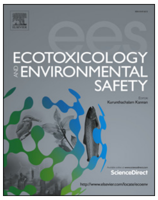
Ecotoxicology and Environmental Safety 161 (2018) 746–754

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Reproduction impairments in metal-polluted environments and parental hormones: No evidence for a causal association in an experimental study in breeding feral pigeons exposed to lead and zinc

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ARTICLE INFO

Keywords:
Ecotoxicology
Urban pollution
Endocrine system
Corticosterone
Prolactin
Testosterone

ABSTRACT

Humans are responsible for land-cover changes resulting in the emission of hazardous chemical elements including metallic trace elements i.e. MTEs. As a consequence, urban wildlife is exposed to high concentrations of MTEs, which exposure is linked to reproductive impairments. MTE effects on reproduction outputs might result from MTE exposure disrupting the endocrine pathways involved in reproductive behaviours. In birds, there is strong evidence that prolactin, corticosterone and testosterone are all involved in the regulation of parental effort during incubation and chick rearing. Endocrine-disrupting chemicals might stimulate or inhibit the production of those hormones and consequently alter parental investment and reproductive success outcomes. We measured baseline corticosterone, prolactin and testosterone plasma levels, and the corticosterone stress response of breeding feral pigeons (*Columba livia*) experimentally exposed to ecologically relevant lead and/or zinc concentrations. Independently of lead and/or zinc exposure, male and female plasma levels of corticosterone and prolactin (but not testosterone) showed temporal variations along the reproduction stages (i.e. incubation, early rearing and late rearing). In addition, both hatching and fledging success were slightly correlated with corticosterone, prolactin and testosterone levels. However, our study did not find any influence of lead or zinc exposure on hormone levels, suggesting that MTE effects on reproductive outputs might not be explained by MTE-induced modifications of corticosterone, prolactin and testosterone-linked behaviours during incubation and rearing. Alternatively, MTE-induced reproductive impairments might result from MTE exposure having direct effects on offspring phenotypes or prenatal indirect effects on the embryo (e.g. maternal transfer of MTEs, hormones or immune compounds).

1. Introduction

Across the globe, anthropogenic activities have led to numerous changes in ecosystems through agricultural activities, deforestation, industrial development or urban land expansion. Amongst other environmental modifications, humans are responsible for land-cover changes resulting in the emission of hazardous chemical elements (e.g. CO_2, NO, metallic trace elements, persistent organic pollutants, organohalogenated contaminants, etc.; Crutzen, 2006). Most of metallic trace elements (MTEs; e.g. lead, cadmium, zinc, copper, etc.) present in the environment originate from anthropogenic sources (Azimi et al., 2005; Bilos et al., 2001; Nriagu, 1989), leading to elevated concentrations nearby metallurgic factories (Dauwe et al., 1999; Derome and Nieminen, 1998; Kiikkilä et al., 2003) but also in urban areas, where lead and zinc are two of the most abundant metals (Azimi et al., 2003; Maas et al., 2010; Manta et al., 2002; Roux and Marra, 2007). In 2030, urban areas are expected to cover 1.5% of the planet's land area (Seto et al., 2012). For this reason, it becomes increasingly essential to better understand MTE effects on urban wildlife.

Nowadays, MTEs are chemical pollutants of prime concern given their implication in several human diseases (reviewed in Jarup, 2003) and their noxious effects on wildlife (Hsu et al., 2006). The relationship between reproductive success and proximity to a metallurgic smelter has been extensively studied in passerine birds from two study regions (in Belgium and in Finland). Birds nesting in the most polluted area consistently exhibited lower reproductive outputs. For instance, proximity to the smelter was associated with higher egg-laying interruptions (in great tits *Parus major* and blue tits *Cyanistes caeruleus*; Janssens et al., 2003; Dauwe et al., 2005), reduced clutch size and hatching success (in great tits and pied flycatchers *Ficedula hypoleuca*; Eeva and

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https://doi.org/10.1016/j.ecoenv.2018.06.043
Received 7 March 2018; Received in revised form 12 June 2018; Accepted 14 June 2018

Lehikoinen, 1995; Janssens et al., 2003; Eeva et al., 2009), lower growth and lower fledging success (in great tits and blue tits; Eeva et al., 2009; Eeva and Lehikoinen, 1996). Moreover, experimental exposure to lead decreased nestling growth, fledgling corpulence and fledging success, while it increased fledgling physiological stress (i.e. heterophile/lymphocyte ratio) (in feral pigeons *Columba livia*; Chatelain et al., 2016a).

Reproductive behaviours including territorial defence, courtship display, incubation and chick rearing are under control of multiple endocrine pathways (Cooke et al., 2004; Eisner, 1960). For instance, there is strong evidence that prolactin, corticosterone and testosterone are all involved in the regulation of parental behaviour in birds (i.e. incubation and rearing, reviewed in Lynn, 2016). First, prolactin secretion is responsible for the initiation and maintenance of incubation (Angelier and Chastel, 2009; Eisner, 1960; Khan et al., 2001; Lormée et al., 2000; Sockman et al., 2000), but also for the expression of parental cares during chick-rearing period (reviewed in Angelier et al., 2016b). For example, experimentally increased prolactin levels can enhance the expression of incubation behaviour, chick-feeding behaviour and chick defense in the rock pigeon (Buntin, 1991; Lea, 1991; Miller et al., 2009; Wang and Buntin, 1999). Importantly, reduced prolactin levels are associated with reproductive failure and abandonment of the chicks in wild birds (Angelier et al., 2015, 2013; Groscolas et al., 2008; Smiley and Adkins-Regan, 2016; Thierry et al., 2013), suggesting that this hormone is linked to reproductive performances. Second, the corticosterone stress response mediates behavioural and physiological adjustments that reduce reproductive activities to promote survival (Breuner et al., 2008; Hau et al., 2010; Landys et al., 2006; Love and Williams, 2008; Wingfield et al., 1998; Wingfield and Sapolsky, 2003). Overall, elevated circulating corticosterone levels are associated with a reduction in parental effort. For instance, experimentally increased corticosterone levels are associated with lower incubation commitment, lower nest attendance, and reduced food provisioning to nestlings in wild birds (Angelier et al., 2009; Silverin, 1986; Spée et al., 2011; Thierry et al., 2014) although the relationship between circulating corticosterone levels and parental behaviour seems context-dependent (Bonier et al., 2009; Ouyang et al., 2015, 2013). Finally, although testosterone is classically involved in the initiation of reproduction (territorial aggression, sexual and courtship behaviour; Wingfield, 1984; Wingfield et al., 1987), it is also known to mediate a trade-off between mating and parental effort in both males and females. For instance, experimental elevation of circulating testosterone levels decreased paternal and maternal care for offspring (Ketterson et al., 1992; Saino and Møller, 1995; Veiga and Polo, 2008, reviewed in Lynn, 2016, 2008) and the time the female spent brooding as well as their nestling defence in wild birds (O'Neal et al., 2008).

In 2002 the World Health Organization (WHO) defined endocrine-disrupting chemicals (EDCs) as "an exogenous substance or mixture that alters functions of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations." (Damstra et al., 2002). EDCs can stimulate (agonists) or inhibit (antagonists) the endocrine system, causing overproduction or underproduction of hormones. Amongst EDCs, endocrine-disrupting metals (EDMs; e.g. arsenic, lead, cadmium, mercury and nickel) have been identified (Georgescu et al., 2011; Lavicoli et al., 2009). For instance, cadmium interferes with the biosynthesis of steroid hormones in rats (Piasek and Laskey, 1994; Sen Gupta et al., 2004). MTEs also induce behavioural impairments including changes in activity level, foraging success, courtship behaviour, nest attentiveness and cognitive performance, which arguably result from endocrine alterations (Clotfelter et al., 2004a, 2004b; Zala and Penn, 2004). However, very few ecological studies have investigated the effects of environmental levels of MTEs on the endocrine system (Baos et al., 2006; Franceschini et al., 2017, 2009; Herring et al., 2012; Meillère et al., 2016; Tartu et al., 2015, 2013; Wada et al., 2009; Wayland, 2002). Most of these studies focused on mercury exposure and demonstrated either negative (Franceschini et al., 2017, 2009; Herring et al., 2012; Tartu et al., 2015; Wada et al., 2009), positive (Franceschini et al., 2017) or non-significant effects (Franceschini et al., 2017; Tartu et al., 2015; Wayland, 2002) of mercury exposure on baseline corticosterone levels and the corticosterone and prolactin stress responses. Interestingly, feather corticosterone levels (i.e. medium-term baseline corticosterone levels) were positively correlated with lead, cadmium and mercury concentrations in the feathers of blackbirds *Turdus merula* along an urbanization gradient (Meillère et al., 2016), while the corticosterone stress response decreased with increasing lead exposure in white storks *Ciconia ciconia* (Baos et al., 2006). While MTEs' disruptive effects on parental investment-linked hormones might explain the lower reproductive outputs of birds exposed to high pollution levels, this potential underlying mechanism has never been experimentally tested but for mercury exposure and therefore remains largely unexplored.

The feral pigeon is an emblematic species of cities and it is therefore exposed to high levels of MTEs (Chatelain et al., 2014; Frantz et al., 2012; Gasparini et al., 2014). To estimate to which extent MTE-induced reproductive impairments are explained by the endocrine disruption properties of these MTEs, we first measured baseline corticosterone, prolactin and testosterone levels, and the corticosterone stress response of feral pigeons experimentally exposed to ecologically relevant lead and/or zinc concentrations. Hormone profiles of both males and females were measured along three reproduction stages: incubation, early chick-rearing and late chick-rearing period. Second, we investigated the effects of hormone profiles, concurrently with lead and zinc exposure, on two proxies of reproduction success: hatching success and fledging success.

2. Methods

2.1. Subjects and housing

Free-living eumelanic feral pigeons were caught in February and March 2014 from several pigeon flocks in Paris (France). Among these birds, we chose an even number of females and males (72 males and 72 females, sexed using discriminant function analysis on weight, caruncle size and wing size. The proportion of correctly classified individuals was estimated by jackknife cross-validation procedure. The discriminant rate was 83%; Dechaume-Moncharmont et al., 2011. Sex was also validated by observations of courtship behaviour) with various eumelanin-based plumage colourations. Pigeons were kept in 12 outdoor aviaries (3.10 m × 2.00 m × 2.40 m) at the CEREEP field station (Centre d'Ecologie Expérimentale et Prédictive-Ecotron Ile-de-France, UMS 3194, Ecole Normale Supérieure, Saint-Pierre-lès-Nemours, France). They were evenly distributed among aviaries according to their sex, flock, and plumage eumelanin-based colouration in such a way that there were no confounding effects between aviaries and these variables (sex: 6 males and 6 females per aviary, flock: $Chi^2 = 165.76$, $df = 165$, $P = 0.469$, and plumage colouration: $F_{1,144} = 0.13$, $P = 0.721$). They were fed ad *libitum* with a mix of maize, wheat, and peas. The aviaries were provided with a bowl of water used for bathing and with branches as perches. Birds were individually identified with a numbered plastic ring. At the end of the experiment (i.e. after 6 months of captivity), birds were released back to the wild at their site of capture.

2.2. Treatments

The aviaries were randomly assigned to one of the 4 following metal exposure treatments: exposed to lead only (*lead* treatment; 10 ppm lead acetate, Sigma-Aldrich), exposed to zinc only (*zinc* treatment; 100 ppm zinc sulphate, Prolabo), exposed to both lead and zinc (*lead+zinc* treatment; 10 ppm lead acetate and 100 ppm zinc sulphate) or control (*control* treatment; tap water with no added metal). Consequently, there were 3 aviaries with 12 pigeons each (36 pigeons in total) per