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Short Communication

First evidence of neonicotinoid residues in a long-distance migratory raptor, the European honey buzzard (*Pernis apivorus*)

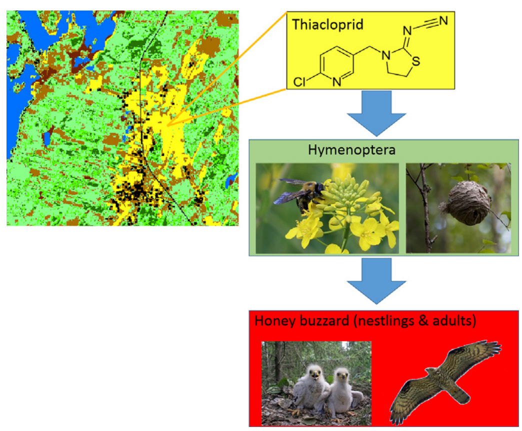
Patrik Byholm [a,*], Sanna Mäkeläinen [b], Andrea Santangeli [b], Dave Goulson [c]

[a] Novia University of Applied Sciences, Raseborgsvägen 9, FI-10600 Ekenäs, Finland
[b] Finnish Museum of Natural History Luomus, P.O. Box 17 (P. Rautatiekatu 13), University of Helsinki, FI-00014, Finland
[c] School of Life Sciences, University of Sussex, BN1 9RH Brighton, United Kingdom

HIGHLIGHTS

- We studied the prevalence of neonicotinoids in a migrant raptor, the honey buzzard.
- Residues of neonicotinoids were present in the majority of blood samples.
- In blood, thiacloprid was the most prevalent of neonicotinoid compounds.
- Presence of oil plants at foraging distances matched with neonicotinoid presence.
- We suggest more investigations of neonicotinoid presence on top of the food chain.

GRAPHICAL ABSTRACT



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ABSTRACT

The evidence of negative impacts of agricultural pesticides on non-target organisms is constantly growing. One of the most widely used group of pesticides are neonicotinoids, used in treatments of various plants, e.g. oilseed crops, corn and apples, to prevent crop damage by agricultural insect pests. Treatment effects have been found to spill over to non-target insects, such as bees, and more recently also to other animal groups, among them passerine birds. Very little is known, however, on the presence of neonicotinoids in other wild species at higher trophic levels. We present results on the presence of neonicotinoid residues in blood samples of a long-distant migratory food-specialist raptor, the European honey buzzard. Further, we investigate the spatial relationship between neonicotinoid residue prevalence in honey buzzards with that of crop fields where neonicotinoids are typically used. A majority of all blood samples contained neonicotinoids, thiacloprid accounting for most of the prevalence. While neonicotinoid residues were detected in both adults and nestlings, the methodological limit of quantification was exceeded only in nestlings. Neonicotinoids were present in all sampled nests. Neonicotinoid presence in honey buzzard nestlings' blood matched spatially with the presence of oilseed plant fields. These are the first observations of neonicotinoids in a diurnal raptor. For better understanding the potential negative sublethal of neonicotinoids in wild vertebrates, new (experimental) studies are needed.

* Corresponding author.
E-mail address: patrik.byholm@novia.fi (P. Byholm).

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1. Introduction

There is accumulating evidence on the negative impacts of agricultural pesticides aimed at particular groups of herbivorous insect pests on non-target organisms (Geiger et al., 2010; Goulson, 2013). For example, declines of grassland bird populations in the United States have been found to be more strongly linked to large-scale use of pesticides rather than to land-use change and intensification in farmland (Mineau and Whiteside, 2013). Similarly, global declines of pollinator species have been related with the extensive use of neonicotinoids, a widely used class of agricultural pesticides globally (Tsvetkov et al., 2017; Woodcock et al., 2017).

Neonicotinoids are used mainly in seed coating of a wide variety of cultivated plants from corn (*Zea mays*) to oilseed rape (*Brassica napus oleifera*) (Jeschke et al., 2011; Simon-Delso et al., 2015). Targeted at sucking and boring insect pests, such as aphids and wireworm larvae, they effectively bind to the neural receptors of insects eventually causing paralysis and death (Tomisawa and Casida, 2003). However, much of the active ingredient does not end up in the crop but instead contaminates soil (Goulson, 2013; Jones et al., 2014), water and non-target foliage, including wild flowers growing in farmland (Botías et al., 2015; David et al., 2016). Neonicotinoids have been found to represent a major threat to bees through increased mortality and decreased colony establishment (e.g. Tsvetkov et al., 2017; Woodcock et al., 2017). Reports on adverse effects in other invertebrate groups are also accumulating (Pisa et al., 2017). It was long assumed that neonicotinoids have negligible impacts on vertebrate species due to their lower toxicity, yet recent studies have reported adverse effects of neonicotinoids on both terrestrial and aquatic vertebrates, even when found in concentrations well below the level causing acute poisoning or lethality (Crosby et al., 2015; Gibbons et al., 2015). To this end, subtle consequences such as impaired migratory ability, decreased body condition and breeding success in granivorous birds following the ingestion of small amounts of neonicotinoid coated grains have been shown (Lopez-Antia et al., 2015; Millot et al., 2017). Moreover, the toxicity level of neonicotinoids may vary greatly among species, and may differentially impact on all or some of the key life-stages (e.g. breeding, survival, migration) of a species (e.g. Gibbons et al., 2015; Eng et al., 2017). In the case of small bird species, even ingestion of a single treated grain can cause acute intoxication and cause adverse effects (Mineau and Palmer, 2013). Moreover, indirect effects of neonicotinoids causing a lack of insects and other invertebrate food will lead to food deprivation in many species that depend on invertebrates for food (cf. Hallman et al., 2014).

In the terrestrial realm, most scientific and political attention on the adverse effects of neonicotinoids has largely focused on species groups that are in direct contact with neonicotinoid-treated crops, such as invertebrates and granivorous birds (EU, 2013; Gibbons et al., 2015; Eng et al., 2017). However, as neonicotinoids are known to spillover into the environment and the food-chain in which they are introduced, there is a risk they may be transported towards the higher levels of the food chain. However, to date investigations of neonicotinoids spilling over to the top of the food-chain, e.g. raptors, is extremely rare (but see Taliansky-Chamudis et al., 2017). There is therefore need for further scientific investigations on the exposure to neonicotinoids of species at different trophic levels of the food-chain beyond those in more direct contact with these pesticides.

Here we report on the prevalence of neonicotinoid residues in European honey buzzards (*Pernis apivorus*; from here onward honey buzzard) breeding in Finland during the Boreal summer. This is a relevant study species because it mainly feeds on the larvae of social Apoidea, especially wasps (*Vespidae*) but also pollinating bumble-bees (*Bombus* sp.), which in turn feed on mass flowering oilseed crops that are often treated with neonicotinoids (Ketola et al., 2015). Being a long-distance migrant, the honey buzzard could also be exposed to neonicotinoids across its passage range as well as on its wintering range in Sub-Saharan Africa. Residues of neonicotinoids have been in fact found in honey collected in countries along the flyway of this raptor species (cf. Mitchell et al., 2017). The objective of this study is to provide the first evaluation of prevalence of neonicotinoids in a wild population of a food-specialist raptor species. To account for local variation in the occurrence of oilseed plant fields (potential neonicotinoid source) between sample sites, the spatial match between neonicotinoid prevalence in sampled birds with that of oilseed rape and turnip rape (*Brassica rapus oleifera*) fields within the hunting range of breeding birds was also investigated.

2. Methods

The honey buzzard is a forest-dwelling migratory raptor breeding in the Palearctic and wintering in Sub-Saharan Africa (Cramp, 1980). In the northern hemisphere, during the breeding season it feeds almost entirely on larvae of wasps and bumblebees, while frogs and small birds constitute alternative food (Itämies and Mikkola, 1972; Gamauf, 1999). Similarly, on the African wintering sites, insects constitute an important food source for honey buzzards (Cramp, 1980).

As a part of a long-term study on forest raptors (Byholm and Nikula, 2007; Vansteelant et al., 2017), honey buzzard nests were sought for opportunistically. From a subset of found nests situated in Norway spruces (*Picea abies*) at 6–12 m height, ten honey buzzards from five families (Table 1) were sampled for blood in Western Finland (latitude 61°14′–63°12′ N, longitude 21°16′–23°31′ E) during the nestling phase in July–August 2013 when the nestlings were approximately 25–35 days old. To minimize disturbance, nest visits involving handling of nestlings never lasted longer than 45 min, but when adults were caught (see below) visits lasted for up to 2 h. Approximately 0.1 ml blood was collected from the brachial vein of one breeding pair and eight nestlings (two being offspring of the same pair) using a needle and a small glass capillary. The blood was placed in an Eppendorf-tube, and moved to a freezer bag containing dry ice for transportation to a super freezer (−80 °C). Four months post-sampling the blood samples were sent to the University of Sussex for analyses of the residues of the neonicotinoids acetamidprid (ACE), imidacloprid (IMC) and thiacloprid (THC). Analysis of neonicotinoid residues in samples was performed using ultra high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) (Waters Acquity UHPLC system, Waters, Manchester, UK). Procedures are described in full in David et al. (2015). Analyte concentrations in blank workup samples were all below the method limit of detection (LOD).

As a part of ongoing work on honey buzzard movement ecology (Vansteelant et al., 2017; P. Byholm et al., unpublished), the parental birds ($n = 4$) at two nests (nest #1 and #5, cf. Table 1) were caught using a dho-gaza (Zuberogoitia et al., 2008) and equipped with solar-powered Argos-GPS platform terminal transmitters (PTTs) (Microwave Telemetry Inc.) or UvABiTS-GPS-trackers (Bouten et al., 2013) using body-loop harnesses made of Teflon ribbon (Kenward, 2004). Tags' weight (22–25 g) corresponded to ca. 3% of the birds' body mass at deployment (833 ± 161; avg ± SD). The amount of delivered GPS-fixes delivered varied depending on tracker model and programming. All work requiring special permits (visiting nests, handling of honey buzzards, collection and storage of blood samples) was performed under special licenses issued by the relevant Finnish authorities to PB (ESAVI/1592/04.10.03/2011, EPOELY/135/07.01.2013, PIRELY/49/07.01/2013, VARELY/73/07.01/2013, VARELY/215/2015).

In order to investigate the spatial relationship between neonicotinoid prevalence and landscape composition, we collated spatial data on the location of field parcels (Agency for Rural Affairs, 2012, 2013) representing crop types typically treated with neonicotinoids in the study region in Finland, i.e. spring turnip rape and oilseed rape (Ketola et al., 2015). Next, we extracted the fields used in cultivation of oilseeds for the year 2013 (i.e. the year when blood sample data on the birds were collected). The area of fields with the above crop types