



# Effects of pollution on land snail abundance, size and diversity as resources for pied flycatcher, *Ficedula hypoleuca*

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## ARTICLE INFO

### Article history:

Received 1 March 2010

Received in revised form 5 May 2010

Accepted 19 May 2010

Available online 9 June 2010

### Keywords:

Air pollution

Calcium availability

*Ficedula hypoleuca*

Heavy metal

Land snail

## ABSTRACT

Passerine birds need extra calcium during their breeding for developing egg shells and proper growth of nestling skeleton. Land snails are an important calcium source for many passerines and human-induced changes in snail populations may pose a severe problem for breeding birds. We studied from the bird's viewpoint how air pollution affects the shell mass, abundance and diversity of land snail communities along a pollution gradient of a copper smelter. We sampled remnant snail shells from the nests of an insectivorous passerine, the pied flycatcher, *Ficedula hypoleuca*, to find out how the availability of land snails varies along the pollution gradient. The total snail shell mass increased towards the pollution source but declined abruptly in the vicinity of the smelter. This spatial variation in shell mass was evident also within a single snail species and could not be wholly explained by spatially varying snail numbers or species composition. Instead, the total shell mass was related to their shell size, individuals being largest at the moderately polluted areas. Smaller shell size suggests inferior growth of snails in the most heavily polluted area. Our study shows that pollution affects the diversity, abundance (available shell mass) and individual quality of land snails, posing reproductive problems for birds that rely on snails as calcium sources during breeding. There are probably both direct pollution-related (heavy metal and calcium levels) and indirect (habitat change) effects behind the observed changes in snail populations.

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## 1. Introduction

Human activities may have detrimental effects on land snail populations. For example, snails are known to be sensitive to acidification or pollution of environment (van Tol et al., 1998; Johannessen and Solhoy, 2001), and they are shown to easily accumulate pollutants, such as heavy metals (Berger and Dallinger, 1993; Jordaens et al., 2006). Land snails have therefore been suggested as sentinel organisms for biomonitoring (Regoli et al., 2006). Snails have also been recognized as one of the most important sources of calcium for many passerine bird species (Graveland and Drent, 1997; Tilgar et al., 1999a; Mänd et al., 2000; Bureš and Weidinger, 2000). Therefore, changes in snail populations are reflected higher up in the food chain. Decreased snail abundance may pose a problem to birds, especially during the breeding period, when birds need to acquire extra calcium for developing egg shells and for a proper growth of the nestling skeleton (Graveland and van Gijzen, 1994). We are, however, not aware of any studies trying to quantify population level changes in terrestrial snail numbers in environments exposed to pollution.

We studied how air pollution affects the abundance, species richness and structure of land snail communities along a pollution gradient of a copper smelter. To get relevant information on pollution-related changes in snail populations from the viewpoint of birds we sampled remnant snail shells from the nests of an insectivorous passerine, the pied flycatcher, *Ficedula hypoleuca*. This species frequently feeds the nestlings with small land snails and a part of them drop to the nest cup, and can be later counted. Earlier studies have shown that egg shells of *F. hypoleuca* were thinner and egg volume and clutch size were smaller in the vicinity of the smelter compared to the background area (Eeva and Lehtikoinen, 1995). *Ficedula hypoleuca* nestlings also had problems with their bones: their leg or wing bones did not develop normally (Eeva and Lehtikoinen, 1996). Thin egg shells and poorly developing bones are likely due to low calcium availability and increased heavy metal concentrations in the birds' diet in heavily polluted sites (Eeva and Lehtikoinen, 2004).

In the current study we want to find out what kind of changes in land snail populations may be behind the calcium-related reproductive problems in *F. hypoleuca*. We expect that availability of snail shells is inferior in the heavily polluted area, where most severe egg shell thinning in *F. hypoleuca* has taken place. Snail shell availability may vary in three ways: 1. the number of snails differs, 2. the size of individuals differs, or 3. the snail species composition differs. First, we explored how the shell numbers and the amount of shell mass in *F. hypoleuca* nests

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vary along the pollution gradient. Second, we tested for the most abundant species whether the individual size (shell mass) of snails varies along the pollution gradient. Third, we wanted to find out whether the variation in total shell mass was related to changes in species composition, i.e. whether smaller sized species dominate in different parts of the pollution gradient than larger sized species.

## 2. Methods

### 2.1. Study area

The data were collected during two breeding seasons (2003 and 2006) in the surroundings of a copper smelter in Harjavalta (61°20' N, 22°10' E), SW Finland. Sulphuric oxides and heavy metals (especially Cu, Zn, Ni, Pb and As) are common pollutants in the area (Kubin, 1990; Jussila and Jormalainen, 1991). Elevated heavy metal concentrations occur in the polluted area due to current and long-term deposition, and metal contents decrease exponentially with increasing distance to the smelter approaching background levels at sites farther than 5 km from the smelter (Jussila et al., 1991; Koricheva and Haukioja, 1995; Eeva and Lehikoinen, 1996). Organic soil copper concentrations along the pollution gradient are shown in Fig. 1. The amount of exchangeable Ca in the organic layer of soil has decreased near the smelter due to leaching but soil pH is the same or even slightly higher than in the background area (Fritze et al., 1989; Jussila, 1997; Derome and Nieminen, 1998).

Twelve study sites, each with 40–50 nest-boxes, were established along the air pollution gradient in the three main directions (SW, SE and NW) away from the copper smelter complex. The forests in the area are dominated by Scotch pine (*Pinus sylvestris*), which forms mixed stands with spruce (*Picea abies*) and birches (*Betula* spp.) with the occasional occurrence of other deciduous trees like aspen (*Populus tremula*), rowan (*Sorbus aucuparia*) and willows (*Salix* spp.). Special attention was paid to selecting study areas so that they would represent the same habitat type, i.e. relatively barren and dry pine dominated forests typical of the study area. Earlier studies have shown that land snail numbers are generally much lower in coniferous and Ca poor than deciduous and Ca rich habitats (Andersen and Halvorsen, 1984; Mänd et al., 2000), and ambient Ca levels may affect their shell morphology (Goodfriend, 1986). Therefore we expect that barren habitat is likely to make the land snail populations of our study area vulnerable to pollution stress.

### 2.2. Counts of snails in nests

*Ficedula hypoleuca* parents feed small land snails to nestlings and drop some of the snails into the bottom of the nest. This species does

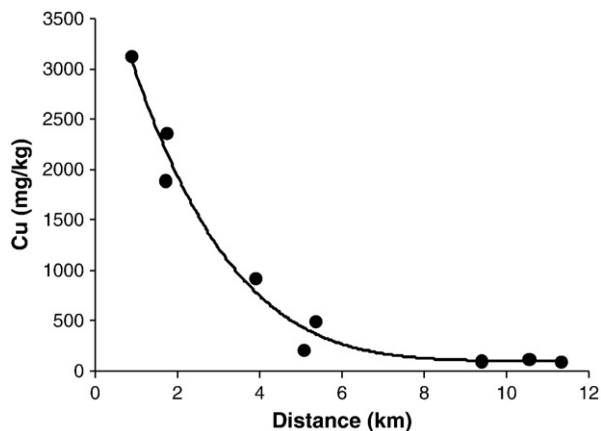


Fig. 1. Organic soil copper concentrations (mg/kg) along the pollution gradient of a copper smelter. Cu concentrations were determined with ICP-MS spectrometer and certified reference material (mussel tissue ERM-CE278) was used for method validation. The recovery from the reference sample was 106%.

not clean dropped items from the nest and the number of snail shells can be counted after fledging. We collected 120 nests from 12 study sites along the pollution gradient (2003:  $n=55$ ; 2006:  $n=65$ ) of *F. hypoleuca* after fledging (30th June–24th July) and searched them for remnant snail shells. Only those nests were collected where at least one nestling fledged. Therefore, territories with worst breeding success (e.g. none of the eggs hatch) are underrepresented in our data but this should only make our analyses more conservative and does not bias comparisons among sites.

Nests were frozen, air dried and sieved to remove most of the nest material. Snail shells were then picked up from the remaining sample. Snail species were identified by OS with the help of the guides by Kerney et al. (1979) and Hutri and Mattila (1991), and shells were individually weighted. Shells containing soil or a dead snail were emptied with a thin needle before weighing. The shell mass and number of individuals were used as an index of snail abundance in birds' territories. Experimental supplementation of snail shells in *F. hypoleuca* territories has confirmed that the availability of snails is really reflected in the numbers of remnant snail shells in nest material (Tilgar et al., 1999b).

### 2.3. Statistics

The variations in total snail shell mass per nest and total shell numbers per nest were analyzed by using generalized linear models (with negative binomial error distribution and log-link function) in a glimmix procedure of SAS statistical software 9.1 (SAS Institute, 2003). Zero observations (nests with no shells) were included in these analyses. Year and first, second and third order terms of the log-distance to the pollution source were used as independent variables. Accounting for possible non-linear responses, the second order term (distance $\times$ distance) corresponds a quadratic and third order term (distance $\times$ distance $\times$ distance) a cubic response to distance. Non-significant terms were dropped from the models one-by-one, starting from higher order terms. Year was, however, retained in the final models to avoid temporal pseudoreplication. Brood size (a number of hatchlings) was first included as a covariate in the models, but it was omitted from final models as a non-significant variable. For evaluating the goodness of fit of the models we always checked the scaled Pearson statistic ( $\chi^2/df$ ), which should optimally be 1. Note, however, that with negative binomial distribution the glimmix procedure always includes this scaling parameter in the model to correct for possible under- or overdispersion in data. The scaled Pearson statistics and other details on specific models are given in Table 2. Since five of the sampled nests contained a few snails from moist/wet habitat (Table 1) we also ran the previous models without those nests to confirm that our analyses are not biased by more moist habitats next to some territories. Omitting those nests, however, did not change the models and the full data was used in all analyses.

Variation in snail size (individual shell mass) was analyzed by using a generalized linear model (with negative binomial distribution and log-link function; SAS Institute, 2003) containing year and first, second and third order terms of the log-distance to the pollution source as independent variables. Individual shell mass variation could only be tested for the most common species, *Discus ruderatus*, because the spatial distribution of this species was representative over the whole pollution gradient and the sample size was adequate to get a satisfactory model fit. Since some of the nests contained multiple *D. ruderatus* shells, nest was used as a random factor in this analysis. We also ran the same model by including the data on all the species, by adding species as a further random factor to account for species specific size variation. We further analyzed the relationship between the average shell mass of a species and the average sampling distance of the species with a linear regression model (REG procedure in SAS). Average shell mass values were log<sub>10</sub>-transformed to make the distribution normal (after transformation, Kolmogorov–Smirnov test:  $p>0.15$ ).

**Table 1**The snail species and frequencies in the nests of *Ficedula hypoleuca* at four distance classes from the pollution source.

Species	Abbr.	Distance to smelter (km)				Total
		0–1.5	1.5–3	3–6	>6	
<i>Bithynia tentaculata</i>	BT	0	4	0	0	4
<i>Clausilia bidentata</i>	CB	0	5	0	10	15
<i>Cochlicopa lubrica</i>	CL	1	0	6	0	7
<i>Cochlicopa lubricella</i>	CU	0	1	12	0	13
<i>Discus ruderatus</i>	DR	8	45	12	12	77
<i>Euconulus alderi</i>	EA	12	2	10	2	26
<i>Euconulus fulvus</i>	EF	6	2	12	5	25
<i>Lymnaea truncatula</i>	LT	0	1	2	0	3
<i>Nesovitrea hammonis</i>	NH	2	3	12	7	24
<i>Nesovitrea petronella</i>	NP	0	2	18	5	25
<i>Succinea putris</i>	SP	0	5	0	0	5
<i>Theodoxus fluviatilis</i>	TF	0	1	0	0	1
<i>Vallonia costata</i>	VC	0	5	0	0	5
<i>Vallonia excentrica</i>	VE	0	8	0	0	8
<i>Zonitoides nitidus</i>	ZN	4	7	8	0	19
Total		33	91	92	41	257
Nests		29	28	34	29	120
Mean shells/nest and its 95% CL		1.14 (0.67–1.93)	3.25 (2.05–5.16)	2.71 (1.76–4.15)	1.41 (0.85–2.36)	2.14 (1.56–2.72)
Species		6	14	9	6	15
Shannon diversity <sup>a</sup> and its 95% CL		1.54 (1.43–1.64)	1.72 (1.29–2.08)	2.00 (1.81–2.13)	1.64 (1.52–1.72)	

<sup>a</sup> Calculated with EcoSim software for the sample size of 30 individuals.

Due to the large differences in the number of individuals in our samples, snail species diversity (Shannon diversity index) at different distances from the pollution source was analyzed by using rarefaction estimation (Gotelli and Colwell, 2001). The number of species was estimated for similar sample sizes ( $n = 30$ ) with 95% confidence limits by using EcoSim 7.7 software (Gotelli and Entsminger, 2004).

### 3. Results

All together 257 individuals belonging to 15 snail species were found from the nests of *F. hypoleuca* (Table 1). The number of species was highest 1.5–3 km from the pollution source, while the Shannon diversity index, which also takes into account the evenness of the species distribution, was highest at the distance of 3–6 km (Table 1). The dominant species was *D. ruderatus*, which was found at all distances, but was by far most abundant at the distance of 1.5–3 km from the pollution source. In addition to land snails, our sample included a few specimens of freshwater species (*Bithynia tentaculata*, *Lymnaea truncatula* and *Theodoxus fluviatilis*), which birds likely picked up from ditches or the nearby river shore at one of the study sites (Table 1).

The total snail shell mass followed a cubic relationship with distance to the pollution source, increasing towards the pollution source, being highest at the distance of 1.5–3 km from the smelter and declining abruptly in the vicinity of the smelter (Table 2, Fig. 2a). Total shell mass was, on average, 1.9 times higher in 2006 than in 2003, but this difference was not quite statistically significant (Table 2). Snail numbers followed a similar pattern but did not vary significantly across the pollution gradient (Table 2; Fig. 2b). Their number however, was on average 2.1 times higher in 2006 (mean  $\pm$  SE:  $2.9 \pm 0.44$ ) than in 2003 ( $1.4 \pm 0.25$ ). When the snail number was added in the model of shell mass as a further independent variable it expectedly explained a great deal of the variation in shell mass (snail number:  $F_{1,114} = 17.7$ ,  $p < 0.0001$ ), but even then the cubic effect of distance remained significant (cubic distance:  $F_{1,114} = 4.8$ ,  $p = 0.030$ ), suggesting that either intraspecific variation in shell size and/or spatially varying species composition may have additional effects on total shell mass.

The individual shell mass of the dominant species, *D. ruderatus*, followed a similar cubic pattern than the total shell mass (Table 2), i.e. the shell mass increased towards the pollution source, was highest at the moderately polluted areas and declined abruptly very close to the smelter (Fig. 2c). This cubic pattern was also found when all the

species were included in the model and species was used as a random factor (cubic distance:  $F_{1,102.1} = 4.5$ ,  $p = 0.036$ ).

We plotted further the average individual shell mass of each snail species against the average sampling distance of the species to see whether smaller sized species dominated in different part of the pollution gradient than larger species. There was no significant relationship between the average shell mass and average sampling distance (Linear regression:  $F_{1,13} = 0.07$ ,  $p = 0.80$ ; Fig. 3).

### 4. Discussion

On the basis of our sample based on *F. hypoleuca* nest material the most diverse and abundant land snail populations were found in the moderately polluted areas, while they were scarce in remote unpolluted areas and in the most heavily polluted areas. This spatial variation in shell mass could not be wholly explained by spatially varying snail numbers or species composition, since we found no clear trends in the number of snails or the distribution of different sized snail species along the pollution gradient. Instead, the total snail shell mass was related to the shell size, individuals being largest at the moderately polluted areas. Abruptly decreasing shell size at the most polluted area suggests an inferior growth of snails possibly due to

**Table 2**

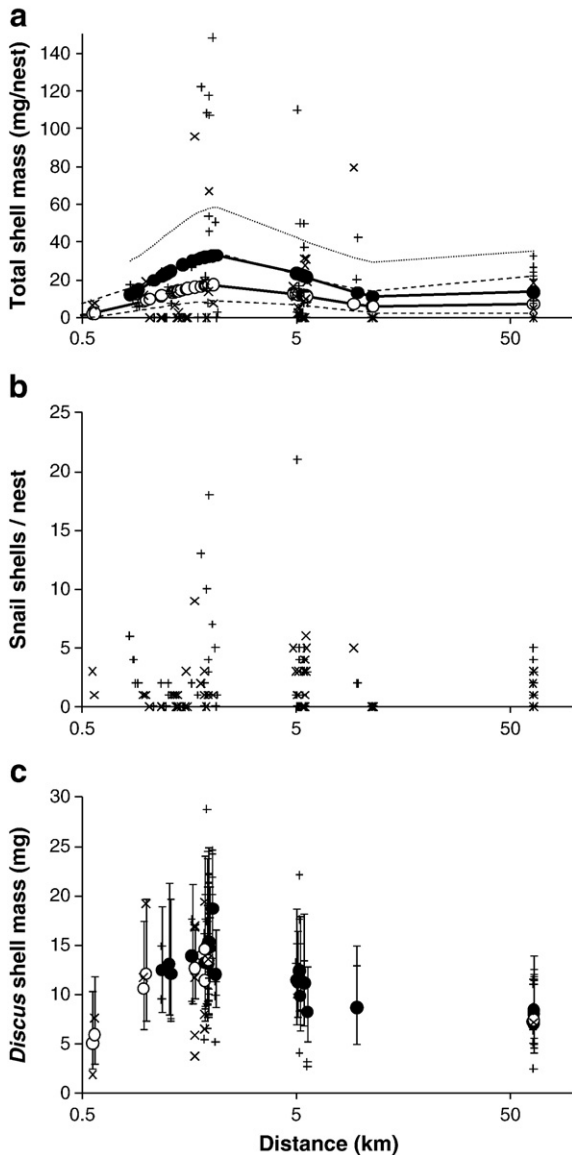
Generalized linear models for the variation in total snail shell mass (mg/nest), total shell numbers (individuals/nest) and individual shell mass (mg) of *Discus ruderatus*. The terms in boldface were retained in the final models.

Effect	Total shell mass <sup>a</sup>			Total shell number <sup>b</sup>			<i>Discus</i> shell mass <sup>c</sup>		
	df	F	p	df	F	p	df	F	p
Year	<b>1,115</b>	<b>2.98</b>	<b>0.087</b>	<b>1,118</b>	<b>9.59</b>	<b>0.0024</b>	<b>1,25.3</b>	<b>0.16</b>	<b>0.69</b>
Brood size	1,114	1.29	0.26	1,116	0.04	0.84	.	.	.
Distance	<b>1,115</b>	<b>6.20</b>	<b>0.014</b>	1,117	0.05	0.83	<b>1,72.0</b>	<b>5.15</b>	<b>0.026</b>
Quadratic distance	<b>1,115</b>	<b>6.13</b>	<b>0.015</b>	1,115	0.85	0.36	<b>1,63.1</b>	<b>7.35</b>	<b>0.0086</b>
Cubic distance	<b>1,115</b>	<b>5.06</b>	<b>0.026</b>	1,114	2.80	0.097	<b>1,56.1</b>	<b>6.39</b>	<b>0.014</b>

<sup>a</sup> GLM with negative binomial error distribution for the shell mass of all species ( $n = 120$  nests). Pearson  $\chi^2/df = 0.68$ .

<sup>b</sup> GLM with negative binomial error distribution ( $n = 120$  nests). Pearson  $\chi^2/df = 1.1$ .

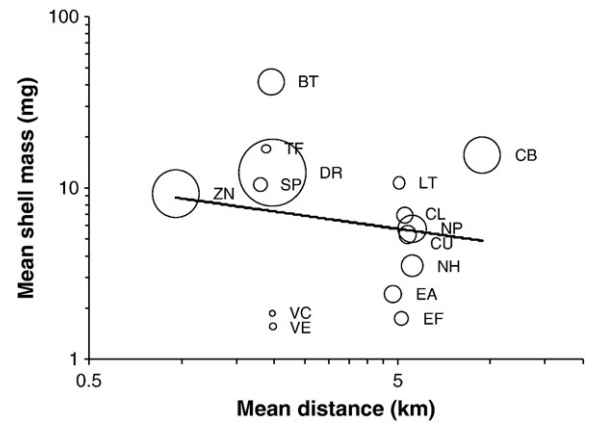
<sup>c</sup> GLMM with negative binomial error distribution for the shell mass of the dominant species, *Discus ruderatus* ( $n = 77$  individuals). Nest ( $n = 41$ ) was used as a random factor and degrees of freedom were calculated with Kenward–Roger method. Pearson  $\chi^2/df = 1.0$ .



**Fig. 2.** a) The total snail shell mass per *Ficedula hypoleuca* nest, b) the number of shells per nest and c) individual shell mass of *Discus ruderatus* at different distances from a copper smelter in 2003 (x) and 2006 (+). Predicted values  $\pm$ 95% confidence limits ( $\circ$  = 2003 and  $\bullet$  = 2006) are also shown for the generalized linear models indicating significant variation along the pollution gradient (see Table 2). Note the logarithmic scale of the x-axis.

decreased Ca availability, increased heavy metal levels or resource limitation due to sparser herbaceous layer in the most polluted sites.

Forest snails generally benefit of relatively calcium-rich environments (Goodfriend, 1986; Wäreborn, 1992). Forest soils in our study area are relatively acidic, pH of organic layer being about 3.6 (Derome and Lindroos, 1998). This likely explains the relatively low snail shell masses even in the unpolluted sites of our study area. Although soil total Ca levels in the vicinity of the pollution source are similar to more distant sites, the amount of exchangeable Ca in the organic layer of soil has decreased near the smelter due to leaching (Derome and Nieminen, 1998). Therefore there may be less Ca available for plants and snails near the pollution source. Snail growth rates and densities are found to correlate positively with Ca availability (Ireland, 1991; Gärdenfors, 1992; Wäreborn, 1992; Mänd et al., 2000; Johannessen and Solhoy, 2001; Skeldon et al., 2007). Heavy metals are further known to inhibit growth in snails (Gomot, 1997; Ebenso and Ologhobo, 2009) and many studies have shown that low calcium



**Fig. 3.** The mean shell mass (mg) of the snail species against the mean sampling distance (km) of a species from the pollution source. Symbol size indicates the proportional shell mass of a species from the total snail shell mass. Note the logarithmic scale of the axes. Linear regression on  $\log_{10}$ -transformed mass values.

availability enhances the uptake of heavy metals in animals (Six and Goyer, 1970; Barton et al., 1988; Fullmer, 1997; Sharma and Bakre, 1998). It is thus possible that decreased Ca availability together with increased heavy metal levels could explain the smaller shell size near the smelter. Jordaens et al. (2006) did not find differences in shell morphology of *Cepaea nemoralis* between heavy metal polluted and unpolluted sites. Their study sites were, however, more Ca rich and not acidic ( $\text{pH} > 7$ ), which likely explains the difference to our results. Adult size has been found to influence strongly the reproductive output in snails, larger snails having larger and more offspring (Anderson et al., 2007). Therefore decreased snail shell mass in the polluted area could reflect inferior growth and be indicative of reduced reproductive output.

The quality of forest and forest floor vegetation are important determinants of snail population sizes (Ström et al., 2009). Land snail fauna is typically more diverse and abundant in habitats with luxurious vegetation (Andersen and Halvorsen, 1984). Although we sampled similar habitats in our study there still remains variation among territories and the long-term pollution itself has changed vegetation close to the copper smelter. In the most heavily polluted sites of our study area the ground layer vegetation is rather sparse or lacking due to long-term pollution (Salemaa and Vanha-Majamaa, 1993), and it is obvious that such an environment does not support abundant land snail populations. However, ground layer vegetation was relatively sparse also in the moderately polluted sites, where the snail numbers in *F. hypoleuca* nests were highest. The snail species composition at the distance of 1.5–3 km from the pollution source suggests that some birds may have acquired snails from more moist and grassy habitats (ditches or river shore) next to their territories (e.g. *Succinea putris*), as well as from more calcareous habitats (e.g. *Vallonia* spp.). In this area some birds may also have acquired snails from nearby gardens, which offer a relatively diverse and luxurious habitat for snails and which also are artificially limed. Some of the nests were just 10–20 m from nearest gardens, which were well within the foraging territory of *F. hypoleuca* (von Haartman, 1956). More distant sites, on the other hand, are less populated and do not offer the same option for birds. Some species like *Clausilia bidentata* are associated with aspen or some other calcium-rich trees (Wäreborn, 1969). Such habitats are in general good ones to most snails, leading to higher abundance and species richness of other species as well (Suominen et al., 2003).

Our data also showed that snail availability varied among years, but we are not aware of any obvious reason for that. Forest snails are known to be sensitive to drought and to be more active in rainy weather (Wäreborn, 1992). Weather condition in summer 2003 and 2006 were generally rather similar. During *F. hypoleuca* nestling time



(average 15th June–14th July) temperatures and rainfall were, however, somewhat higher in 2006 (18.1 °C, 54 mm) than in 2003 (15.7 °C, 36 mm). Higher activity abundance of snails in summer 2006 might therefore explain the observed difference in snail numbers in *F. hypoleuca* nests.

It must be recalled that our sample was collected by *F. hypoleuca* parents and it is not likely to be a random sample from snail populations. Supposedly, *F. hypoleuca* parents have no reason to avoid taking any snail species of suitable size, but interspecific differences in snail behavior could easily affect the probability of being captured by birds. Therefore, the community structure shown by our sample may differ from the actual one. As well, the yearly variation in numbers might also reflect the behavior of birds, which might change e.g. according to availability of other calcium-rich invertebrates. Despite these uncertainties the strong decline in the snail abundance, shell mass and species richness at the vicinity of the smelter seems very obviously pollution induced. On the other hand, our sample is most relevant from the birds' viewpoint and the observed trends in snail numbers and shell mass support the hypothesis that decreased availability of calcium-rich snail shells in the heavily polluted areas is one of the most important factors causing calcium limitation and related problems in calcium metabolism of birds (Eva and Lehtikoinen, 2004). Most likely there are both direct (heavy metal and calcium levels) and indirect (habitat change) pollution-related effects behind the observed changes in snail populations. Collecting snails from bird nests is a noteworthy and cost-effective method for monitoring land snail populations but warrants further studies to reveal how closely they indicate the actual variation in snail population sizes. Understanding the mechanisms leading to human-induced calcium limitation in birds will be important in planning actions to reduce pollution-related detrimental effects.

## Acknowledgements

We thank Tuija Koivisto for measuring the snail shells and Prof. Pekka Niemelä for the heavy metal analyses. Biology students of Turku University helped us in screening of the nest material. Two anonymous referees gave valuable comments on the manuscript. Our study was financed by the Academy of Finland (project 8119367).

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