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Snail consumption and breeding performance of pied flycatchers (*Ficedula hypoleuca*) along a pollution gradient in the Middle Urals, Russia

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HIGHLIGHTS

- Pied flycatchers consume less snail shells in the heavily polluted sites
- Diversity of snails collected by birds decreased in polluted sites
- The closer the smelter, the higher proportion of deserted clutches and abnormal eggs
- Brood size decreased in the polluted area, especially if snail supply was low

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ABSTRACT

During the years 1989–91, 1997–2003, and 2005–07, we studied how emissions from the Middle Urals copper smelter affect snail availability and reproduction of free-living pied flycatchers (*Ficedula hypoleuca*). We counted snail shells dropped in nests and analysed food samples of nestlings. Pied flycatchers brought to nestlings fewer shells in heavily polluted sites compared to background sites, resulting in reduced Ca intake. Species diversity of snails collected by birds decreased with decreasing distance from the pollution source. The pattern was the same both in deciduous and coniferous forests. In sites closest to the smelter, 20–50% of breeding females suffered from Ca deficiency, which resulted in an increased proportion of deserted clutches and clutches with defective eggshells. Number of fledglings per nest decreased in heavily polluted sites, especially in broods with decreased snail supply. This study demonstrated that pollution can cause both direct effect of toxicants to birds and indirect effects via reduced Ca availability.

1. Introduction

Birds need calcium (Ca) as a constructional material for eggshell and nestling skeleton. This is why Ca consumption by birds increases during breeding (Simkiss, 1967). Calcium deficiency in adult birds results in clutch desertion, eggshell defects, reduced clutch size and hatching success, retarded laying date, and irregular laying (review: Reynolds and Perrins, 2010). Snail shells are considered one of the main sources of Ca for free-living passerines (Graveland and Van Gijzen, 1994; Graveland, 1996; Bureš and Weidinger, 2000). This explains why shell availability can affect breeding success and should be taken into account when analysing bird reproduction.

The abundance and spatial distribution of land snails depend on chemistry and humidity of soil (litter) and vegetation characteristics (Wäreborn, 1992; Götmark et al., 2008). These factors determine food supply of snails and availability of Ca required for shell construction. Land snail abundance is low in ecosystems on Ca-poor soils (Drent and Woldendorp, 1989; Tilgar et al., 1999; Graveland and van der Wal, 1996; Mänd et al., 2000). Soil Ca concentrations are low in acidified areas and near industrial enterprises emitting acidifying compounds (e.g., sulphur and nitrogen oxides). The snails disappear in forests and meadows near such polluters (Vorobeichik et al., 2012; Eeva et al., 2010; Nesterkov, 2013). However, relationships between industrial pollution, snail availability, and reproduction of free-living birds have not been studied sufficiently. As regards the pied flycatcher, there were only few studies in southwest Finland (Eeva and Lehikoinen, 2004; Eeva et al., 2010).

In this study, we analysed snail consumption and some effects of Ca deficiency in local populations of pied flycatchers along a pollution gradient in the Middle Urals, Russia. We tested two hypotheses: 1) snail shell consumption is affected both by pollution and habitat; 2) breeding

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output of birds depends on availability of snails representing an important source of Ca. If snails are sensitive to acidification and vegetation change we predicted that birds in polluted sites consume less shells compared to undisturbed areas. We expected that snail abundance and diversity in the bird diet is reduced in coniferous habitat compared to deciduous forest, which is considered to be more favourable for snails. If reproduction of birds depends on calcium availability, then we predicted reduced breeding output in polluted areas.

2. Material and methods

2.1. Study area

The study was performed during the years 1989–91, 1997–2003, and 2005–07 in the vicinity of the Middle Urals copper smelter (Russia, Revda, 56°51′N, 59°53′E), which is a strong source of sulphur dioxide and polymetallic dust. Total emissions varied from 140,700 t in 1989 to 24,500 t in 2007 (Vorobeichik et al., 1994; DNRSO, 2008). Metal (Cu, Pb, and Cd) concentrations in the soil (horizon A1, extracted with 5% HNO_3 for 24 h with a soil-to-acid ratio of 1:10 by weight) decreased exponentially with increasing distance to the smelter (Fig. 1).

Zones with different levels of pollution and degradation of forest ecosystems were distinguished in the vicinity of the plant, based on investigations of soil quality and microbiological activity (Kaigorodova and Vorobeichik, 1996; Vorobeichik, 2007), vascular plants (Vorobeichik and Khantemirova, 1994), epiphytic lichens (Mikhailova and Vorobeichik, 1995), soil-dwelling (Vorobeichik et al., 2012) and epigeic invertebrates (Zolotarev and Belskaya, 2012), leaf-eating insects (Belskaya and Vorobeichik, 2013), birds (Belskii and Lyakhov, 2003) and small mammals (Mukhacheva, 2007). The zone of high pollution (impact zone) extends westward up to 2.5 km from the smelter. Copper and Pb concentrations in the soil (horizon A1) exceed regional background levels by 43.4 and 9.5 times, respectively (Belskii et al., 2005), and pH_{water} is 4.37–5.17 (Kaigorodova and Vorobeichik, 1996). The moderately polluted (buffer) zone extends 3–15 km to the west of the smelter. Soil Cu and Pb concentrations exceed regional background levels by 9.9 and 4.4 times, respectively, and soil pH_{water} is 4.48–5.77. The relatively unpolluted (background) zone is situated ≥16 km to the west of the smelter, where soil pH_{water} is 5.04–6.24.

Study sites, each with 14–81 nestboxes, were established in two habitats along the pollution gradient to the west of the smelter, within a 1–27-km range (Fig. 2). Both habitats were represented in each pollution zone. There were 1) aspen-birch (*Populus tremula* and *Betula verrucosa* + *Betula pubescens*) forests with some admixture of conifers and 2) fir-spruce (*Picea obovata* and *Abies sibirica*) forests with an admixture of pine (*Pinus sylvestris*), birch, and aspen. The forest was rarefied near the smelter, with large amounts of dead wood and depressed young growth due to the long-term (starting from 1940) effects of pollution.

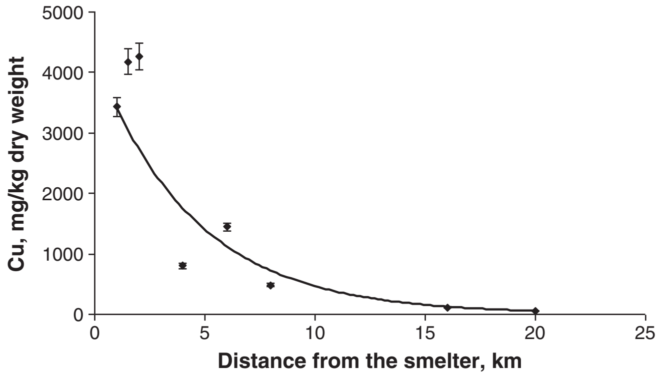

Fig. 1. Organic soil copper concentrations (±SE; extracted from humus layer with 5% HNO_3) at different distances from the Middle Urals copper smelter ($y = 4285.1 \times e^{-0.22 \times \text{distance}}$, $R^2 = 0.94$).

2.2. Model species

Nestboxes were commonly occupied by pied flycatchers (*Ficedula hypoleuca* Pall). They are small (~12 g) passerines and long-distant migrants with winter grounds in West Africa, south of the Sahara. Pied flycatchers inhabit different forest types and readily occupy nestboxes. They feed on different arthropods taken from tree crowns, on the ground, or in the air by darting out from a perch (Cramp and Perrins, 1993). Pied flycatchers breed in the study area in May–July; nestlings usually fledge at age 15 days.

2.3. Sampling

The nestboxes were checked regularly to record dates of the egg laying and hatching, number of eggs, hatchlings, and fledglings. Special attention was paid to eggs, which desiccated during incubation because of defective shells. Nests with at least one egg laid were included in the analysis.

Adult birds bring small snail shells to nestlings, which are an important source of Ca required for proper growth of nestling skeletons (review: Reynolds and Perrins, 2010). Some shells drop and build up in the bottom of nests. Numbers of spilled shells in nests reflect snail abundance in breeding territories and their consumption by birds (Graveland and van der Wal, 1996; Tilgar et al., 1999). After fledging, nests were collected in plastic bags, transported to the laboratory, and searched for dropped snail shells. Only those nests were collected where at least one nestling fledged. Only undamaged (or slightly damaged) snail shells were sampled. In total, 1113 shells in 388 nests were sampled. Snail species were identified by Maxim Grebennikov with the help of the guide by Sysoev and Schileyko (2009).

The diet of *F. hypoleuca* nestlings (6–11 days old) was studied using neck collars made of fishing line (Kuligin, 1981) at the same sites in deciduous forest in 2000, 2003, and during 2005–07. In total, 1567 food boluses were collected in 104 nests of six sites (two sites per pollution zone). Sampled snails ($n = 80$) were preserved in alcohol.

To estimate contaminant exposure, nestling faeces was sampled during 2002–03 and 2006 in 10 nests each in the background and impact zones.

2.4. Metal analyses

Faecal samples (on average 100 mg dry mass weighed to the nearest 0.1 mg) were digested in a mixture of 7 mL supra-pure HNO_3 + 1 mL de-ionised H_2O in Teflon bombs in a microwave system MWS-2 (Berghof, Germany). Copper, Zn, Cd, and Pb concentrations were measured with an AAS 6 Vario atomic absorption spectrometer (Analytik Jena, Germany) and Ca with an ICP-atomic SPECTRO Genesis emission spectrometer (SPECTRO Analytical Instruments, Germany). Certified reference material (bovine liver CRM-185R) was used for method validation. The recovery from the reference sample was as follows: Cu, 95%, Zn, 99%, Cd, 101%, and Pb, 107%.

2.5. Statistical analyses

Most statistical analyses were performed with STATISTICA v.8.0 (StatSoft, Inc., 2008). Proportion of nests with snail shells was calculated for each site as a yearly mean. The number of shells per nest was calculated only for nests with ≥1 shell. When analysing species composition of snails stored in nests, 14 sites were grouped by three pollution zones and two habitats. Species richness (number of species) was estimated per minimal sample (26 shells in nests and 11 shells in food samples) by using individual rarefaction procedure in PAST, v.1.92 (Hammer et al., 2001). Shannon diversity indices and 95% confidence limits

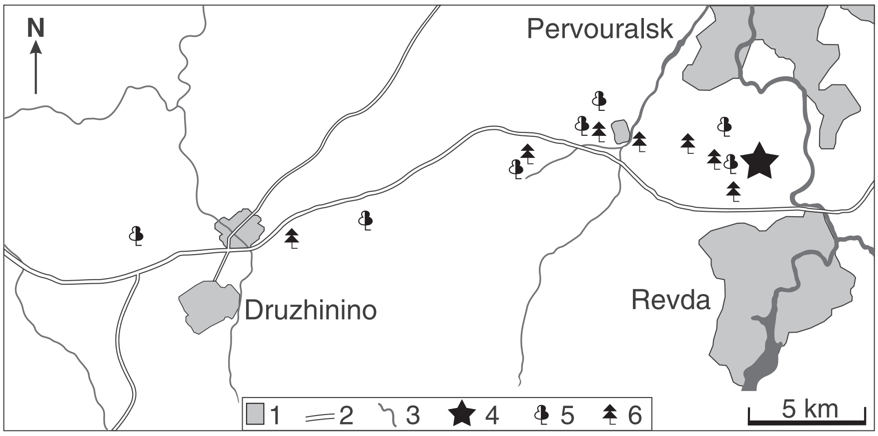

Fig. 2. Location of study sites along the pollution gradient in the study area. Legend: 1, settlements; 2, roads; 3, rivers; 4, Middle Urals copper smelter; 5 and 6, study sites in deciduous and coniferous forests.

were calculated with the same software. The similarity of different shell samples was calculated using the index (Schoener, 1970):

$$I = 1 - 0.5\sum_i \left| p_{ij} - p_{ik} \right|$$

where p_{ij} and p_{ik} are proportions of *i*th species in *j*th and *k*th samples.

The dependencies of shell number per nest on the distance to the smelter ($\log_{10}$), number of hatchlings, hatching date (June 1st = 1), and weather in each habitat were analysed with multiple linear regression using data from the years 1997–99, 2002, and 2006–07. Nests with no shells were included in this analysis. To remove effect of the year, the number of shells in each nest was divided by the largest value among all nests (all sites combined) of a particular year (x_i / x_{max}). This allowed for presentation values of different years in the same range, 0–1. The hatchling numbers and hatching dates were standardised as follows: $y_i = (x_i - x_{mean}) / SD$, where x_i is the value in *i*th nest, x_{mean} is the mean for all nests of a particular year, and *SD* is standard deviation. Since snail activity depends on weather (Wäreborn, 1992), the sum of mean daily temperatures and sum of precipitation during a 15-day period from hatching to fledging for each nest were included in the analysis (data from Revda weather station).

The relationship between bird breeding performance, zone of pollution, and snail availability was analysed with analysis of covariance (ANCOVA). The dependent variable was standardised number of fledglings per nest, and the independent categorical factors were zone (background, buffer, and impact), habitat (deciduous and coniferous), and number of shells per nest grouped into three classes: none (zero), few (1–3), and many (>3). The continuous predictors were standardised first egg date (May 1st = 1), sum of mean daily temperatures, and sum of precipitation over a 15-day period from hatching to fledging. Post-hoc comparisons were performed with a Scheffe test.

Table 1
Metal concentrations (mean ± SE, mg/kg dry weight) in faeces of *F. hypoleuca* nestlings in two zones near the Middle Urals copper smelter (n = 10 nests per zone).

Metal	Zone		Effect of zone [a]	
	Background	Impact	*F*	*p*
Cu	41.7 ± 3.6	254.6 ± 42.7	82.9	<0.001
Zn	424.9 ± 44.8	777.7 ± 92.9	11.1	0.004
Cd	6.4 ± 0.7	23.8 ± 3.6	34.9	<0.001
Pb	13.7 ± 1.9	186.5 ± 25.0	136.1	<0.001
Ca	3200.6 ± 943.5	1758.2 ± 479.6	2.7	0.12

[a] One-way analysis of variance (ANOVA), metal concentrations were $\log_{10}$-transformed.

3. Results

Concentrations of Cu, Zn, Cd, and Pb in nestling faeces were 2–14 times higher in the impact zone compared to the background zone, indicating higher contaminant exposure (Table 1).

Among shells spilled in nests, *Discus ruderatus* was the most abundant in all pollution zones, followed by *Perpolita hammonis* (Table 2). In deciduous forest of the impact zone, *Zonitoides nitidus* was as numerous as *D. ruderatus*. Dietary proportion of *Euconulus fulvus* and *Z. nitidus* (in the deciduous habitat) increased along the pollution gradient in contrast to *Fruticicola fruticum* and *Cochlicopa lubricella*. Similarity of snail composition between two habitats was strong in the background (0.85) and buffer (0.90) zones but moderate in the impact zone (0.53).

In food samples, *F. fruticum* and *D. ruderatus* were dominant species in the background zone, *Z. nitidus* and *D. ruderatus* in the buffer zone, and *Z. nitidus* in the impact zone (Table 3). Species richness of shells spilled in nests was higher compared to food samples due to a larger number of rare species. At the same time, one species (*Columella edentula*) was registered only in food samples. Similarity of snail composition between nest and food samples was not strong: I (±SE) = 0.63 ± 0.03 (n = 4 sites).

Species richness of snails in nest and food samples decreased with increasing pollution (Tables 2, 3). Calculation per minimal sample (26 and 11 shells, respectively) gave the same results. Shannon diversity index tended to decrease close to the pollution source.

The proportion of nests with shells decreased with decreasing distance from the smelter in both habitats (Fig. 3A) and was 2.5–4 times less in the impact zone compared to other zones. The number of shells per nest varied similarly (Fig. 3B), being lower within 4 km from the polluter. The incidence of snails in food samples decreased with increasing pollution, as well, namely by six times (Table 3). Spearman rank correlation between percentages of nests and boluses with snails was rather high (0.70) but non-significant ($p > 0.05$), probably due to small sample size (n = 5 sites).

The regression analysis showed that in deciduous forest the number of shells per nest increased with the distance from the smelter and the sum of precipitation during the chick-rearing period. At the same time, the number of shells decreased as the season progressed (Table 4). In coniferous forest, the number of shells per nest was positively related to the distance from the smelter and number of hatchlings.

Calcium concentrations in nestling faeces tended to be lower in the impact zone compared to the background zone (Table 1), indicating lower Ca supply. It is probable that not only nestlings but also breeding females suffer from Ca deficiency, resulting in production of eggs with defective shells. Content of such eggs desiccates during incubation. Proportion of nests with desiccated eggs increased with decreasing distance from the smelter (Fig. 4A). Another effect of Ca deficiency in

Table 2
Species composition and relative abundance (% of total number of shells) of snails collected in nests of *F. hypoleuca* in three zones near the Middle Urals copper smelter. Species are ordered alphabetically.

Species, indices	Deciduous forest			Coniferous forest		
	Zone of pollution					
	Background	Buffer	Impact	Background	Buffer	Impact
Aplexa sp.	0	0.5	0	0	0	0
Cochlicopa lubrica	5.9	5.7	6.4	10.8	2.1	6.9
Cochlicopa lubricella	8.4	1.1	0	0.9	2.1	0
Cochlicopa nitens	0	0.5	0	1.5	0.7	0
Cochlicopa sp.	0	0.5	0	0.3	0	10.4
Discus ruderatus	47.2	44.3	38.3	45.4	48.2	44.8
Euconulus fulvus	1.6	1.1	8.5	2.4	2.1	17.2
Euomphalia strigella	0.5	0	0	1.2	0	0
Fruticicola fruticum	5.4	2.6	0	10.2	0.7	0
Lymnaea truncatula	0.8	1	0	2.4	3.6	0
Perpolita hammonis	27.5	27.6	0	24.3	27.7	20.7
Succinea putris	0.3	0	0	0.3	0	0
Vallonia costata	1.9	0	0	0.3	1.4	0
Vallonia pulchella	0	0	8.5	0	0	0
Zonitoides nitidus	0.5	15.1	38.3	0	11.4	0
Number of shells	371	192	47	333	141	29
Number of species [a]	11	10	5	11	10	4
Number of species (*SD*) per minimal sample (26 shells)	5.78 (1.10)	5.36 (1.06)	4.85 (0.37)	5.86 (1.10)	5.70 (1.18)	4 (0)
Shannon diversity index (95% conf. limits)	1.50 (1.49–1.73)	1.46 (1.44–1.75)	1.33 (1.20–1.82)	1.55 (1.49–1.73)	1.45 (1.39–1.77)	1.20 (0.98–1.82)
Number of nests	95	55	57	101	38	42
Number of nests with shells	68	41	17	85	31	9
% nests with shells ± SE	71.6 ± 4.6	74.5 ± 5.9	30.0 ± 6.1	84.2 ± 3.6	81.6 ± 6.3	21.0 ± 6.3

[a] *Cochlicopa* sp. was not taken into account when calculating species number.

females is nest desertion during egg laying. Proportion of deserted clutches increased near the smelter (Fig. 4B) and equaled (±SE) 3.8 ± 1.1% (n = 316 nests) in the background zone, 5.7 ± 2.1% (124) in the buffer zone, and 12.9 ± 3.3% (101) in the impact zone.

The ANCOVA showed that the number of fledglings per nest of *F. hypoleuca* was negatively related to the pollution level and positively to snail availability, when the effects of timing of breeding and weather conditions were controlled for (Table 5). There was a significant interaction between zone and number of shells. Nests with no or few shells produced less fledglings in the impact zone compared to buffer and background zones (Scheffe-test, $p < 0.02$). However, nests with a high snail supply produced as many fledglings in the impact zone as in other zones (Fig. 5). Effects of habitat and other interactions were insignificant. The final model (after non-significant terms were dropped) explained 41.5% of the variance in standardised number of fledglings.

4. Discussion

The proportion of broods receiving snail shells and consumption of the latter were negatively related to the distance from the polluter, confirming observations near a Cu–Ni smelter in southwest Finland (Eeva and Lehikoinen, 2004; Eeva et al., 2010). These results agree with censuses near the Middle Urals copper smelter, which detected no snails in the forest litter up to a 3-km distance from the smelter and in some sites up to 6 km (Vorobeichik et al., 1994, 2012) and no snails in the grass layer on meadows at a distance 1 km from the smelter (Nesterkov, 2013). The drastic decline of snail populations in heavily polluted areas can be attributed to reduced Ca availability in acidified soil (Kaigorodova and Vorobeichik, 1996), transformation of the grass layer, and microclimatic conditions. Calcium is the main building material for snail shell and its leaching from soil

Table 3
Species composition and relative abundance (% of total number of shells) of snails in food samples of nestlings *F. hypoleuca* in deciduous forest in three zones near the Middle Urals copper smelter. Species are ordered alphabetically.

Species, indices	Zone of pollution		
	Background	Buffer	Impact
Cochlicopa lubrica	0	8.7	0
Cochlicopa lubricella	2.2	0	0
Cochlicopa nitens	2.2	0	0
Columella edentula	2.2	0	0
Discus ruderatus	39.1	30.4	9.1
Euconulus fulvus	0	4.4	9.1
Fruticicola fruticum	43.5	0	0
Perpolita hammonis	6.5	8.7	0
Vallonia costata	4.3	0	0
Zonitoides nitidus	0	47.8	81.8
Number of shells	46	23	11
Number of species	7	5	3
Number of species (*SD*) per minimal sample (11 shells)	3.71 (0.95)	3.95 (0.75)	3 (0)
Shannon diversity index (95% conf. limits)	1.29 a (1.31–1.82)	1.28 a (1.12–1.81)	0.60 b (0.91–1.72)
Number of boluses	568	450	549
Number of boluses with shells	40	13	6
% boluses with shells ± SE	7.0 ± 1.1	2.9 ± 0.8	1.1 ± 0.4
Number of broods examined	52	22	30

Shannon diversity index: values with the same letter are not significantly different.

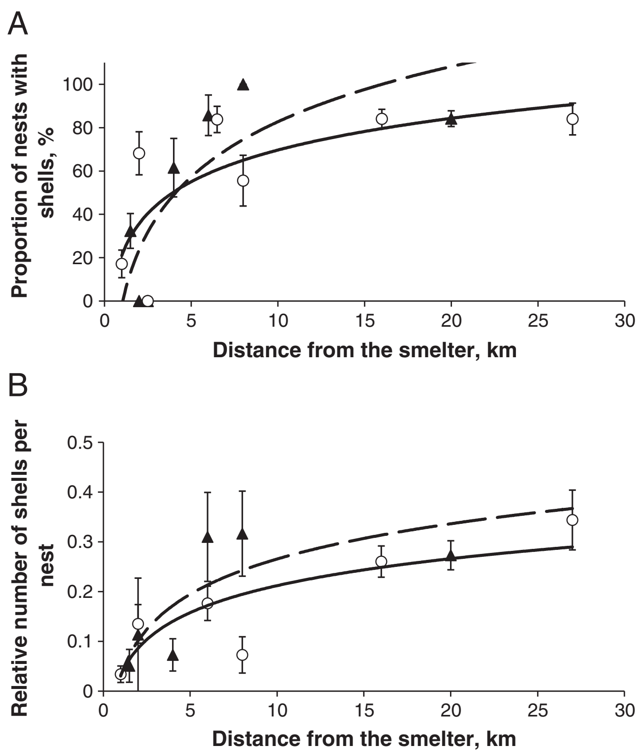

Fig. 3. Mean frequency (±SE) of nests with shells (A) and relative number of shells per nest (x_i / x_{max}, nests with no shells excluded) (B) in deciduous (solid lines and circles; in panel A y = 20.9 × Ln(distance) + 21.5, R^2 = 0.51; in panel B y = 0.08 × Ln(distance) + 0.03, R^2 = 0.70) and coniferous (dashed lines and triangles; in panel A y = 36.8 × Ln(distance) − 2.0, R^2 = 0.63; in panel B y = 0.10 × Ln(distance) + 0.03, R^2 = 0.62) forest.

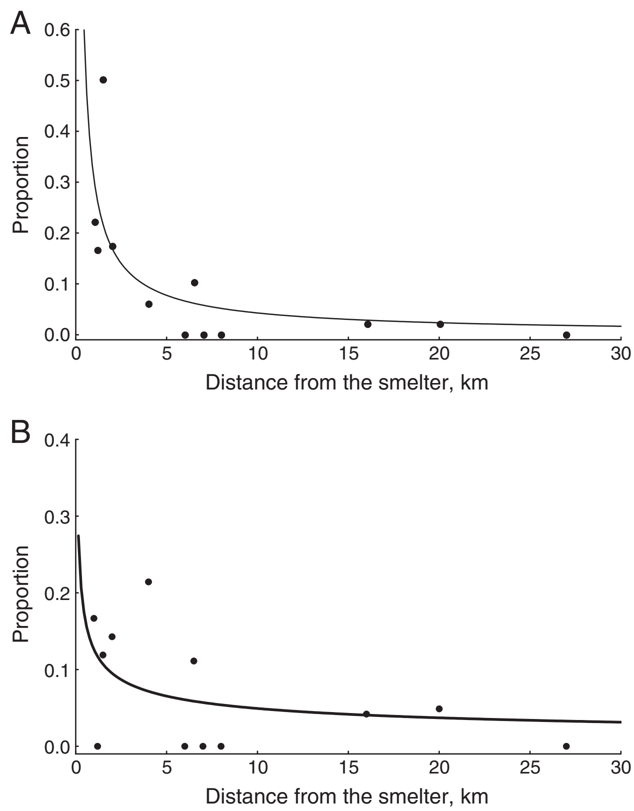

Fig. 4. Proportions of *F. hypoleuca* nests with desiccated eggs (A, y = 0.31 × $(\text{distance})^{-0.86}$, R^2 = 0.54) and deserted nests (B, y = 0.13 × $(\text{distance})^{-0.41}$, R^2 = 0.20) at different distances to the Middle Urals copper smelter. Points denote site means.

results in decreased abundance of molluscs (Wäreborn, 1992). Species diversity of vascular plants decreases in polluted areas (Kozlov et al., 2009; Trubina and Vorobeichik, 2012), resulting in reduced food supply for snails. In polluted areas, the replacement of herbs with grasses with weak branching of stems and narrow leaf blades results in a simplified three-dimensional structure of the field layer. Such changes in vegetation promote greater fluctuations of temperature and humidity in the ground layer, which are unfavourable for molluscs (Nesterkov, 2013).

Interestingly, birds found snails more efficiently than researchers; ~20% of nests contained shells in the impact zone where no molluscs were counted in the forest litter and in the grass layer in meadows. Despite apparent selectivity, birds consumed fewer snails in polluted areas. The analysis of food samples confirmed the results based on examination of nests. Unfortunately, our data allowed comparison of nest and food samples only within sites and zones, not nests. The proportion of nests with shells exceeded the proportion of boluses with snails (Tables 2, 3). An explanation may be that dropped items are stored in nests during the whole feeding period unlike food samples, which reflect short-time foraging efforts. Nevertheless, proportions of nests with shells and boluses with snails varied along the pollution gradient in a similar way and decreased as the pollution increased.

Snails were the main source of Ca for flycatchers in our study area unlike in Central Europe (Bureš and Weidinger, 2003). Snail shortage in polluted sites resulted in some dietary shifts to other Ca-rich items (i.e., isopods and soil). In the background zone, 40 of 568 boluses contained snails, and two boluses contained soil. In the impact zone, six of 549 boluses contained snail shells, four boluses contained woodlice, and four boluses soil. Apparently, only some pairs managed to find Ca-rich items close to the smelter, resulting in high inter-nest variability of Ca concentrations in nestling faeces. Nevertheless, low mean Ca level in faeces suggests that birds in heavily polluted sites are Ca-limited.

Studies near non-ferrous smelters revealed a number of effects of Ca deficiency on nestlings *F. hypoleuca*. Calcium concentration in nestling bones near a sulphide ore smelter in Sweden was 20–25% lower compared to the background area (Nyholm, 1995). In such nestlings, wing and leg bones become fragile and can break or

Table 4
Results of the multiple regression of relative number of shells per nest (x_i / x_{max}) on the distance to the smelter, number of hatchlings, hatching date, sum of temperatures, and sum of precipitation during a 15-day period from hatching to fledging in deciduous (n = 155 nests) and coniferous (n = 102) habitats. Boldface indicates significant effects.

Variable	Deciduous forest			Coniferous forest		
	β	SE	*p*	β	SE	*p*
Log_{10} distance to the smelter	0.38	0.08	**<0.001**	0.28	0.10	**0.006**
Standardised number of hatchlings	−0.04	0.08	0.620	0.27	0.11	**0.017**
Standardised hatching date	−0.18	0.08	**0.031**	0.02	0.10	0.863
Sum of precipitation during nestling period	0.21	0.08	**0.010**	−0.05	0.09	0.565
Sum of temperatures during nestling period	0.01	0.08	0.926	−0.13	0.09	0.145

Table 5
Results of the analysis of covariance (ANCOVA) with the standardised number of fledglings per *F. hypoleuca* nest as the dependent variable, zone of pollution (background, buffer, and impact), habitat type (deciduous and coniferous forests), and number of shells per nest (zero, 1–3, and > 3) as categorical variables (factors), and standardised first egg date (May 1st = 1), sum of mean daily temperatures, and sum of precipitation during a 15-day period from hatching to fledging as continuous variables (covariates).

Source of variation	SS	D.f.	MS	F	*p*
Date of first egg, standardised	34.66	1	34.66	54.08	<**0.001**
Sum of temperatures during nestling period	0.99	1	0.99	1.54	0.22
Sum of precipitation during nestling period	1.00	1	1.00	1.55	0.22
Zone	27.00	2	13.50	21.07	<**0.001**
Habitat	0.12	1	0.12	0.18	0.67
N shells	12.00	2	6.00	9.36	<**0.001**
Zone × habitat	1.87	2	0.94	1.46	0.23
Zone × N shells	7.67	4	1.92	2.99	**0.019**
Habitat × N shells	0.90	2	0.45	0.71	0.49
Zone × habitat × N shells	2.29	4	0.57	0.89	0.47
Error	214.07	334	0.64		

Boldface indicates significant effects.

bend. Proportion of broods with bone defects equaled 27% near a Cu–Ni smelter in Finland (Eeva and Lehikoinen, 1996). In our study area nestlings with bent legs were also observed.

Breeding females also evidently suffer from Ca deficiency. Part of the Ca required for eggshell formation is mobilised from the female skeleton. The bones become fragile if dietary Ca intake is insufficient to cover its loss with eggshell. In 2007, a dead female was found in a nest with seven eggs in the impact zone. Both its tibiotarsi were broken at the distal ends. In this female, Ca losses were high enough to cause fragility of bones, which could not withstand body weight. However, this is an extreme case. More often, Ca-deficient females lay eggs with thin shells and/or supernormal porosity (Drent and Woldendorp, 1989; Graveland and van der Wal, 1996). Content of such eggs desiccates before or during incubation, and embryos perish. Incidence of nests with desiccated eggs reflects the proportion of Ca-deficient females, which increased up to 20–50% in the impact zone (Fig. 2). If deserted clutches are taken into account, the proportion of Ca-deficient birds is still higher.

An important result was significant interaction of “zone of pollution” and “shell availability” factors on reproductive output of birds. Breeding performance in the impact zone was lower than in background zone only in those pairs which could not provide nestlings with large amount of snail shells. In other words, reproductive losses in heavily polluted sites were greater in Ca-deficient birds. This can be attributed to increased intestinal absorption of toxic metals like Pb and Cd in birds on a low-Ca diet (Scheuhammer, 1996; Snoeijs et al., 2005). In contrast,

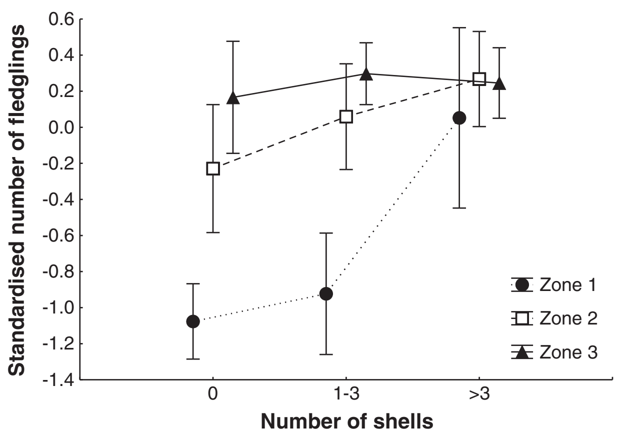

Fig. 5. Breeding performance of *F. hypoleuca* (standardised number of fledglings / nest ± SE) in the impact (1), buffer (2), and background (3) zones by different snail supply (number of spilled shells in nests).

high dietary Ca supply diminishes toxic effects of heavy metals in birds (Eeva and Lehikoinen, 2004). Therefore, Ca deficiency is an important cause of increased reproductive failures near non-ferrous smelters affecting both breeding females and their offspring.

5. Conclusions

Our study showed that censuses of snail shells spilled in nests are suitable for assessing dietary shell consumption in *F. hypoleuca*. Pied flycatchers brought to nestlings fewer shells in the heavily polluted zone compared to background zone, resulting in reduced Ca intake. Species diversity of snails collected by birds decreased with decreasing distance from the pollution source. The pattern was the same both in deciduous and coniferous forests. In the impact zone, 20–50% of breeding females suffered from Ca deficiency, which resulted in an increased proportion of deserted clutches and clutches with defective eggshells. The number of fledglings per nest decreased in heavily polluted sites, especially in nests with decreased snail supply. This study demonstrated that pollution can cause both direct effects of toxicants to birds and indirect effect via reduced Ca availability.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Belskaya EA, Vorobeichik EL. Responses of leaf-eating insects feeding on aspen to emissions from the Middle Ural copper smelter. Russ J Ecol 2013;44(2):108–17.
Belskii EA, Lyakhov AG. Response of the avifauna to technogenic environmental pollution in the southern taiga zone of the Middle Urals. Russ J Ecol 2003;34(3):181–7.
Belskii EA, Lugas'kova NV, Karfidova AA. Reproductive parameters of adult birds and morphophysiological characteristics of chicks in the pied flycatcher (*Ficedula hypoleuca* Pall.) in technogenically polluted habitats. Russ J Ecol 2005;36(5):342–50.
Bureš S, Weidinger K. Estimation of calcium intake by meadow pipit nestlings in an acidified area. J Avian Biol 2000;31:426–9.
Bureš S, Weidinger K. Sources and timing of calcium intake during reproduction in flycatchers. Oecologia 2003;137:634–41.
Cramp S, Perrins CM. The birds of the Western Palearctic. , 7. Oxford: Oxford Univ. Press; 1993.
DNRSO (Department of Natural Resources of Sverdlovsk Oblast).. Public report on the state of environment and effects of ecological factors on human health in Sverdlovsk oblast in 2007. In: Pakhalchak GY, editor. Yekaterinburg: USU Publishing; 2008. [in Russian].
Drent PJ, Woldendorp JW. Acid rain and eggshells. Nature 1989;339:431.
Eeva T, Lehikoinen E. Growth and mortality of nestling great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. Oecologia 1996;108:631–9.
Eeva T, Lehikoinen E. Rich calcium availability diminishes heavy metal toxicity in pied flycatcher. Funct Ecol 2004;18:548–53.
Eeva T, Rainio K, Suominen O. Effects of pollution on land snail abundance, size and diversity as resources for pied flycatcher, *Ficedula hypoleuca*. Sci Total Environ 2010;408:4165–9.
Götmark F, Von Proschwitz T, Franc N. Are small sedentary species affected by habitat fragmentation? Local vs. landscape factors predicting species richness and composition of land molluscs in Swedish conservation forests. J Biogeogr 2008;35:1062–76.
Graveland J. Avian eggshell formation in calcium-rich and calcium-poor habitats: importance of snail shells and anthropogenic calcium sources. Can J Zool 1996;74:1035–44.
Graveland J, Van der Wal R. Decline in snail abundance due to soil acidification causes eggshell defects in forest passerines. Oecologia 1996;105:351–60.
Graveland J, Van Gijzen T. Arthropods and seeds are not sufficient as calcium sources for shell formation and skeletal growth in passerines. Ardea 1994;82:299–314.
Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 2001;4:1–9.
Kaigorodova SY, Vorobeichik EL. Changes in certain properties of grey forest soil polluted with emissions from a copper-smelting plant. Russ J Ecol 1996;27:177–83.

Kozlov MV, Zvereva EL, Zverev VE. Impacts of point polluters on terrestrial biota. Dordrecht, Heidelberg, London, New York: Springer; 2009.

Kuligin SD. A new method of neck ligation in nestlings of insectivorous birds for intravital studies on their feeding. Ornithologia 1981;16:174–5. [in Russian].

Mänd R, Tilgar V, Leivits A. Calcium, snails, and birds: a case study. Web Ecol 2000;1:63–9.

Mikhailova IN, Vorobeichik EL. Epiphytic lichenosynusia under conditions of chemical pollution: dose–effect dependencies. Russ J Ecol 1995;26(6):425–31.

Mukhacheva SV. Spatiotemporal population structure of the bank vole in a gradient of technogenic environmental pollution. Russ J Ecol 2007;38(3):161–7.

Nesterkov AV. Reaction of mollusk population to emissions from the Middle Ural copper smelter. Contemp Probl Ecol 2013;6:667–73.

Nyholm NEI. Monitoring of terrestrial environmental metal pollution by means of free-living insectivorous birds. Ann Chim 1995;85:343–50.

Reynolds SJ, Perrins CM. Dietary calcium availability and reproduction in birds. In: Thompson CF, editor. Curr Ornithol, 17. ; 2010. p. 31–74.

Scheuhammer AM. Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminum in birds. Environ Pollut 1996;94:337–43.

Schoener T. Nonsynchronous spatial overlap of lizards in patchy habitats. Ecology 1970; 51:408–18.

Simkiss K. Calcium in reproductive physiology. London and New York: Chapman and Hall; 1967.

Snoeijs T, Dauwe T, Pinxten R, Darras VM, Arckens L, Eens M. The combined effect of lead exposure and high or low dietary calcium on health and immunocompetence in the zebra finch (*Taeniopygia guttata*). Environ Pollut 2005;134:123–32.

Sysoev A, Schileyko A. Land snails and slugs of Russia and adjacent countries. Pensoft: Sofia-Moscow; 2009.

Tilgar V, Mänd R, Leivits A. Effect of calcium availability and habitat quality on reproduction in pied flycatcher *Ficedula hypoleuca* and great tit *Parus major*. J Avian Biol 1999; 30:383–91.

Trubina MR, Vorobeichik EL. Severe industrial pollution increases the β-diversity of plant communities. Dokl Biol Sci 2012;442:17–9.

Vorobeichik EL. Seasonal changes in the spatial distribution of cellulolytic activity of soil microflora under conditions of atmospheric pollution. Russ J Ecol 2007;38(6):398–407.

Vorobeichik EL, Sadykov OF, Farafontov MG. Ecological standardization of technogenic pollution of terrestrial ecosystems. Yekaterinburg: Nauka Publishing; 1994. [in Russian].

Vorobeichik EL, Ermakov AI, Zolotarev MP, Tuneva TK. Changes in diversity of soil macrofauna in industrial pollution gradient. Russ Entomol J 2012;21(2):203–18.

Wäreborn I. Changes in the land mollusc fauna and soil chemistry in an inland district in southern Sweden. Ecography 1992;15:62–9.

Zolotarev MP, Belskaya EA. The effects of industrial pollution and habitat characteristics on epigeic invertebrate abundance. Eurasian Entomol J 2012;11(1):19–28.