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

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 (Vorobechik 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...
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) at different distances from the Middle Urals copper smelter (y = 0.1 mg) were digested in a mixture of 7 mL supra-pure HNO3 + 1 mL de-ionised H2O) 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%.

2.3. 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 suprapure HNO3 + 1 mL de-ionised H2O 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.
were calculated with the same software. The similarity of different shell samples was calculated using the index (Schoener, 1970):

\[ I = 1 - 0.5 \sum_{i} |p_{ij} - p_{ik}| \]

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 number of shells with snails was rather high \((0.70)\) but non-significant. Difference among classes \((\text{buffer}, \text{impact})\) was in the background zone, the number of shells per nest was positively related to the distance from the smelter and number of hatchlings.

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 over a 15-day period from hatching to fledging. Post-hoc comparisons were performed with a Scheffe test.

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 of the pollution source. The incidence of snails in food samples decreased with increasing pollution, as well, namely by six times \((Table 3)\). 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: \(I = 0.63 \pm 0.03\) \((n = 4 \text{ sites})\).

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

Table 3

<table>
<thead>
<tr>
<th>Metal</th>
<th>Zone</th>
<th>Effect of zone *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Background</td>
</tr>
<tr>
<td>Cu</td>
<td>41.7 ± 3.6</td>
<td>2546 ± 42.7</td>
</tr>
<tr>
<td>Zn</td>
<td>4249 ± 44.8</td>
<td>7777 ± 92.9</td>
</tr>
<tr>
<td>Cd</td>
<td>6.4 ± 0.7</td>
<td>238 ± 3.6</td>
</tr>
<tr>
<td>Pb</td>
<td>13.7 ± 1.9</td>
<td>1865 ± 25.0</td>
</tr>
<tr>
<td>Ca</td>
<td>32006 ± 943.5</td>
<td>17582 ± 479.6</td>
</tr>
</tbody>
</table>

* One-way analysis of variance (ANOVA), metal concentrations were log_{10}-transformed.

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.

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 Eucomis 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: \(I = 0.63 \pm 0.03\) \((n = 4 \text{ sites})\).

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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 \((n = 5 \text{ sites})\).

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 spilled increased 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 hatchings.

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
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 were negatively affected compared to buffer 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.

### 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.

<table>
<thead>
<tr>
<th>Species, indices</th>
<th>Zone of pollution</th>
<th>Deciduous forest</th>
<th>Coniferous forest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Background</td>
<td>Buffer</td>
<td>Impact</td>
</tr>
<tr>
<td><strong>Number of species</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of nests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of shells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shannon diversity index (95% conf. limits)</strong></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

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

### 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.

<table>
<thead>
<tr>
<th>Species, indices</th>
<th>Zone of pollution</th>
<th>Deciduous forest</th>
<th>Coniferous forest</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td><strong>Number of species</strong></td>
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<tr>
<td><strong>Number of nests</strong></td>
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</tr>
<tr>
<td><strong>Number of shells</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Shannon diversity index (95% conf. limits)</strong></td>
<td></td>
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</table>

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...
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 simplification 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 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.

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 ($\frac{x}{x_{\text{max}}}$, nests with no shells excluded) 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Deciduous forest</th>
<th>Coniferous forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log_{10} distance to the smelter</td>
<td>0.38</td>
<td>0.28</td>
</tr>
<tr>
<td>Standardised number of hatchlings</td>
<td>−0.04</td>
<td>0.27</td>
</tr>
<tr>
<td>Standardised hatching date</td>
<td>−0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Sum of temperatures during nesting period</td>
<td>0.21</td>
<td>−0.05</td>
</tr>
<tr>
<td>Sum of temperatures during nestling period</td>
<td>0.01</td>
<td>−0.13</td>
</tr>
</tbody>
</table>
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 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**


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).


