404. <https://doi.org/10.1007/s00244-003-2195-6>
- Sures, B., Nachev, M., Selbach, C., & Marcogliese, D. J. (2017). Parasite responses to pollution: What we know and where we go in 'Environmental parasitology'. *Parasites & Vectors*, 10(1), 65. <https://doi.org/10.1186/s13071-017-2001-3>
- Tietz Marques, S. M., Marinho De Cuadros, R., Jardim Da Silva, C., & Baldo, M. (2007). Parasites of pigeons (*Columba livia*) in urban areas of lages, southern Brazil. *Parasitologia Latinoamericana*, 62(3–4), 183–187. <https://doi.org/10.4067/S0717-77122007000200014>

How to cite this article: Jeantet, A., Audebert, F., Agostini, S., Decencière, B., Lorang, C., Jamet, L., Giner, C., Flandi, E., Nardou, N., Lemaire, B., Federici, P., Rozen-Rechels, D., & Gasparini, J. (2024). Crossed effects of helminth infection and lead exposure on fitness: An experimental study in feral pigeons (*Columba livia*). *Journal of Animal Ecology*, 93, 1996–2009. <https://doi.org/10.1111/1365-2656.14211>