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Original research article

Is the lesser horseshoe bat (*Rhinolophus hipposideros*) exposed to causes that may have contributed to its decline? A non-invasive approach

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HIGHLIGHTS

- Twenty lesser horseshoe bat maternity colonies were measured for habitat availability, genetic differentiation, and exposure to chemical pollutants and a protozoan parasite.
- Colonies exhibited a low genetic differentiation, suggesting a non-fragmentedd population.
- 7 colonies were exposed to lindane at concentrations comparable to those reported in the literature for bat guano.
- Prevalence for the parasite *Eimeria hessei* ranged from 0% to 80% among colonies.
- Concentrations of cadmium and zinc in droppings were positively related to *E. hessei* prevalence.

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ABSTRACT

While the lesser horseshoe bat (*Rhinolophus hipposideros*) was common in most western and Central Europe, this species endured a dramatic decline in the 1950s–1970s. The causes are thought to comprise the extensive spread of chemical pollutants affecting both bat and prey populations, changes in landscape composition and structure leading to population fragmentation or roost deterioration, and infectious diseases. While lesser horseshoe bat populations recently increased after nearly thirty years of decline in the Franche-Comté region (eastern France), it is unclear whether bats are currently confronted with causes that may have contributed to former demographic disturbances. Although stressors are generally studied separately, we simultaneously measured several variables directly or indirectly reflecting the supposed causes of bat decline: availability of woodland habitats around bat roosts, genetic differentiation amongst colonies, and exposure to chemical pollutants and to a protozoan parasite, *Eimeria hessei*. Twenty maternity colonies were sampled using a non-invasive approach based on droppings. Maternity roosts were located in buildings mainly surrounded by woodland and shrubland (48% of a 2500 m radius

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buffer around the colonies), which are preferentially used by the lesser horseshoe bat as foraging areas. Low genetic differentiation ($F_{ST} = 0.023$–0.028, $D = 0.076$–0.097) and no genetic clusters were found among the 20 sampled colonies, in which 449 individuals were distinguished over two sampling sessions (2010 and 2011). This suggests that no obvious physical barrier acts as a constraint with respect to gene flow, and we conclude that all individuals sampled belong to a unique non-fragmented population. Among the 66 chemical substances measured in bat droppings, the insecticides endosulfan alpha (0.035 μg/g dm) and lindane (from 0.013 to 0.112 μg/g dm) were the only organic compounds found at detectable concentrations in one and 7 colonies, respectively. Fourteen metals were measured in the guano. Metal concentrations were lower than or of the same order of magnitude as concentrations reported in bats in the literature. The overall faecal prevalence of *E. hessei* was 42% (range: 0%–80%) and did not differ between the two sampling sessions. Faecal prevalence was not related to lindane concentrations in droppings. Despite the low concentrations of metals, a positive relationship was found between cadmium and zinc concentrations and *E. hessei* faecal prevalence. Although it is difficult to conclude on the risks of adverse effects due to the lack of both toxicological references and knowledge on *E. hessei* pathogenicity, this study stresses out the interest of systemic approaches in the study of ecology of wildlife species, especially via the investigation of multiple exposures.

1. Introduction

The populations of many European bat species experienced a dramatic decline in the latter half of the twentieth century, and most of them are currently considered to be vulnerable or endangered species (IUCN, 2012). The lesser horseshoe bat (*Rhinolophus hipposideros*) is comparably the bat species that endured the most spectacular decline in most of western and Central Europe in the 1950s–1970s, becoming locally extinct (Bontadina et al., 2000; Stebbings, 1989). The causes of this decline have not formally been identified but bat experts attributed it to a combination of various factors, such as the extensive spread of chemical pollutants, habitat destruction, changes in landscape structure, disturbance and destruction of roost sites (through deforestation and/or urban expansion), climate change, declines in insect prey, competition for prey with pipistrelles, genetic inbreeding, and/or diseases (Arlettaz et al., 2000; Bontadina et al., 2000; Motte and Libois, 2002).

Since the 1990s, the number of hibernation sites of the lesser horseshoe bat has increased across European countries, suggesting that populations may have partly recovered from their former massive decline (Van der Meij et al., 2015). Van der Meij et al. (2015) argue that this trend could be a consequence of global environmental regulations like restrictions of pesticide use and to specific bat conservation efforts engaged in the 1990s, such as the protection of roost sites (EUROBATS, EU Habitats Directive). In western France, the number of maternity roosts and the effective size of maternity colonies of the lesser horseshoe bat have increased since 1998 (Farcy et al., 2009; Petit et al., 2014). In the Franche-Comté region (eastern France), the effective size of hibernating colonies has increased of almost 200% since 1990 after nearly thirty years of population decrease (Roué, 2005). More than 100 maternity roosts are currently recorded in the region, which might represent a population of approximately 3000 individuals (Commission de Protection des Eaux, du Patrimoine, de l'Environnement, du Sous-sol et des Chiroptères (CPEPESC) Franche-Comté, unpublished data). The exposure of lesser horseshoe bat populations in Franche-Comté to potential stressors is largely unknown. However, although the number of roosts and individuals has increased, it is not clear whether these colonies are currently confronted with causes that may have contributed to former demographic disturbances. In that context, the aim of the present study was to evaluate the potential exposure of lesser horseshoe bats to some of the stressors that may have led to their former decline. While the vast majority of studies on bat species focussed on one potential stressor, we simultaneously searched for population fragmentation, pollutant and pathogen exposure, as well as their potential interactions.

The lesser horseshoe bat is a sedentary species, with winter and maternity roosts located less than 30 kilometres apart (Gaisler and Chytil, 2002). Individuals hibernate during the winter in mixed-sex groups within roosts commonly located in caves (Crucitti and Cavalletti, 2002). Females join maternity roosts at the end of spring to give birth and raise their single offspring throughout the summer, while males generally live as isolated individuals or gathered in small groups of three or four individuals (Gaisler, 1963). Because this species is known to forage along linear wooded elements, such as tree lines and well-structured hedgerows, landscape connectivity is assumed to be a key-factor for population sustainability (Bontadina et al., 2002; Motte and Libois, 2002; Zahn et al., 2008). The loss of connectivity between maternity roosts and foraging areas due to the fragmentation of wooded habitats is thus expected to parcel out lesser horseshoe bat populations. Population fragmentation and restricted gene flow can lead to genetic differentiation between sub-populations, loss of genetic variation through genetic drift, and loss of fitness due to inbreeding, which are predicted to enhance the risk of local extinction, especially in populations that have recently declined in size (Freeland, 2009; Mayer et al., 2009). Yet, the distribution of maternity colonies of the lesser horseshoe bat in the Franche-Comté region does not depend on wooded elements connecting the maternity roost with the foraging areas, and instead depends on the spatial integration of maternity roosts into a connected network of wooded patches (Tournant et al., 2013). This suggests that individual movements are little