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Short Communication

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

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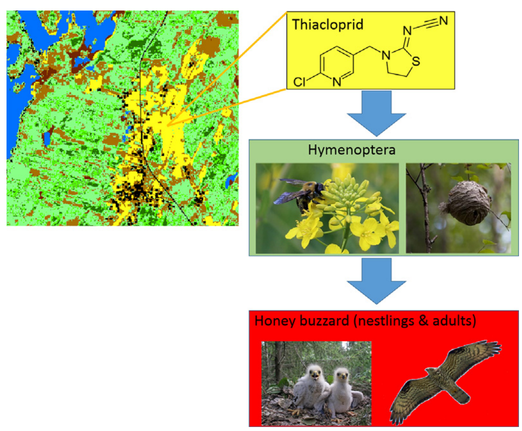
HIGHLIGHTS

- 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.

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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 acetamidpride (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

Table 1
Residues of neonicotinoid compounds (ACE = acetamidprid, IMC = imidacloprid and THC = thiacloprid) measured as picograms/ml (values in the respective cells) in blood samples of ten honey buzzard individuals. LOD = amount in blood is lower than limit of detection figure given. LOQ = analyte is present in blood at a concentration higher than the LOD but lower than limit of quantitation (LOQ) figure given. Also age (ad = adult; pull = nestling), nest identity, and areas (ha) and proportions (%) of turnip and rape fields at different spatial scales from the nest (0.5 to 5 km) are presented.

Bird id	Age	Nest id	ACE	IMC	THC	Area				Proportion			
						0.5 km	1 km	2 km	5 km	0.5 km	1 km	2 km	5 km
1	ad	1	<LOD (7.1)	<LOQ (>13 <39)	<LOD (2.0)	0.19	16.4	82.97	162.1	0.24	5.52	7.07	2.11
2	ad	1	<LOD (5.5)	<LOD (11)	<LOD (1.6)	0.19	16.4	82.97	162.1	0.24	5.52	7.07	2.11
3	pull	1	<LOD (0.6)	8.9	<LOQ (>0.2 <1.3)	0.19	16.4	82.97	162.1	0.24	5.52	7.07	2.11
4	pull	1	<LOD (6.7)	<LOD (13)	31	0.19	16.4	82.97	162.1	0.24	5.52	7.07	2.11
5	pull	2	<LOD (6.9)	<LOQ (>13 <39)	<LOQ (>2.1 <6.3)	0	0	4.55	88.89	0	0	0.36	1.14
6	pull	3	<LOD (6.8)	<LOD (13)	12	0	0	0	54.34	0	0	0	0.70
7	pull	3	<LOD (6.5)	<LOD (13)	<LOD2)	0	0	0	54.34	0	0	0	0.70
8	pull	4	<LOD (6.4)	<LOQ (>13 <39)	17	0	0	7.06	43.73	0	0	0.63	0.59
9	pull	4	<LOD (7.1)	<LOD (15)	23	0	0	7.06	43.73	0	0	0.63	0.59
10	pull	5	<LOD (7.5)	<LOD (15)	25	0	0	2.37	28.51	0	0	0.19	0.37

were then calculated within circular buffers of radius 500 m, 1 km, 2 km and 5 km centered on each of the five honey buzzard nest site. Disregarding night fixes (10:00 PM-5:00 AM) and fixes ≤80 m from the nest, the distances between GPS-locations delivered by the GPS-trackers and the nests were calculated for the four adult honey buzzards during whole breeding season (May 20th-August 29th). This material was then used as a proxy for understanding over which distances from the nest the parents move while hunting for their young. Finally we investigated whether (a) the amounts of neonicotinoids in honey buzzard nestlings were correlated with the area of oilseed plant fields around honey buzzard nests, and (b) whether presence of neonicotinoids in nestlings' blood was related to the presence of the oil plant fields at different spatial scales. The latter was performed by Fisher's exact test. Here, when the combined area of oil plant field parcels within a certain radius was below one hectare (nest #1 at 500 m), this was approximated to zero, based on its assumed negligible effects given the area considered. Spatial data handling was done in ArcGIS 10.1 SP1 for Desktop (ESRI, 2012) and statistical tests in R software for statistical computing, version 3.2.2 (R Core Team, 2015).

3. Results

Neonicotinoid residues were detected in the majority (8/10) of blood samples, adults and young combined, at the methodological limit of detection (LOD; Table 1). Of these, 60% contained neonicotinoids above the methodological limit of quantification (LOQ), with the amount of compounds ranging from 8.9 to 31 pg/ml (average of 14.6 ± SD 11.5, Table 1). Among the nestlings ($n = 8$), residues of imidacloprid or thiacloprid exceeding LOD and LOQ were found in seven and six individuals, respectively. Among the two adults, imidacloprid exceeded the LOD in one individual, whereas no neonicotinoid residues were found in the blood of adults above the LOQ. Thiacloprid accounted for most of the quantified neonicotinoids prevalence, whereas imidacloprid was less common and residues of acetamiprid were totally absent. Neonicotinoids were detected in all of the five nests (Table 1).

Oilseed plant fields around the five different nest sites showed considerable variation at all landscape scales (500 m–5 km). Some nests completely lacked oilseed plant fields ≤2 km from the nest, but all nests had oilseed plant fields within a 5-km distance (Table 1). The overall proportion of oilseed plant fields over the whole circular buffer area considered was generally small (max. 7% of the total area in each case).

No correlation between the absolute area, or the percentage, of oil plant fields and the quantified neonicotinoid levels in honey buzzard blood was found at any spatial scale (Pearson correlation coefficients ranging from −0.11 to −0.27 and p-values from 0.46 to 0.78). However, when neonicotinoid residues (of any compound) found in nestlings and the coverage of oil plant fields around their nest were categorized into presence and absence, the presence/absence of neonicotinoid residues matched with the presence/absence-pattern of oil plant fields at the scales of 2 and 5 km (Fisher's exact test, LOQ and LOD analyzed separately, all $p \geq 0.79$). No such spatial match was found at the 1 km (LOQ: $p = 0.07$; LOD: $p = 0.02$) or 500-m scale (LOQ: $p = 0.007$, LOD: $p = 0.007$). When comparing this result with the distance arrays describing how far from the nest parental honey buzzards move when foraging, there is a fit with the spatial match observed for neonicotinoid residue and oil plant field presence/absence. In other words, although adults differ in respect to how far from the nest they forage, they typically forage at distances 2–5 km (or more) than at 500 m-1 km from the nest (average median distance: 2.70 km; Fig. 1).

4. Discussion

This is one of the first studies documenting the presence of neonicotinoid residues in a higher trophic level consumer in a natural food web (but see Taliansky-Chamudis et al., 2017). Of eight blood sampled honey buzzard nestlings, residues of imidacloprid or thiacloprid

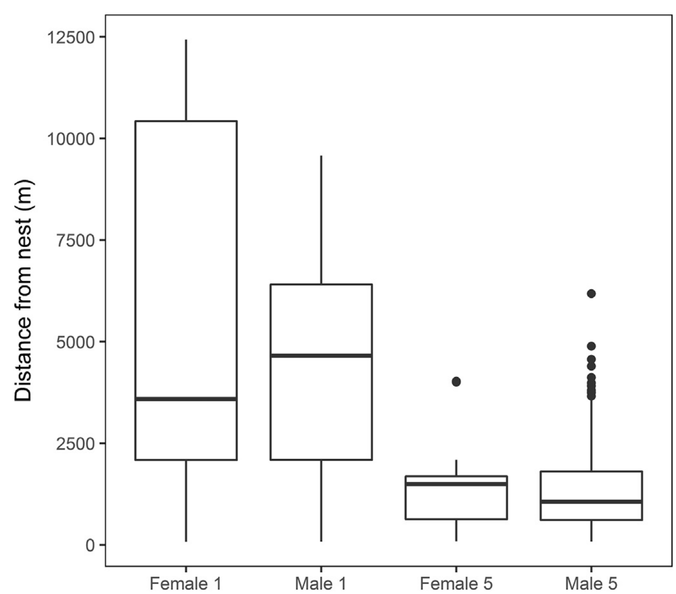

Fig. 1. Box and whiskers - plot of the distances of four GPS-tracked parental honey buzzards from their nests (nests 1 and 5, see Table 1) during the 2013 breeding season (May 20th – August 29th). Box denotes the 25% and 75% quantiles of the distances of individual locations from the nest ($n_{female1} = 812$, $n_{male1} = 4312$, $n_{female5} = 34$, $n_{male5} = 510$) and thick horizontal line denotes the median value. The average median distance for all four birds is 2.70 km. Outlying observations are shown by dots. Since both night time and nest-related locations were excluded (see Methods) these distributions describe the distances traveled to forage by the honey buzzards in different sites.

exceeding LOD and LOQ were found in seven and six individuals, respectively. LOD was also exceeded for imidacloprid in the blood of one of the two sampled adults. No residues were found in the blood of adults above the LOQ. Although there is limited knowledge about the persistence of neonicotinoids in non-target animal species, the neonicotinoid in vivo metabolism observed in most animal species is fast (Nishiwaki et al., 2004; Dick et al., 2005; Shao et al., 2013; Kapoor et al., 2014). Thus, because neonicotinoids are water soluble (Goulson, 2013) and assuming the neonicotinoid compounds in the honey buzzard blood circulation system in common with other species are metabolized fast, the result that neonicotinoids were found in the majority of the blood samples of nestlings implies that neonicotinoid exposure is of local origin rather than caught on the wintering grounds or *en route* by adults during migration. If this inference is correct, it does not mean that an individual testing negative for neonicotinoid residues could not have been in contact with the pesticides during other parts of the annual cycle, but merely that testing for neonicotinoid residues as sampled on the breeding grounds is an inadequate approach for identifying wintering ground exposure. Thus, if aiming at understanding to what extent migratory birds are exposed to neonicotinoids during the whole annual cycle, sampling must be organized regularly over wider areas.

Although the sample size for this study is limited and absolute residue levels remain low, the prevalence of neonicotinoid residues observed in this study is still higher than in previous studies based on blood samples collected from other wild predators (Taliansky-Chamudis et al., 2017). Since honey buzzards prefer wasps and bumble-bees (*Bombus* sp.) as food, it seems most likely that the reason for the neonicotinoid contamination is connected to this food source. Neonicotinoid residues have commonly been found in the tissues of wild bees (Botías et al., 2017). Because neonicotinoids in Finland in practice are used only to seed-coat and spray turnip rape and oilseed rape (Ketola and Hakala, 2015), we can infer that the Apoidea species have been visiting these fields for foraging and have come in direct contact with the neurotoxic pesticides. This conclusion is further supported by the finding that the presence of neonicotinoid residues in honey buzzard nestlings' blood matches strongly with the presence of oilseed plant fields within the home range at distances of 2–5 km from the nest. These distances include the area of the home-range that is most intensively used by foraging adult honey buzzards during the nestling provisioning phase (Fig. 1). Consequently, provisioning parents likely deliver food that is contaminated by neonicotinoid pesticides to their chicks, thereby explaining the presence of these substances found in the blood of nestlings.

Species at the top of the food-chain, such as raptors, are in general highly sensitive to environmental change, including the increasing prevalence of environmental pollutants, due to the well-known process of bioaccumulation (Newton, 1976). Pollution, particularly through the use of pesticides aimed at increasing crop yields, is pervasive across the cropland areas worldwide, with seed-coating pesticides, such as neonicotinoids, being used across many countries in Europe, Africa and elsewhere (www.fao.org/faostat). Most of these regions are very important for long-distance Palearctic migrant birds breeding in Europe, such as the honey buzzard. Although the residue levels reported here for honey buzzards are low, it should be noted that even a low dose may have negative long-term effects (Goulson, 2013; Rondeau et al., 2014). Populations of long-distance Palearctic migrants have been recently shown to be facing rapid declines, often driven by anthropogenic pressures such as habitat loss and degradation, and overexploitation, outside of their breeding grounds in Europe (Vickery et al., 2014; Laaksonen and Lehikoinen, 2013). Threats often act in synergy in their adverse impacts on species (Brook et al., 2008). Exposure to pesticides, such as neonicotinoids, may thus represent an additional pressure imposed on already declining migratory species. Such added pressure may act on the non-breeding ground of the life of migratory species (Eng et al., 2017), with potential consequences on survival, or on the breeding ground, impacting on reproductive success (e.g. Lopez-Antia et al., 2015; Millot et al., 2017).

The findings presented here reinforce the scarce evidence so far available that neonicotinoid substances can move up the top levels of the food-chain. However, neonicotinoid exposure of predator species remains largely unquantified, and so are its possible consequences. Because the rate of metabolism of neonicotinoids in birds is poorly understood, we cannot predict the dose of neonicotinoids these honey buzzards were exposed to. Impacts of neonicotinoid exposure on birds have been identified, ranging from direct mortality (Lopez-Antia et al., 2015; Millot et al., 2017) to impaired migratory ability (Eng et al., 2017), compromised body condition and breeding success. All such impacts are plausible to be present, and can lead to cascade effects, on species at the top of the food-chain that become exposed to such pesticides. While our results are based on a small sample size and cannot be generalized, they should sound as an early warning. We thus call the scientific community to engage in collecting samples and assessing the exposure and its consequences on species at the top levels of the food chain, both on the breeding and wintering grounds. This endeavor is likely to be challenging methodologically, and would require long-term studies to ultimately quantify the impacts of exposure on the population demography of long-lived species. A first and relevant step, however, would be to quantify the extent of the potential problem. That is, what species may be exposed to neonicotinoids. This study contributes to fill the above gap in knowledge.

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Conflict of interest

Authors state that there is no conflict of interest.

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