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Polluted environment does not speed up age-related change in reproductive performance of the pied flycatcher

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Abstract

Environmental pollution could enhance deterioration of fecundity with advancing age, directly via toxic effects of pollutants or indirectly via pollution-related resource (e.g. dietary antioxidants) limitation. Since there are very few studies on age-related changes in reproduction as regards to pollution, we analyzed a long-term (25yr) data set on reproduction of a small insectivorous and migratory passerine bird, the pied flycatcher *Ficedula hypoleuca*, to explore if female birds show faster age-related decrease of average breeding parameters in a metal-polluted area around a copper-nickel smelter than in the control area. In our population level analysis, all the breeding parameters (clutch size, hatching success, fledging probability, and fledgling number) showed generally lower levels in the polluted area but aside that, none of them indicated faster decrease with age in the polluted area. Clutch size and fledgling number increased after the first breeding, but showed no significant change later on. Hatching probability decreased slightly after the second breeding while fledging probability showed no significant age-dependent variation. Our results suggest that moderate long-term pollution does not reduce the viability of our study population via faster age-related decrease in fecundity.

Key words: Age-related fecundity, environmental pollution, heavy metals, insectivorous passerines

Introduction

Small and relatively short-lived passerines may show deterioration of fecundity with advancing age, already after the age of three years (Gustafsson and Pärt 1990; Sanz and Moreno 2000; Balbontin et al. 2007; Vleck et al. 2011). Anthropogenic stress, such as environmental pollution and urbanization, may speed up age-related decrease in fecundity, as was the case with metal exposed white storks *Ciconia ciconia* after a toxic spill (Baos et al. 2012). In small passerines, reduced maternal nutrient allocation to egg yolk, growth retardation, decreased plasma vitamins, increased levels of oxidative stress and shortening of telomeres have been documented in polluted environments (Koivula et al. 2011; Espín et al. 2016; Stauffer et al. 2016; Ruiz et al. 2017). Such effects are partly indirect, due to inferior food quality (e.g. lower antioxidant levels) in polluted areas (Eeva et al. 2005; Eeva et al. 2009; Koivula et al. 2011). Chronic oxidative stress or inflammation can speed up the decline of fecundity with age by increasing cellular and tissue damages and eventually leading to lower reproductive output (Alonso-Álvarez et al. 2010; Losdat et al. 2011; Vleck et al. 2011; Isaksson 2015). Several pollutants (e.g. some metals and fat-soluble organic pollutants) accumulate in the body with age (Scheuhammer 1987; Gochfeld et al. 1996; Hogstad 1996; Sakamoto et al. 2002; but see Bustnes et al. 2003; Agusa et al. 2005; Vives et al. 2005; Berglund et al. 2011; Tartu et al. 2015). Therefore, higher tissue levels of pollutants and more negative impacts can be expected in old individuals, although accumulating tissue damage and age-related decrease in fecundity would be possible even with constant, age-independent internal pollutant levels. On the other hand, old individuals might be the best to cope with pollutants if pollutants represent a strong selective agent.

So far, age-related decrease in fecundity relative to environmental pollution has been studied very little (Baos et al. 2012). We therefore analyzed a long-term (25yr) data set on reproduction of a small insectivorous and migratory passerine bird, the pied flycatcher *Ficedula hypoleuca*, to explore if female birds show accelerated population-level decrease in fecundity in a metal-polluted area around a copper-nickel smelter in Harjavalta, SW Finland. Long-term monitoring of breeding parameters of a *F. hypoleuca* population around this emission source has revealed increased dietary metal exposure, increased proportion of thin-shelled eggs, smaller egg size and clutch size, decreased hatchability, increased number of growth abnormalities, increased nestling mortality, and lower fledgling production as compared to more remote reference areas (Eeva and Lehikoinen 1995; Eeva and Lehikoinen 1996). Despite considerable reductions in emissions and improvement of breeding parameters over this long period, clutch size and number of fledglings still remain lower in the polluted area (Eeva and Lehikoinen 2015).

Migratory passerines have been considered especially prone to senescence because of their yearly physiologically-demanding migratory journey (Sanz and Moreno 2000; Wikelski et al. 2003). For this reason and because of their relatively high metabolic rates (Bennett and Harvey 1987) and fast accumulation of pollutants at their breeding grounds (Berglund et al. 2010), *F. hypoleuca* females should be a good study model to explore possible decline of fecundity with age relative to pollution. In the case of pollution-related effects, we expect to find an earlier decline of the reproductive output in the pollution-exposed bird population as compared to the one living in an unpolluted area.

Materials and methods

Study species

Ficedula hypoleuca is a small, relatively short-lived, insectivorous and migratory passerine wintering in Western Africa and breeding in a large range across Europe and Russia (Lundberg and Alatalo 1992). They arrive to their breeding sites in Finland in the beginning of May and start to lay eggs in the end of May. *Ficedula hypoleuca* breed numerously in nest boxes, making it an ideal species to study reproductive parameters in polluted environments.

Study area and data collection

The data were collected in 1991–2016 (2001 missing) around a copper-nickel smelter (61°20’ N, 22°10’ E) in Harjavalta, southwestern Finland (Figure 1). Sulphur oxides (SO_x) and heavy metals (especially As, Cu, Ni, Pb and Zn) are common pollutants in the area (Kiikkilä 2003; Kozlov et al. 2009). Elevated heavy metal concentrations occur in soil, vegetation, insects and birds of the polluted area due to current and historical deposition (since 1945), and metal contents decrease exponentially with increasing distance to the smelter (Koricheva and Haukioja 1995; Eeva and Lehikoinen 1996; Eeva et al. 1997; Eeva et al. 2010; Berglund et al. 2012). For example, organic soil Cu (5799 ppm, dry weight [d.w.]) and Pb (314 ppm, d.w.) concentrations near the smelter have been found to be, respectively, 39 and 5 times higher than at background sites, 8 km from the smelter (Derome and Nieminen 1998). Arsenic concentrations in *F. hypoleuca* nestling feces have been c.a. 13 times higher in the polluted area as compared to the background, indicating dietary exposure (Eeva et al. 2005). Especially non-essential (or ultra-trace essential) elements (As, Cd, Pb) have been found to accumulate in the liver tissue of *F. hypoleuca* females and nestlings in the polluted area of Harjavalta (Berglund et al. 2011). Heavy metal and SO_x emissions from the smelter decreased considerably during 1990s and the Harjavalta smelter was removed from a ‘hot spot’ list of top Baltic polluters in 2003 (Kozlov et al. 2009; Berglund et al. 2015). At the same time, metal levels in *F. hypoleuca* nestlings have decreased with a simultaneous increase in breeding success (Eeva and Lehikoinen 2000; Eeva and Lehikoinen 2015).

Twenty-four study sites, each with 20–80 nest boxes (see Lambrechts et al. 2010), were established in the pollution gradient in three main directions (southwest, southeast and northwest; i.e. to get wide spatial coverage and replicate sites in different distances), in a range of 0.4–73 km from the smelter (Figure 1). The number of active sites varied in different years (Appendix 1). We captured and ringed females from nest boxes during the incubation and nestling periods. Nest boxes were further checked weekly to record final clutch size, number of hatchlings and number of fledglings, and to ring nestlings. Final clutch size denotes the number of eggs during the incubation phase. Hatchling number was determined from the numbers of recently hatched nestlings and unhatched eggs. Fledgling numbers were determined from the numbers of nestlings prior to fledging and those found dead in the nest after fledging. To compare the breeding parameters in different parts of the pollution gradient, we split the data in two parts: the area less than 2.5 km from the pollution source is hereafter called ‘polluted’, whereas the area beyond 2.5 km from the source (median distance 10.3 km) is called ‘control’, as emission levels approach the background values beyond the distance of 2.5 km (Berglund et al. 2012).

Age determination

Females were aged by their plumage characteristics into two age-classes, one year old (hereafter young) or older (hereafter old), mainly on the basis of the shape of the primary coverts, primaries and tail feathers (Karlsson et al. 1986; Svensson 1992). Because differences in plumage characteristics are relatively small and aging is not always easy (in 8.3% of captures it was not possible to determine age) we calculated two figures to estimate the reliability of our age determinations: 1. proportion of erroneous determinations among the individuals that were ringed as nestlings (i.e. their age was known), and 2. proportion of old (on the basis of capture history) birds erroneously determined as young. The former proportion was 4.6% (5 out of 109 individuals) and the latter 4.6% (14 out of 303 individuals). Although we corrected the known erroneous determinations in the data for the further analyses, we need to accept that <5% of the age determinations may be wrong. This could slightly weaken the estimated age effects on reproductive parameters since in some cases old birds may have been determined as young at first capture. On the other hand the bias should be very small because the older age classes, which are more critical for our analyses due to their smaller sample size, cannot contain young birds. Age classes in our data denote calendar years (i.e. 1 = year of birth, 2 = the year following birth year, etc.).

For the current analyses we used all individuals for which we knew their year of birth. This applies to nestlings (born recently) and females determined as ‘young’ on the basis of their plumage characteristics (born in the previous season). When we later recapture one of these birds we know from their ringing history how old they are. Often, the same individual was captured more than once per breeding season and sometimes age determinations differed. If there were more than two determinations we relied on the age determined in majority of the cases. When these were equal (e.g. 1 young vs. 1 old) we considered the age as unknown. Because there were relatively few individuals in the age classes of 5 (n = 21) and 6 (n = 4; the maximum age in our data) years, we used in the analyses a combined age class “≥5 years”. In this class we also included those 25 old birds for which the exact age was not known but which were known to be at least 5 years old on the basis of their capture history (i.e. they were determined as old in their first capture and were retrapped again after at least two years). The final data contains 2502 observations on 2224 individuals, of which 90% were trapped just once. Some individuals may change their breeding location between polluted and control areas over years but on the basis of our known cases (3.95% of birds which were trapped in more than one year), we consider their number low.

Statistical analyses

We studied four reproductive parameters for their potential age dependence: clutch size, hatching success (probability of an egg to hatch), fledging probability (probability of a hatchling to fledge) and fledgling number. These four parameters represent important life-history variables (i.e. offspring size, mortality and fitness). These were analyzed with generalized linear mixed models (GLMMs, Glimmix procedure) with the statistical software SAS 9.4 (SAS Institute Inc. 2013). The values of breeding parameters affected by predation, human disturbance or manipulations were not included in the analyses. However, if individual chicks are taken from the nest by a predator with no other signs on predation, which we consider rare, we cannot separate these cases from ‘normal’ mortality because parents may also remove small dead nestlings from the nest. Explanatory factors in the models were area (polluted vs. control), age (2, 3, 4 and ≥5) and area × age (significant interaction would be indicative of pollution-related age dependence). Because pollution levels decreased and some of the breeding parameters increased during this long-term study (Eeva and Lehikoinen 2000; Eeva and Lehikoinen 2015) we further included in the models two factors to take account of the possible confounding effect of temporal trends in breeding parameters: year (continuous variable) and year × area. However, temporal trends in breeding parameters will not be dealt with in detail here because a more detailed analysis on them is recently given in Eeva and Lehikoinen (2015). For clutch size and fledgling number we used Poisson error distribution. For hatching and fledging probabilities we modelled binomial proportions (events/trials syntax of the Glimmix procedure) with binary error distribution. In all models year (class variable) and study site were used as random factors to control for the non-independence of the observations within years and sites. Model residuals were further used as a random factor to control for overdispersion in the models. In this bird species, laying date is known to affect breeding parameters like clutch size and it is also known to depend on age, young birds (age class 2) laying 2 − 3 days later than the older ones (Lundberg and Alatalo 1992). However, we considered timing of breeding as just one of the correlates of individual quality among many others and therefore we did not try to include it in our models.

Besides reproductive senescence (i.e. the within-individual decline in reproductive success with increasing age), any differences in reproductive parameters among age classes at population level could be related to phenotype-dependent survival (i.e. selective disappearance; van de Pol and Verhulst 2006; Bouwhuis et al. 2009; Rebke et al. 2010), good quality individuals likely living longer than lower quality individuals, which could change population mean for reproductive parameters along the age classes. In our population-level study (i.e. cross-sectional analysis) this could mask the effect of reproductive senescence (see Bouwhuis et al. 2009). To take account of this possibility, we ran the above mentioned models again by including only those birds that were known to live long, i.e. ≥4 calendar years (n = 219 observations on 92 individuals; hereafter called 'long-lived birds'). This further allowed for more balanced analyses as regards to sample sizes because in the previous models the number of observations in the youngest age class was disproportionally large (87%) as compared to the older age classes. For these models, where most individuals were captured more than once, we added individual as a random factor to control for the non-independence of the multiple observations on the same individual. The average of individual mean time intervals between observations is 1.5 years (n = 88 individuals, SD = 0.74; excluding four individuals which were ringed as nestlings and captured once as breeding).

Because reproductive parameters include some missing values, the final sample size varies among the different models. The effects with $p<0.05$ were considered statistically significant. Non-significant terms were dropped out from the models one by one, starting from interactions, but we always retained the main terms (area, age and area × age) in the final models. The degrees of freedom were adjusted with the Kenward-Roger method.

Results

Full dataset

All the breeding parameters showed generally lower levels in the polluted area but aside that none of them showed significantly different age dependence between the two areas (Table 1, Figure 2). Overall significant age dependence was found for clutch size, hatching probability and fledgling number (Table 1). Clutch size increased 0.54 eggs and fledgling number 0.50 chicks from the age class 2 to the age class 3, but neither of them showed a significant change after that (Figure 2). Hatching probability decreased 9.0% from age class 3 to the age class ≥5

(Figure 2). Fledging probability did not show any significant age-dependent variation (Figure 2). Clutch size and fledgling number showed their overall peak value at the age class 4 (Figure 2). Significant interactions between year and area (Table 1) indicate that clutch size and fledgling numbers increased in the polluted area over the study period (log scale model estimates ±SE for polluted and control areas, respectively, for clutch size: 0.00097 ±0.00084 vs. 0.0059 ±0.0011; and for fledgling numbers: 0.0042 ±0.0051 vs. −0.0023 ±0.0046).

Long-lived birds

In general, the subset of long-lived birds showed relatively similar patterns along the age groups than the full dataset (Table 1, Figure 3). However, except for fledgling number, the differences between areas were not significant, which was due to slightly smaller effect size and much smaller sample size (Table 1). Unlike in the full dataset, the fledgling number did not significantly vary with age (Table 1), which was mainly because the subgroup of long-lived birds produced slightly (10%) more fledglings in their first breeding season (age class 2) than the rest of the population. This difference was, however, not statistically significant (GLMM with area [polluted vs. control] and bird subgroup [long-lived vs. others] as explanatory factors: $F_{df} = 2.92_{1,1763}$, $p = 0.088$, $n = 1801$). For an unknown reason, fledging probability was 12% lower in age class 3 than in age class 4 (Table 1, Figure 3) but, like in the full data, there was no clear indication of age-related decrease. Temporal trends were not statistically significant in this dataset (Table 1).

Discussion

Although all of the reproductive parameters showed generally lower values in the polluted environment, we found no indication of faster age-related decrease there. According to the society of European Union for Bird Ringing (EURING) statistics, the maximum known age for *F. hypoleuca* is 10.9 years (Euring 2017) and one could speculate that our sample had too few birds in the oldest age classes to demonstrate any effect. However, even in the case that pollution would decrease fecundity only at very old age, this would have a minimal effect on the production at population level because in our population only less than 1% of females will reach their 6th calendar year. This migratory species also shows extensive natal dispersal (Lundberg and Alatalo 1992) and a great deal of females breeding in the polluted area were not likely born there. Growing in an unpolluted environment and spending most of the year away from the polluted area may alleviate the effect of pollution on senescence around point sources of pollution, though other sources of pollution are possible during migratory and wintering seasons (Raja-aho et al. 2012). Taken together, environmental pollution does not reduce the viability of our study population via faster age-related decrease in fecundity at population level. Despite that fledgling production in the polluted area has been and still remains smaller than in the control area, the population densities have increased over our long-term study (Eeva and Lehikoinen 2015).

Clutch size and fledgling number of *F. hypoleuca* females increased after their first breeding, after which there was no significant change and, hence, no strong evidence of age-related decrease of fecundity at population level, though decreasing estimates for the fledgling number in the oldest age class could be indicative of that. Bouwhuis et al. (2010) found improved reproductive performance (recruit production) by great tit *Parus major* females up to the age of 3 years (= 4th calendar year) due to improved skills or optimization of reproductive effort, after which performance declined, most likely due to senescence. However, in agreement with our results, Sanz and Moreno (2000) found no deterioration in population level clutch size or fledgling number of *F. hypoleuca* females before the age of 5 years (= 6th calendar year). Increased reproductive performance with age is a general pattern in birds, often explained by selective disappearance (due to mortality or dispersal) of lower quality individuals and/or by true age-related improvement in competence or effort (Forslund and Pärt 1995; Bouwhuis et al. 2010). Several studies, however, suggest that selective disappearance alone cannot explain age-related changes in a population, but increasing individual competence has an important role (Forslund and Pärt 1995; Balbontin et al. 2007; Rebke et al. 2010). Our analyses for the subclass of long-lived *F. hypoleuca* females suggest that selective disappearance did not cause any major bias as regards to the effect of aging.

Hatchability of eggs slightly decreased after the second breeding (age class 3). This could be indicative of decreasing fertility or increasing embryonic mortality of eggs with age, e.g. because of increasing oxidative damage, behavioral changes and/or changing maternal input (Alonso-Álvarez et al. 2010). However, a recent study in our same study area found no evidence of age-related increase of oxidative stress markers, and some of the antioxidant enzymes (catalase) even showed lower activities in old *F. hypoleuca* females (age class $\geq$3) than in young (age class 2) ones, despite that old females produced larger broods (Berglund et al. 2014). On the other hand, lower catalase activities could suggest decreased response to oxidative stress (either due to aging or as a trade-off between antioxidant activity and brood size). Remeš et al. (2011) observed that older ($\geq$3 calendar years) *P. major* females deposited higher concentrations of nutrients (carotenoids and vitamin E) in yolks than the first-time breeders (2nd calendar year). This agrees with a general observation of improved breeding success of older birds compared to novel breeders (Saether 1990). No age effect, however, was found on yolk carotenoid levels of the collared flycatcher *F. albicollis*, a closely related species to *F. hypoleuca* (Török et al. 2007).

The overall decreased reproductive output in the polluted environment is more likely a consequence of pollution-related changes in food chains and consequent resource limitation for insectivorous birds than direct toxic effects of pollutants, invertebrate food abundance and quality being lower in the polluted area (Eeva et al. 1997; Eeva et al. 2005). The body mass of incubating *F. hypoleuca* females in our study area showed faster seasonal decrease in polluted than in unpolluted area, which may indicate more drastic decrease in food abundance in polluted area (Rainio et al. 2017). After the early years of this long-term study (i.e. the beginning of 1990's) the average metal levels measured in flycatchers of our study area have generally been moderate or low and not considered toxic (Berglund et al. 2012). Furthermore, metal exposure levels in territories of *F. hypoleuca* females have neither shown clear associations with levels of antioxidants (e.g. glutathione, carotenoids) or antioxidant enzymes (e.g. glutathione peroxidase, glutathione-S-transferase, superoxide dismutase or catalase), which are considered as indicators of oxidative stress (Eeva et al. 2012; Berglund et al. 2014), nor with yolk vitamin levels or egg characteristics such as size or eggshell index (Espín et al. 2016). Therefore, we consider indirect effects (e.g. food quality) a more likely explanation for decreased reproductive output than direct toxic effects. For example, *F. hypoleuca* egg yolks were found to contain 26% less food-derived carotenoids (lutein) in the same polluted area as compared to eggs in the unpolluted area (Espín et al. 2016).

Recent studies have found that DNA telomeres of another insectivorous passerine, *P. major,* have been shorter in the nestlings grown in the polluted or urban areas as compared to the control areas (Salmón et al. 2016; Stauffer et al. 2016). The nestlings of this species also showed increased mutation rates in a metal polluted area (Eeva et al. 2006). However, neither of these effects were found in *F. hypoleuca* nestlings in our study area (Eeva et al. 2006; Stauffer et al. 2016), suggesting that this species may better resist pollution-related senescence. This is in agreement with the view that, due to their efficient detoxification capacity, insectivorous and migratory birds would be less sensitive to environmental contaminants than granivorous and non-migratory birds when exposed to similar levels (Rainio et al. 2012). Interspecific comparisons on this topic would therefore be valuable.

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Table 1. Effects of area (polluted vs. control) and age (2, 3, 4 or ≥5 calendar years) of *F. hypoleuca* females on four breeding parameters. Generalized linear mixed models (GLMM)[1] for full data and for a subset of data containing only long-lived birds (≥4 calendar years). Year (continuous variable) was included in the models to account for temporal trends in breeding parameters. Final models are shown in bold.

	Clutch size[2]		Hatching probability[3]		Fledging probability[3]		Fledgling number[2]	
Full data	F_{df}	*p*	F_{df}	*p*	F_{df}	*p*	F_{df}	*p*
Area	**21.7 $_{1,1810}$**	**<0.0001**	**6.66 $_{1,156}$**	**0.011**	**7.70 $_{1,129}$**	**0.0063**	**4.1 $_{1,1198}$**	**0.043**
Age	**25.6 $_{3,2441}$**	**<0.0001**	**2.67 $_{3,2238}$**	**0.046**	**1.60 $_{3,1949}$**	**0.19**	**5.51 $_{3,2015}$**	**0.0009**
Area × Age	**2.05 $_{3,2434}$**	**0.11**	**0.21 $_{3,2238}$**	**0.89**	**0.64 $_{3,1945}$**	**0.59**	**0.13 $_{3,2013}$**	**0.94**
Year	**17.7 $_{1,17.7}$**	**0.0002**	0.35 $_{1,25.7}$	0.56	0.00 $_{1,21.5}$	0.97	**0.04 $_{1,22.1}$**	**0.84**
Area × Year	**21.4 $_{1,1804}$**	**<0.0001**	0.07 $_{1,717}$	0.80	0.20 $_{1,779}$	0.65	**3.94 $_{1,1191}$**	**0.047**
Long-lived	F_{df}	*p*	F_{df}	*p*	F_{df}	*p*	F_{df}	*p*
Area	**2.33 $_{1,14.7}$**	**0.15**	**1.10 $_{1,11.1}$**	**0.32**	**2.56 $_{1,15.4}$**	**0.13**	**6.42 $_{1,175}$**	**0.012**
Age	**3.05 $_{3,158}$**	**0.030**	**4.18 $_{3,118}$**	**0.0075**	**4.06 $_{3,140}$**	**0.0085**	**1.08 $_{3,171}$**	**0.36**
Area × Age	**0.69 $_{3,160}$**	**0.56**	**0.13 $_{3,109}$**	**0.94**	**0.74 $_{3,138}$**	**0.53**	**0.41 $_{3,171}$**	**0.75**
Year	1.26 $_{1,31.8}$	0.27	0.05 $_{1,26.8}$	0.83	0.15 $_{1,23.8}$	0.70	0.76 $_{1,19.1}$	0.39
Area × Year	0.10 $_{1,70.6}$	0.76	1.48 $_{1,44.3}$	0.23	0.27 $_{1,77.2}$	0.61	0.11 $_{1,143}$	0.74

[1] Final model estimates (±95% CI) and sample sizes for each group are shown in Fig. 2 and Fig. 3.

[2] GLMM with Poisson error distribution and log link function. Year as a categorical variable and study site were used as random factors. For the subset of long-lived birds individual was further included as a random factor.

[3] GLMM with binary error distribution and logit link function. Year as a categorical variable and study site were used as random factors. For the subset of long-lived birds individual was further included as a random factor. Hatching probability = probability of an egg to hatch. Fledging probability = probability of a hatchling to fledge.

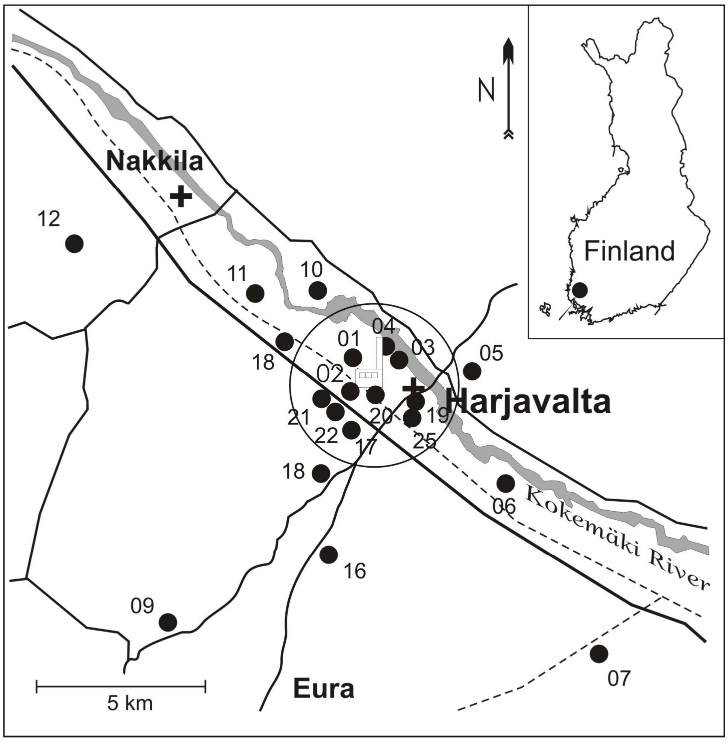

Figure 1. Map of the study area, showing 20 out of 24 study sites where data were collected for this study around a copper-nickel smelter (in the middle). Four more distant sites locate 47, 60, 64 and 73 km SW from the smelter. Sites within the circle (radius 2.5 km) are considered heavily polluted. Sample sizes and distances to the smelter are shown in Appendix 1.

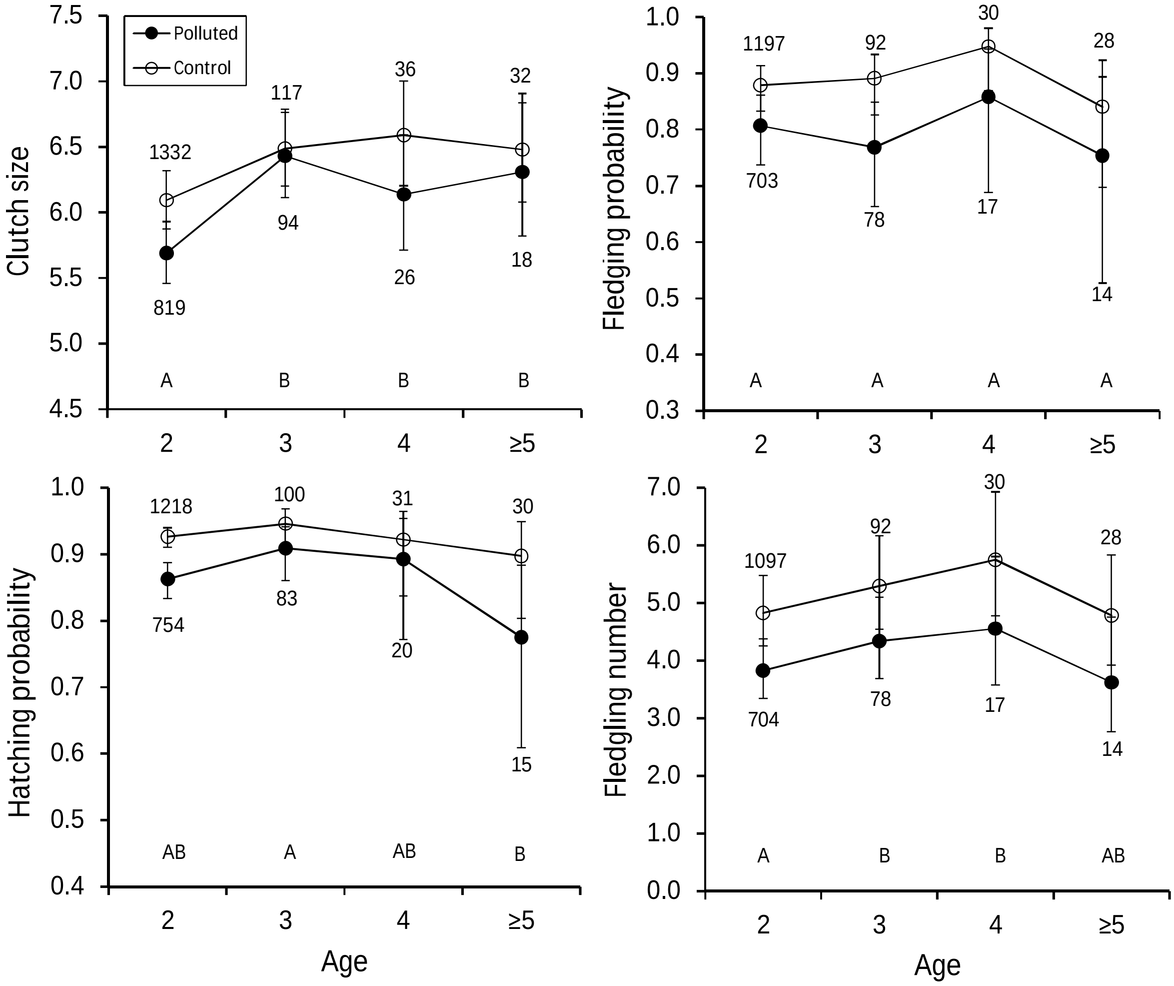

Figure 2. Four reproductive parameters of *F. hypoleuca* in relation to the female age (calendar years; 1 = year of birth) in a metal polluted area and a control area. Combined data from 1991 – 2016. Values are estimates (±95% CI) from the final models in the Table 1. The lettering indicates the pairwise differences among the age groups (Tukey's test; groups with the same letter are not significantly different; p values adjusted with the number of comparisons). Numbers denote the sample size for the breeding data.

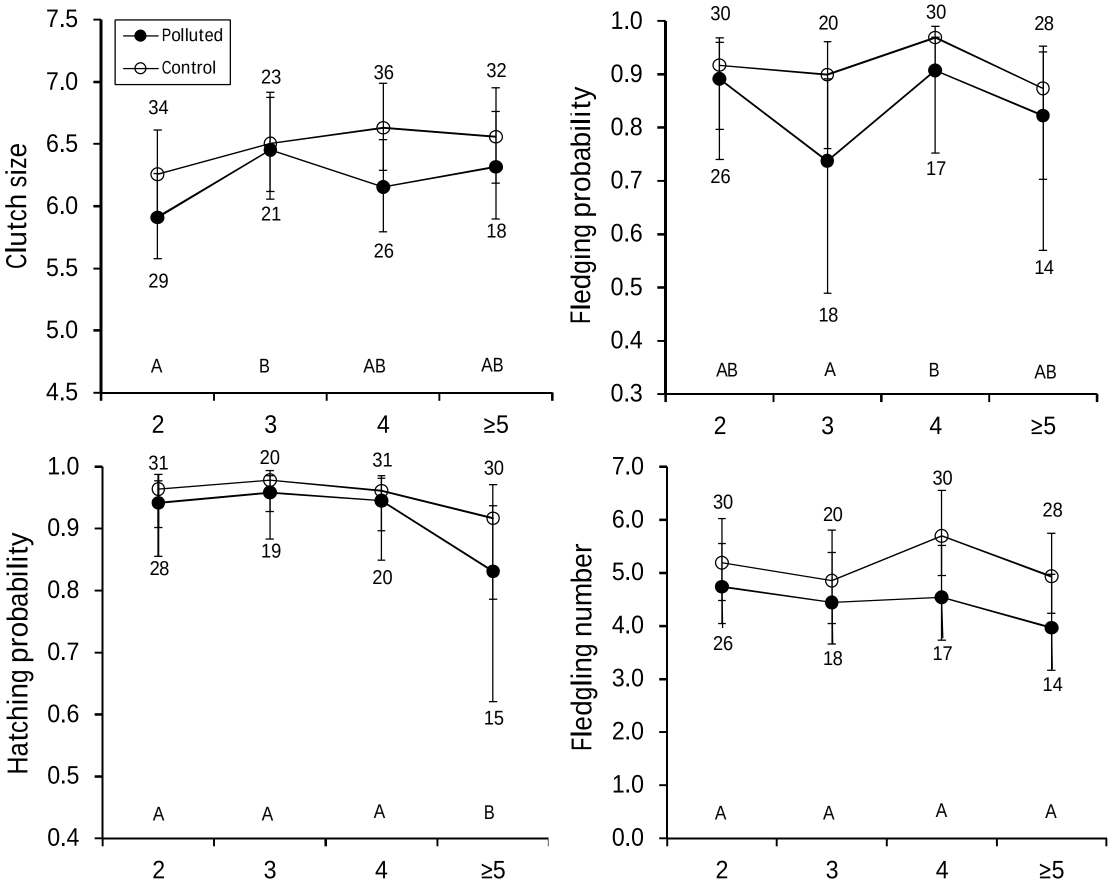

Figure 3. Four reproductive parameters in the subgroup of long-lived *F. hypoleuca* females in relation to the female age (calendar years; 1 = year of birth) in a metal polluted area and a control area. Combined data from 1991 – 2016. Values are estimates (±95% CI) from the final models of long-lived birds in the Table 1. The lettering indicates the pairwise differences among the age groups (Tukey's test; groups with the same letter are not significantly different; p values adjusted with the number of comparisons). Numbers denote the sample size for the breeding data.

Appendix 1. Yearly numbers (N) of captured *F. hypoleuca* females per each study site. Distances of study sites to the pollution source (dist) are shown in kilometers.

	Site number																							
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	N
Dist	1.1	0.4	1.7	1.8	3.3	5.4	11	2.8	9.4	3.4	4.0	10	47	73	64	5.0	1.4	1.9	2.0	0.9	0.5	0.9	60	2.0	
1991	2	1	5	4	4	9	5	2	3	7	4	7	5	0	0	0	0	0	0	0	0	0	0	0	58
1992	2	10	4	4	7	10	8	12	12	9	12	9	6	8	6	0	0	0	0	0	0	0	0	0	119
1993	2	6	3	4	10	15	10	3	10	9	10	10	15	11	5	0	0	0	0	0	0	0	0	0	123
1994	3	7	2	1	9	14	13	2	9	5	8	7	12	3	5	0	0	0	0	0	0	0	0	0	100
1995	4	7	7	0	0	8	3	4	4	6	5	6	13	0	5	10	12	7	0	0	0	0	0	0	101
1996	6	7	4	0	3	12	3	0	2	12	7	2	3	0	3	9	6	9	3	0	0	0	0	0	91
1997	8	3	6	0	0	12	3	0	2	5	3	0	9	0	0	8	3	8	2	0	0	0	0	0	72
1998	10	8	6	0	1	7	5	1	9	6	9	9	10	0	3	10	7	11	6	2	0	0	0	0	120
1999	7	4	3	0	0	7	4	0	4	3	2	1	10	0	0	2	0	8	2	0	0	0	0	0	57
2000	12	0	10	0	1	10	5	0	2	7	3	8	12	0	7	4	6	6	4	0	3	0	0	0	100
2002	1	0	4	0	0	1	5	0	4	0	0	0	7	0	1	3	1	0	0	0	2	0	0	0	29
2003	3	3	5	0	0	7	6	0	4	0	0	1	1	0	5	6	2	0	1	0	1	0	0	0	45
2004	6	1	2	0	0	8	3	0	4	0	0	6	11	0	7	1	6	1	4	0	2	3	0	0	65
2005	4	2	3	0	0	5	9	0	1	0	0	1	4	0	6	9	9	0	1	0	8	3	0	0	65
2006	8	2	5	0	0	2	2	0	3	0	0	4	9	0	7	7	3	0	6	0	6	1	0	0	65
2007	13	6	11	0	0	10	9	0	9	0	0	12	9	0	3	10	13	0	6	0	12	7	0	0	130
2008	14	5	15	0	0	12	10	0	10	10	0	8	5	0	4	11	5	0	4	0	7	7	4	0	131
2009	16	7	14	0	0	11	6	0	13	8	0	7	12	0	5	11	0	0	7	0	13	6	8	0	144
2010	10	9	9	0	0	10	9	0	6	10	0	9	8	0	3	10	0	0	10	0	12	3	5	0	123
2011	13	6	10	0	0	15	11	0	9	6	0	11	16	0	0	16	0	0	9	0	22	9	7	0	160
2012	23	10	14	0	0	20	5	0	9	8	0	16	13	0	0	18	0	0	0	0	16	12	16	11	191
2013	19	0	16	0	0	18	0	0	7	0	0	9	15	0	0	11	0	0	0	0	18	8	17	9	147
2014	7	0	8	0	0	11	5	0	6	0	0	8	14	0	0	11	0	0	0	0	12	9	9	7	107
2015	9	2	4	0	0	8	5	0	2	0	0	6	5	0	0	8	0	0	0	0	9	8	4	5	75
2016	7	5	6	0	0	13	3	0	3	0	0	3	8	0	0	11	0	0	0	0	11	4	4	6	84
N	209	111	176	13	35	255	147	24	147	111	63	160	232	22	75	186	73	50	65	2	154	80	74	38	2502