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Short Communication

Contamination by neonicotinoid insecticides in barn owls (*Tyto alba*) and Alpine swifts (*Tachymarptis melba*)

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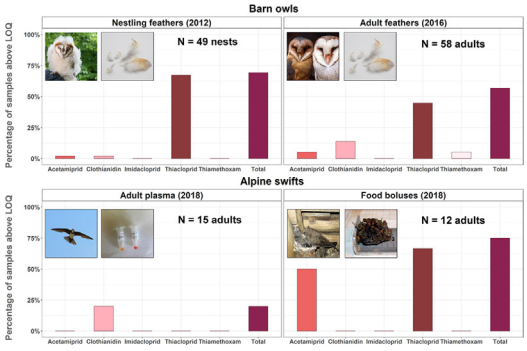
HIGHLIGHTS

- Exposure to neonicotinoid insecticides (NNIs) is poorly studied in non-granivorous birds.
- NNIs quantified from feathers of nestlings and adult's Barn owls.
- NNIs quantified from nestlings' feathers, food boluses and plasma of adults' Alpine swifts.
- In Barn owls, quantifiable concentrations in 69% of nestling feathers and 57% of adult feathers.
- No NNIs in Alpine swift nestling feathers, but in 75% of food boluses and 20% of plasma.

GRAPHICAL ABSTRACT



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ABSTRACT

Monitoring the extent to which wildlife is exposed to the broadly used neonicotinoid insecticides (NNIs) is essential to assess their potential negative effects on biodiversity. Birds are good subjects to assess such exposure, because they inhabit various habitats and they feed at different trophic levels. However, so far, most studies have focused on the contamination of granivorous species. In this study, we assess the concentrations of five NNIs (acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam) in the carnivorous Barn owl (*Tyto alba*), and the insectivorous Alpine swift (*Tachymarptis melba*). NNIs were measured in the Barn owl in feathers collected from nestlings in 2012 ($n = 49$ broods) and adults in 2016 ($n = 58$ individuals), and in the Alpine swift from feathers collected from 50 pooled nestling samples from 50 nests between 2004 and 2017 (nestlings raised in five different nests over ten years; $n = 50$ broods), plasma samples from adults in 2018 ($n = 15$), and food boluses collected from nestling provisioning adults in 2018 ($n = 12$). We found that 69% and 56.9% of Barn owl feathers from nestlings and adults respectively contained at least one NNI at measurable concentration. Mean ± SE and median concentrations (in ppb) of total NNIs were 0.66 ± 1.13 and 0.42 for nestlings, and 0.17 ± 0.57 and 0.04 for adults. In the Alpine swift, although we detected no NNI in nestling feathers, we found that 75% of food boluses and 20% adult plasma samples contained at least one NNI at measurable

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concentration. Mean ± SE and median concentrations (in ppb) of total NNIs were 0.24 ± 0.20 and 0.24 in food boluses, and 0.06 ± 0.13 and 0 in plasma. In view of these results, further research is warranted to determine the extent of contamination in non-granivorous birds and their potential effects.

1. Introduction

Since the introduction of imidacloprid in 1994, neonicotinoid insecticides have been a worldwide commercial success (Simon-Delso et al., 2015). The reasons for their popularity have been manifold: their prophylactic use in the form of seed coatings, allowing farmers not to worry about the dosage, but also their effectiveness on a wide variety of crops against insect pests (Goulson, 2013; Simon-Delso et al., 2015). Moreover, because they are buried in the soil with the seeds after sowing, they are deemed more environmental-friendly with a low risk of contamination of adjacent fields. However, about a decade ago, suspicions about their adverse effects on non-target invertebrates, in particular pollinators, arose and they were blamed as a major factor for the bee colony collapse disorder (Godfray et al., 2014; Pisa et al., 2021; van der Sluijs et al., 2013). They act by strongly binding to nicotinic acetylcholine receptors in the central nervous system of insects, with little species selectivity (Tomizawa and Casida, 2005). These insecticides are highly water soluble, which allows them to migrate into all parts of the plant, reaching the flowers and pollen that are then visited by pollinating insects (Goulson, 2013; Jeschke et al., 2011; Wintermantel et al., 2020). Although the NNI properties are an asset regarding their use, it also raises concerns about the possible contamination of adjacent environment and effects of non-target species. In response to these concerns, measures have been taken by the European Union and Switzerland in the form of a moratorium on the use of three molecules (imidacloprid, clothianidin and thiamethoxam) on selected crops that came into force in 2013 (EU Regulation No 485/2013). Since January 2019, a new regulation stated a ban of these three molecules in all outdoor crops, restricting them to greenhouses (Bundesamt für Landwirtschaft, 2018) Yet, recent studies have established that, due to high contamination risks from the treated fields to the surrounding areas (Bonmatin et al., 2019; Botías et al., 2016; Humann-Guilleminot et al., 2019b) combined with long persistence in the agroecosystems, NNIs continue to expose a large range of non-target species even years after the ban (Humann-Guilleminot et al., 2019b; Wintermantel et al., 2020).

Among the non-target species likely to be affected by neonicotinoid insecticides, birds appear to be exposed via multiple routes. Insectivorous species are thought to be mostly indirectly impacted by pesticides application through the diminution of their food supply (Boatman et al., 2004; Hallmann et al., 2014), while strictly granivorous species are at the forefront by eating neonicotinoids coated seeds (Botha et al., 2018; Hao et al., 2018; MacDonald et al., 2018; Millot et al., 2017). It is mostly on these two routes that research has focused in recent years, although it may be hypothesised that exposure to neonicotinoids may also occur through the ingestion of contaminated arthropods, contaminated drinking water or through direct skin and feathers contact. In fact, some non-granivorous birds or birds at higher trophic levels may also be exposed to NNIs through the consumption of contaminated nectar or wild bees (European honey buzzards: Byholm et al., 2018; rufous and Anna's hummingbirds: Bishop et al., 2018; California hummingbirds: Graves et al., 2019). These studies suggest that NNIs are likely to affect a much larger spectrum of species than just granivorous birds. Overall, regardless of their diet, birds living in agricultural environments are the most exposed to a direct contamination by neonicotinoids, sometimes at a large scale (Humann-Guilleminot et al., 2019a). However, we may still underestimate the extent of the contamination to other species that would not be suspected of being in direct contact with NNIs.

In this context, we assessed the concentrations of five neonicotinoid insecticides in different tissues collected from two bird species in Switzerland. The first species is the carnivorous Barn owl (*Tyto alba*) in which we measured the concentrations of five NNIs (acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam) in feathers of nestlings from 49 broods monitored in 2012 and in feathers of 58 adults collected in 2016. The second species is the insectivorous Alpine swift (*Tachymarptis melba*) in which we measured NNI concentrations in pooled feathers of nestlings from five nests collected over ten years between 2004 and 2017 ($N = 50$ broods). Finally, in 2018, we collected food boluses from 12 adult Alpine swifts while they were provisioning their nestlings and blood samples from 15 different adults. Feathers and blood samples can be obtained without killing the birds and are thus valuable tissues to assess the exposure of wild birds to contaminants (Dauwe et al., 2009; Hao et al., 2018), while food boluses are good indicators of potential ways of contamination of insectivorous nestlings and adults. Although these two bird species are classified as least concern in the IUCN list on a global scale, they are both classified as "near threatened" in Switzerland (Keller et al., 2010).

2. Material and methods

2.1. Model species

Barn owls are non-migratory nocturnal raptors found in various environments and climates and are strongly associated with human activities. They feed mainly on rodents and small mammals, but bats, birds, lizards, amphibians and insects are also occasionally part of their diet. In contrast, Alpine swifts are migratory and strictly insectivorous birds capturing their prey (mostly Diptera and Hymenoptera) on the wing over long-distances and a wide range of habitats. They mainly inhabit cliffs, but occasionally live in colonies in urban areas as it is the case for the colony studied in Switzerland. While Barn owls can easily be in contact with neonicotinoid insecticides by ingesting contaminated prey or via deposition onto their feathers, Alpine swifts are supposedly less likely to be exposed because they eat insects on the wing.

2.2. Field protocols

Feathers of Barn owl nestlings were collected between May and August in 2012 in 49 different broods. Feathers were plucked from several nestlings of the same brood and pooled to reach enough material per brood (ca. 15 mg) for chemical analyses. Feathers of adult Barn owls were collected in May and August in 2016 from 37 males and 21 females breeding in 45 different nest boxes. Barn owls nest boxes were located within a radius of 50 km, thus expanding over an area of over 7850 km^2 (Fig. 1). Adult and nestling Barn owls were captured by hand directly in the nest boxes. Feathers were plucked on the belly, the breast and/or the back of the adults and pooled for each individual (Table 1).

Feathers of Alpine swift were collected in two colonies located in clock-towers in the cities of Solothurn and Biel (Switzerland). Adult and nestling swifts were captured by hand while visiting their nest in the towers. Nestling feathers were collected in July between 2004 and 2017 in Solothurn on 50-day old nestlings. Four feathers from two chicks per nest were pooled together to obtain a minimum of 15 mg of feathers per sample and a total of five nests over ten years from 2004 to 2017, with some missing years (total of 50 samples from 50 different nests; Table 1). Feathers were plucked on the breast and kept in

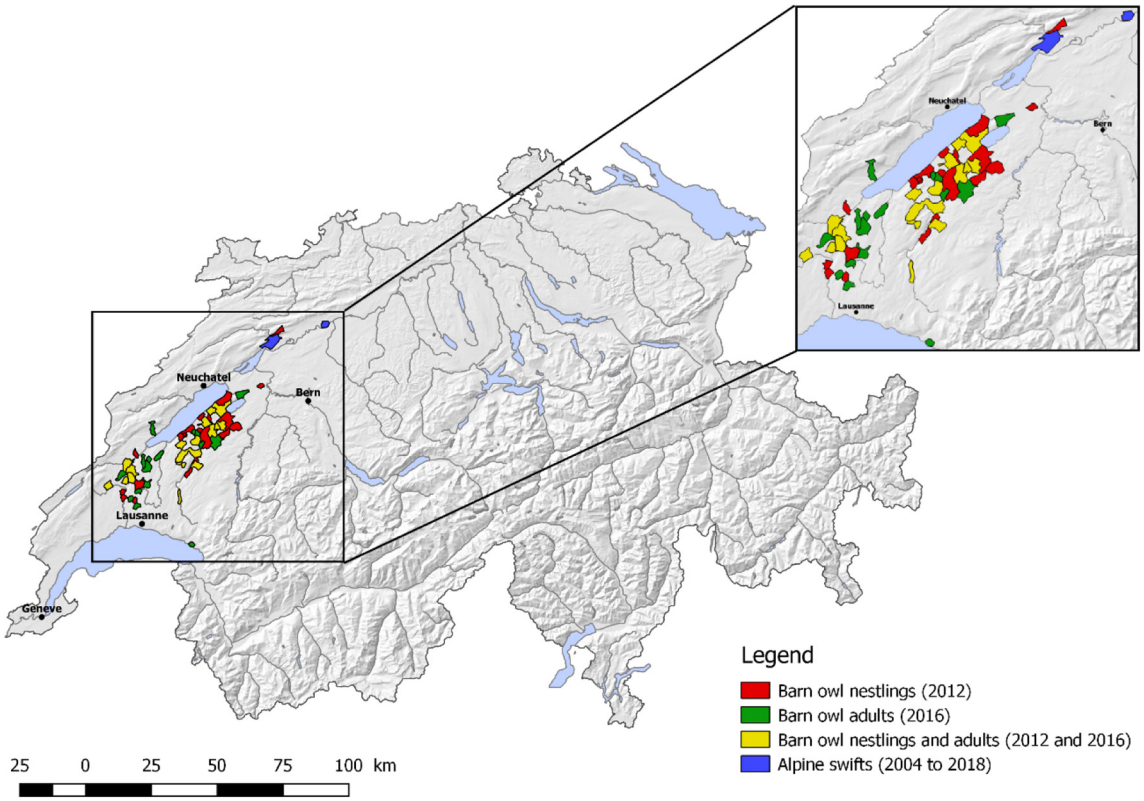

Fig. 1. Sampling location of Barn owl nests and Alpine swift colonies. The map shows limits of communes. Some communes have more than one nest box in their territory.

Table 1
Summary statistics, limits of quantification (LOQ), percentage of samples above the LOQ, and percentage of samples between the limits of detection (LOD) and LOQ calculated for each neonicotinoid measured in each type of sample.

			Neonicotinoids					
			Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam	Total NNIs
Barn owl	Nestling feathers	% > LOQ	2.0%	2.0%	0%	67.3%	0%	69.4%
	N = 49 nests	% > LOD < LOQ	12.2%	2.0%	0%	16.3%	0%	14.3%
	2012	*Maximum [ppb]*	6.17	0.58	0	3.13	0	7.01
		Median [ppb]	0	0	0	0.41	0	0.42
		Average [ppb]	0.13	0.01	0	0.53	0	0.66
		s.e.m [ppb]	0.88	0.08	0	0.66	0	1.13
		LOQ [ppb]	0.1	0.3	0.6	0.05	0.12	
	Adult feathers	% > LOQ	5.2%	13.8%	0%	44.8%	5.2%	56.9%
	N = 58 ind.	% > LOD < LOQ	34.5%	19.0%	0%	29.3%	8.6%	31.0%
	2016	*Maximum [ppb]*	4.01	0.14	0	1.33	0.22	4.08
		Median [ppb]	0	0	0	0	0	0.04
		Average [ppb]	0.08	0.01	0	0.07	0.01	0.17
		s.e.m [ppb]	0.53	0.03	0	0.19	0.03	0.57
		LOQ [ppb]	0.1	0.15	0.6	0.05	0.1	
Alpine swift	Food boluses	% > LOQ	50.0%	0%	0%	66.7%	0%	75.0%
	N = 12 ind.	% > LOD < LOQ	50.0%	0%	0%	33.3%	8.3%	25.0%
	2018	*Maximum [ppb]*	0.36	0	0	0.56	0	0.68
		Median [ppb]	0.05	0	0	0.18	0	0.24
		Average [ppb]	0.08	0	0	0.16	0	0.24
		s.e.m [ppb]	0.10	0	0	0.16	0	0.20
		LOQ [ppb]	0.005	0.03	0.08	0.005	0.02	
	Adult plasma	% > LOQ	0%	20.0%	0%	0%	0%	20.0%
	N = 15 ind.	% > LOD < LOQ	33.3%	40.0%	6.7%	40.0%	0%	66.7%
	2018	*Maximum [ppb]*	0	0.34	0	0	0	0.34
		Median [ppb]	0	0	0	0	0	0
		Average [ppb]	0	0.06	0	0	0	0.06
		s.e.m [ppb]	0	0.13	0	0	0	0.13
		LOQ [ppb]	0.01	0.05	0.05	0.01	0.03	
	Nestling feathers	% > LOQ	0%	0%	0%	0%	0%	0%
	N = 50 nests	% > LOD < LOQ	0%	0%	0%	2.0%	0%	2.0%
	2004–2017							
	(except 2007, 2008, 2010 and 2011)							

envelops in the dark at room temperature until analysis. Food boluses were collected in July in 2018 in Biel from parents while they were provisioning their nestlings. Boluses were stored in Ziplocs bag at −80 °C until analysis. We obtained 12 food boluses collected from 12 different adults (Table 1). In 2018, in the Biel colony, we took blood samples from provisioning adult birds using heparinized tubes. Blood samples were kept on ice until centrifugation less than 8 h after sampling. Plasma was collected and stored at −80 °C until analysis. We obtained blood samples from 15 adult Alpine swifts (Table 1).

2.3. Chemicals

Acronyms are defined in Table S1. Solvents for the preparation of standard solutions and samples were milli-Q water, HPLC grade acetonitrile (ACN) and methanol (MeOH) from VWR International GmbH (Dietikon, Switzerland). For UHPLC-MS/MS analyses, water, acetonitrile, formic acid (FA) and ammonium formate (NH_4FA) of ULC/MS grade were obtained from Biosolve. All salts used for QuECheRS were obtained from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). Isolute PSA bulk phase was purchased at Biotage. C18 (ZeoPrep 90) bulk phase was obtained from ZeoChem AG (Rüti, Switzerland). Certified standards of thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid were all obtained from Sigma-Aldrich Chemie GmbH. Isotopically labelled standards (thiamethoxam-D3, clothianidin-D3, imidacloprid-D4, acetamiprid-D3 and thiacloprid-D4) were obtained from CDN Isotopes. All standard pesticide (native and deuterated) stock solutions were prepared in ACN.

2.4. Sample preparation

2.4.1. Feathers

We extracted neonicotinoid insecticides following the protocol described in Humann-Guilleminot et al. (2019a). Fifteen mg of feathers were weighed and cut in a 2-mL tube and ground with three 5-mm metal beads using a Retsch mill for 6 min at 26 Hz. Feathers were not rinsed prior to extraction. To each tube were added 1.5 mL of ACN and 8 μL of internal standard solution (125 ng/mL in MeOH). The mix was then shaken again for 5 min at 26 Hz with the three 5 mm metal beads and centrifuged at 4000 rpm for 5 min. As much supernatant as possible was collected and added to a 15-mL falcon tube filled with 2 g of MgSO4, 0.5 g of NaCl, 0.5 g of trisodium citrate and 0.25 g of sodium hydrogencitrate sesquihydrate. The pellet was re-extracted once by adding 1.5 mL of ACN, shaken for 5 min at 26 Hz and centrifuged, and both supernatants were combined. 4.5 mL of milli-Q water were added to the "salt tubes" and tubes were vigorously shaken by hand for about 2 min until the salt pellet detached from the tube. After centrifugation (4000 rpm for 5 min), the epiphase (ca. 5 mL of ACN) was collected and put in a 15 mL "purification" tube containing 100 mg of PSA, 150 mg of $MgSO_4$ and 100 mg of C18. The tubes were shaken, centrifuged (4000 rpm for 5 min) and the supernatant collected in a 13 × 100 mm glass tube. The glass tubes were evaporated to dryness under vacuum using a Centrivap (Labconco). The samples were finally reconstituted in 200 μL of milliQ water:MeOH (75:25, v/v), filtered using a 13 mm PTFE filter (BGB Analytik) and transferred in an HPLC vial containing a 250 μL conical insert. Two blank samples (i.e. solvent without matrix submitted to the entire extraction procedure) per batch of 16–36 samples was included and injected into the UHPLC-MS/MS to ensure that no contamination occurred during sample preparation.

2.4.2. Food boluses

Food boluses were first washed with distilled water in order to remove as much saliva as possible. Remaining insects were then lyophilized with a freeze dryer for 24 h and freeze-dried insects were ground using a mortar and pestle. Forty mg of insects were weighed in a 2-mL tube. The same procedure was then applied as for the feather samples.

2.4.3. Plasma

Twenty μL of ACN containing isotopically labelled internal standards (25 ng/mL) were added to the 10 μL of plasma previously aliquoted on the day of collection. The tubes were vortexed for a few seconds before being placed in an ultrasonic bath for 5 min, then 970 μL of milli-Q water were added and the mixture was centrifuged for 3 min at 14′000 rpm. Extraction of neonicotinoids from the plasma was performed using a polystyrene-divinylbenzene SPE cartridge (Oasis HLB 30 mg 1 cc, WATERS, Ireland). The cartridges were conditioned with 1 mL of 100% MeOH and then equilibrated with 1 mL of milli-Q water:MeOH (98:2 v/v). Sample supernatants were loaded into the cartridges, and the cartridges were washed with 1 mL milli-Q water:MeOH (98:2 v/v) and eluted with 1 mL 100% MeOH in 100 × 13 mm glass tubes. The methanolic solutions were evaporated using a Centrivap (Labconco) for 5 h at 35 °C and stored at −20 °C pending further analysis for up to 7 days. On the day of analysis, the samples were resuspended with 100 μL milli-Q water:MeOH (75:25 v/v) water and then centrifuged. Finally, the samples were transferred to an HPLC vial containing a 250 μL conical insert.

2.5. Sample analysis

We quantified acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam in all samples, but not their metabolites. The quantification of neonicotinoids was carried out by UHPLC-MS/MS using a method adapted from Lachat and Glauser (2018). In brief, analyses were performed in multiple reaction monitoring (MRM) mode using an UPLC system (Waters) coupled to a TQ-S triple quadrupole mass spectrometer (Waters). The separation was achieved on a Cortecs UPLC C18 column (50 × 2.1 mm i.d., 1.6 μm particle size, Waters) using a temperature of 25 °C and a flow rate of 0.4 mL/min. Mobile phase A consisted of H_2O + 0.05% FA + 5 mM NH_4FA and mobile phase B of ACN + 0.05% FA. The following gradient program was used: 2–26.5% B in 4.25 min, 26.5–100% B in 0.75 min, holding at 100% B for 2 min and returning to initial conditions at 2% B for 1.5 min. The injection volume was 5 μL. The system was controlled by Masslynx 4.1 (Waters) and data processing was performed using the Quanlynx software (Waters). Neonicotinoids were quantified by internal calibration using calibration solutions prepared in MeOH 25% at 0.005, 0.05, 0.5, 2, 20 and 50 ng/mL, each containing internal standards at a concentration of 5 ng/mL. Linear or quadratic regressions weighted by 1/x were applied.

3. Results and discussion

Prevalence, concentrations, limits of quantification (LOQ) and percentage of samples between the limit of detection (LOD) and the LOQ of the five NNIs measured in all samples are reported in Table 1. LOD and LOQ were determined as the concentrations giving signal-to-noise (S/N) ratios of 3 and 10 respectively.

3.1. Neonicotinoids in barn owls

14% and 31% of Barn owl feathers from chicks and adults contained at least one neonicotinoid at a concentration between the LOD and the LOQ. 69% and 57% of Barn owl feathers from chicks and adults contained at least one neonicotinoid at a concentration above the LOQ. In total, 84% and 88% of Barn owl feathers from chicks and adults contained at least one neonicotinoid above the LOD. Concentrations ranged from 0.02 to 3.13 ppb in chicks and adults taken together. The mean ± SE and median concentrations of total neonicotinoids in the feathers of Barn owl nestlings were 0.66 ± 1.13 ppb and 0.42 ppb (N = 49 nests; Table 1). The mean ± SE and median concentrations of total neonicotinoids in the feathers of adult Barn owls were 0.17 ± 0.57 ppb and 0.04 ppb (N = 58 individuals; Table 1). Thiacloprid clearly dominated in terms of presence in all samples. However, acetamiprid reached the maximum

concentration in feathers of chicks and adults even though it was not found in one nestling sample and three adult samples (Table 1, Fig. 2).

Barn owls live mainly in rural environments and close to agricultural fields, and several modes of exposure are possible for these birds. One possibility is that contamination comes through secondary exposure via the consumption of contaminated prey (e.g. rodents living in cultivated fields). Other possibilities for birds to be exposed are by inhalation of aerosolized NNIs or by direct contact through the deposition of NNIs on their feathers, which is rendered possible by the production of a toxic dust during the sowing of NNIs coated crop seeds (Girolami et al., 2012; Krupke et al., 2012) or by coming in contact with foliage sprayed with NNIs (Rogers et al., 2019; Bishop et al., 2020). Future studies comparing washed and unwashed feathers would address the question of whether individuals may get contaminated via the deposition of NNIs onto feathers and through preening, as is the case for other types of organic pollutants (e.g. Jaspers et al., 2008). Concerning nestlings, they remain in the nest for 8 to 10 weeks after hatching and are fed exclusively by the parents, so their contamination is likely through the ingestion of contaminated prey. In fact, it has been shown that small mammals that enter the diet of Barn owls, such as mice and voles, consume grains treated with NNIs and are therefore exposed to these insecticides (Roy and Coy, 2020). One of the reasons why the concentrations found in nestlings were higher than in adults might be due to a year effect. By the time feathers were collected on nestlings in 2012, the Swiss moratorium had not yet come into force. Consequently, NNIs were still widely used in all types of crops with no restrictions on flowering crops compared to the study conducted on adults in 2016 when the moratorium had already been applied for three years. However, the NNIs detected in owlets and adults were qualitatively the same (i.e. mostly acetamiprid and thiacloprid) and were concerned by neither the European nor the Swiss moratorium. Therefore, the sampling year relative to the enforcement of the moratorium is unlikely to explain the differences in concentration between nestlings and adults. Alternatively, these differences could be explained by the fact the periods when nestlings and adults grow their feathers may coincide with the different periods of application of the insecticides. Barn owlets hatch between ca. mid-March and late August, with a mean hatching date around mid-May (Frey et al., 2010), so that the period of feather growth extends from April to August. Thiacloprid, which shows the highest prevalence, is mainly used as a spray suspension or oil dispersion shortly before the crops flower, i.e. from March to June. This application period therefore coincides with the hatching period. In contrast, acetamiprid is used on all types of crops in the form of suspension or granules in winter and in spring-summer (e.g. winter and spring cereals), which could also explain why we found it at a substantial concentration in one of the nestling feather sample. In contrast, adult Barn owls moult their feathers between June and November (Géroudet, 2000; Martínez et al., 2002), a period when pesticides may be applied at lower rates.

3.2. Neonicotinoids in Alpine swifts

25% and 67% of the food boluses and plasma samples collected in Alpine swifts contained at least one neonicotinoid at a concentration between the LOD and the LOQ. We found measurable concentrations (>LOQ) of neonicotinoids in 75% of the food boluses collected from adults provisioning their nestlings and in 20% of the adult plasma

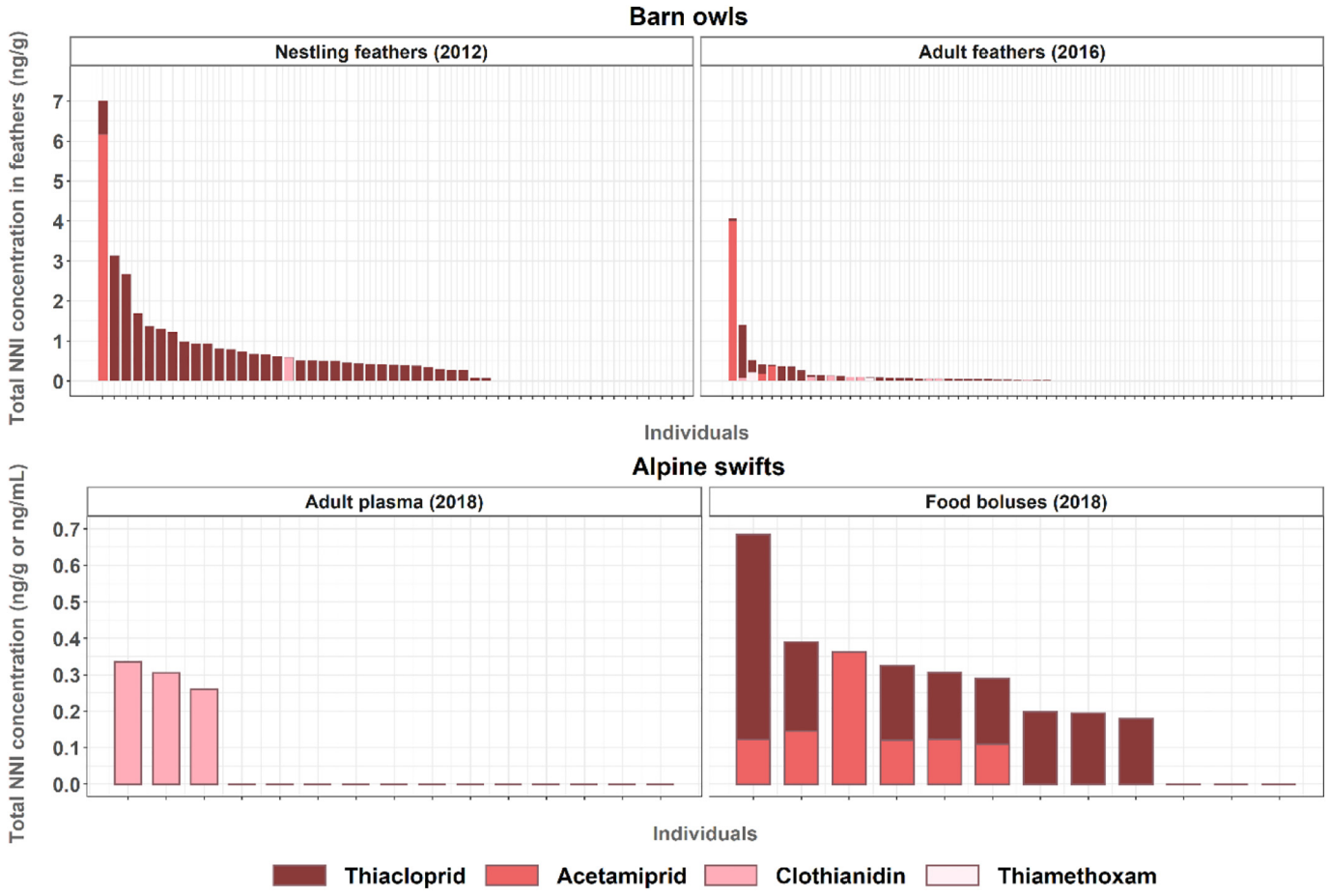

Fig. 2. Distribution of the concentrations in neonicotinoid insecticides (NNIs) measured in each sample. Individual samples are ranked in descending order of concentration. Limits of quantification (LOQ) are provided in Table 1.

samples. In total, 100% and 87% of the food boluses and plasma samples collected in adult Alpine swifts contained at least one neonicotinoid above the LOD. In Alpine swift nestlings, we found only one feather sample (2%) that contained thiacloprid at a concentration between the LOD and the LOQ. We found no measurable concentrations of neonicotinoids in the nestling feathers sampled over the 2004–2017 period (all samples <LOQ; $N = 50$ nests). The mean ± SE and median concentrations of total neonicotinoids in food boluses were 0.24 ± 0.20 ppb and 0.24 ppb ($N = 12$ individuals; Table 1). The mean ± SE and median concentrations of total neonicotinoids in plasma were 0.06 ± 0.13 ppb and 0 ppb ($N = 15$ individuals; Table 1). Only acetamiprid and thiacloprid were found in food boluses, but thiacloprid dominated in terms of presence and concentration (Table 1, Fig. 2). However, clothianidin only was found in three (20%) plasma samples (Table 1, Fig. 2).

The restriction on the use of the three NNIs imidacloprid, clothianidin and thiamethoxam in all outdoor crops has been implemented in Switzerland in January 2019 (Bundesamt für Landwirtschaft, 2018). At the time of sample collection in July 2018, the restriction on the three NNIs was not yet implemented, which could explain the prevalence of thiacloprid and acetamiprid in food boluses, but also clothianidin in plasma. Alpine swifts are strictly insectivorous birds staying aloft in the air for a long period of time and eating insects on the wing. NNIs are agonists of the acetylcholine receptors in the central nervous system of insects, and cause death through paralysis (Goulson, 2013). Hence, insects captured on the wing by Alpine swifts likely harbour supposedly low and non-lethal amounts of NNIs, and, at first glance, Alpine swifts may be considered at low risk of being exposed. However, we observe here that 100% of the food boluses delivered to the nestlings contained NNIs at detectable concentrations (>LOD). Measurable concentrations (>LOQ) are comprised between 0.18 and 0.68 ppb. A plausible explanation for the high detection rate of NNIs in food boluses would be that the contamination comes through the inhalation of aerosolized NNIs from toxic dust or after a drench application (Girolami et al., 2012; Krupke et al., 2012; Rogers et al., 2019). However, these relatively low concentrations in food boluses, compared to the reported lethal concentrations for a variety of arthropods (Humann-Guilleminot et al., 2019b), may be due to the fact that insects weakened by lethal or sublethal doses of NNIs may be unlikely to enter the diet of Alpine swifts, whose hunting behaviour protects them from being exposed to substantial amounts of NNIs. This low contamination by ingestion is further reflected by the low prevalence of neonicotinoids in the plasma of adults. Although 87% of the plasma samples were contaminated, only clothianidin was found at measurable concentrations (>LOQ), which contrasts with the NNIs found in food boluses. The presence of clothianidin in the plasma of adults, but not in the food boluses, may be explained by the rapid excretion of NNIs from the body and the very transient presence of these molecules in the blood, as exemplified with imidacloprid in adult male Japanese quails; Bean et al., 2019). The presence of clothianidin in the birds' plasma could be a one-off event during which exposed individuals may have sought food near crops containing clothianidin, such as beetroots crop in which clothianidin was still allowed at this time in Switzerland. Analysis of NNIs in bird feces would enable a complementary comparison of the concentration found in the food boluses and a better understanding of the birds' exposure, because it allows detection in the longer term than in plasma where NNI presence may be ephemeral (example with imidacloprid in Roy et al., 2020). Additionally, it also suggests a possible alternative route of exposure, besides food consumption, either through feather preening after deposition of toxic dust onto feathers or through the ingestion of contaminated water. Nevertheless, we are aware that samples sizes are small and that the two samples were collected from different birds, which limits our interpretation of the results and calls for further research.

We expected that concentrations found in the food boluses would be reflected in the concentrations found in nestling feathers. However, none of the feather samples contained measurable concentrations of NNIs, and only one sample contained thiacloprid above the LOD. Nestlings continuously grow their feathers, but they are also continuously fed by the two parents, so in theory NNIs could accumulate in feathers during their growth. One explanation for the absence of NNIs in nestling feathers may be that Alpine swifts have the ability to slow down or speed up their metabolism depending on the environmental conditions. For instance, they can slow down their metabolism to a state of torpor when the weather is too bad and insects are lacking. In contrast, they can speed up their metabolism when environmental conditions are favourable and insects are plentiful (Bize et al., 2007; Bize et al., 2010). Such acceleration of metabolism is enabled by rapid food digestion and may potentially lead to fast degradation and/or excretion of NNIs, which then would not accumulate in the feathers. Low NNI concentrations in the food boluses combined with rapid degradation and/or excretion would explain why nestling feathers contain no neonicotinoid insecticides.

3.3. Potential implications on birds' health

Our results agree with those of Byholm et al. (2018) showing that birds of prey can be exposed to neonicotinoid insecticides. Our results also show that strictly insectivorous birds may be exposed to NNIs. Recent research strongly suggests that wild birds may be vulnerable to NNIs exposure with potential adverse effects (reviewed in Gibbons et al., 2015 and Pisa et al., 2021). First, granivorous birds can suffer sublethal effects (López-Antia et al., 2015) or even be poisoned (MacDonald et al., 2018; Millot et al., 2017) when ingesting coated seeds. Second, a single non-lethal dose of NNI (10% or 25% of the LD_{50}: Eng et al., 2017; Eng et al., 2019) may have physiological and behavioural consequences. Lastly, the ingestion of very low, chronic amounts of NNIs (0.07% of the LD_{50} every third day over 21 days: Humann-Guilleminot et al., 2019c; 0.5% of the LD_{50} every day over 30 days: Pandey and Mohanty, 2015; Mohanty et al., 2017) may impair the synthesis of thyroid and sexual hormones, disrupt testicular functions and lower sperm quality. For these reasons, we believe that, even at low concentrations, neonicotinoid insecticides may affect the reproduction and survival of species studied here if birds are chronically exposed.

3.4. Conclusion

This field study provides additional evidence that birds are exposed to NNIs. Recent research shows that non-strictly granivorous birds can be contaminated by NNIs (Humann-Guilleminot et al., 2019a; Lennon et al., 2020). Yet, data on the presence of neonicotinoid residues in birds higher up in the food web is very scarce. Overall, our results substantiate the wide contamination of birds having various diets and living in different habitats by NNIs, and incidentally highlight their ubiquitous presence in the environment. Although a ban on imidacloprid, clothianidin and thiamethoxam has been implemented in Europe and Switzerland for all outdoor crops (with possible derogation for beetroot cultures), we believe that this restriction should be extended to the two others NNIs acetamiprid and thiacloprid that have been found in most of our samples and have the potential to negatively impact bird health.

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CRediT authorship contribution statement

FH, SL designed the study; AR initiated and manages the long-term barn owl project and collected barn owl feathers; PB initiated and manages the long-term Alpine swift project and collected Alpine swift feathers, plasma samples and food boluses; SL, GG analysed the samples; SHG, SL, FH analysed the data; SHG, FH, SL wrote the manuscript and all authors commented and approved the final version.

Ethical note

This work was conducted under licences of the Veterinary Services of the Cantons Berne and Solothurn for the Alpine swift and of the Canton Vaud for the Barn owl. Ringing permits were provided by the Swiss Federal Office for the Environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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