Variation in prevalence and intensity of two avian ectoparasites in a polluted area

TAPIO EEVA* *and* TERO KLEMOLA

Section of Ecology, Department of Biology, FI-20014 University of Turku, Finland

SUMMARY

We counted the numbers of pupae of two ectoparasitic flies (*Protocalliphora* sp. and *Ornithomyia* sp.) in the nests of a passerine bird, the pied flycatcher (*Ficedula hypoleuca*) to find out if their prevalence or intensity are affected by long-term environmental pollution by a copper smelter and whether such an interaction would have impacts on birds' breeding success. Fecal metal concentrations of *F. hypoleuca* nestlings were used to explore direct association between metal levels and parasite prevalence, but we also included other explanatory factors in our analysis, such as timing of breeding, brood size, ambient temperature, habitat quality and host population density. We found that environmental pollution decreased the prevalence of *Protocalliphora* via changed habitat quality but did not affect the prevalence of *Ornithomyia*. The prevalence of neither ectoparasite was, however, directly related to ambient metal levels. Both ectoparasites showed higher prevalence when ambient temperature during the nestling period was high, emphasizing the potential of climate change to modify host–parasite relationships. The prevalence of *Ornithomyia* was further highest in dense *F. hypoleuca* populations and late broods. Nestling survival decreased with increasing infestation intensity of *Ornithomyia* while no association was found for *Protocalliphora*. Despite relatively low numbers and overall weak effect of parasites on survival, the possible delayed and/or sublethal effects of these ectoparasites call for further studies. Our results suggest that pollution-related effects on avian ectoparasite numbers are species-specific and reflect habitat changes rather than direct toxic effect of heavy metals.

Key words: Air pollution, ectoparasites, habitat quality, insectivorous passerines, population density, weather.

INTRODUCTION

Environmental pollution has potential to change ecological interactions between species (Brotons *et al.* 1998; Eeva *et al.* 2005; Lürling and Scheffer, 2007; Weis *et al.* 2011). One such interaction could be the relationship between a parasite and its host. Host–parasite relationships are well known to interact with environmental pollution in plants (Flückiger *et al.* 2002), and there are plenty of examples of such interactions in fish (e.g. Khan and Thulin, 1991; Williams and Mackenzie, 2003) but much less information is available on terrestrial animals. In aquatic systems parasite numbers have generally decreased in polluted environments and especially so at metal polluted sites and for ectoparasites (Blanar *et al.* 2009). Avian host–parasite interactions have been intensively studied in free-living terrestrial bird populations during the past decades (Loye and Zuk, 1991; Clayton and Moore, 1997) but only rarely in the context of environmental pollution. One of the few case studies in birds reports increased ectoparasite numbers in a tree swallow population foraging at a wetland area contaminated by aromatic hydrocarbons (Gentes *et al.* 2007). Metal mining and smelting sites are typical point sources of heavy metals and sulphuric acid (Kozlov *et al.* 2009), and they have been found to have detrimental impacts on terrestrial birds at individual, population and community levels (Janssens *et al.* 2003; Belskii *et al.* 2005; Berglund *et al.* 2010; Eeva *et al.* 2012*a*). Case studies at such sites have shown increased abundance for some ectoparasite taxa while decreased numbers for another taxa, suggesting that responses of parasite populations to pollution are species-specific (Eeva *et al.* 1994; Belskii *et al.* 2005). Pollution–parasite interactions in terrestrial avian populations are currently poorly known and more studies are needed on variable host and parasite taxa to explore the consequences of pollution and to improve the general knowledge of the avian ectoparasites.

The aim of our study was to assess if the prevalence or intensity of avian ectoparasites are affected by long-term environmental pollution and whether such an interaction would have impacts on birds' breeding success. We counted the numbers of ectoparasitic flies in the nests of a small passerine bird, the pied flycatcher (*Ficedula hypoleuca*), during 7 consecutive years in the polluted sites and unpolluted control sites around a point source of heavy metals and sulphuric oxides (copper smelter). We also wanted to take into

* Corresponding author: Section of Ecology, Department of Biology, FI-20014 University of Turku, Finland. E-mail: tapio.eeva@utu.fi

account some other potential factors that could explain the prevalence of parasitic flies in *F. hypoleuca* nests. On the basis of earlier studies we included timing of breeding, brood size, ambient temperature during the nestling period, habitat and population density of *F. hypoleuca* as possible explanatory factors in our analyses. Note, however, that all of these variables potentially interact with pollution and may partly reflect alternative pathways whereby pollution may affect bird–parasite interactions. For one breeding season we also have data on actual heavy metal exposure of *F. hypoleuca* nestlings. This enabled us using the fecal metal concentrations to explore direct association between metal levels and parasite prevalence. Finally we analysed the potential effects of parasite intensity on nestling survival. Since some earlier studies have shown that breeding success of *F. hypoleuca* is inferior in the polluted area due to resource limitations (e.g. food quality) (Eeva *et al*. 1997), we expect that further stressors such as ectoparasites may have stronger negative effect on breeding at the copper smelter.

MATERIALS AND METHODS

Study area

Our study area was the surroundings of a copper smelter in the town Harjavalta (61°20′N, 22°10′E), SW Finland. Sulphuric oxides and metals (especially As, Cd, Cu, Ni, Pb and Zn) are common pollutants in the area, due to long-term emissions from the smelter and adjoining industry (Kiikkilä, 2003; Kozlov *et al*. 2009). Study sites (n = 17) with nest boxes (20–60 boxes per site) were established since 1991 along the air pollution gradient in three main directions (SW, SE and NW) away from the copper smelter complex. A description of the nest boxes is given in Lambrechts *et al*. (2010). Metal emissions from the smelter have considerably decreased over the study period (for example, approximately 99% reduction in dust emissions), but still metal levels in bird feces and tissues show increased values in the vicinity of the smelter (Berglund *et al*. 2011, 2012). Sites closer than 2·5 km from the smelter are hereafter referred to as zone 1 ('polluted area') and sites over 2·5 km from the smelter as zone 2 ('unpolluted area'), emission levels approaching the background values beyond the distance of 2·5 km (Berglund *et al*. 2012). Daily temperature data were collected by the Finnish Meteorological Institute, the weather station locating within our study area (Peipohja, Kokemäki, 61°16′N, 22°15′E).

Bird species and breeding parameters

Ficedula hypoleuca is a small (12–13 g), insectivorous and migratory passerine bird (Lundberg and Alatalo, 1992). It breeds in a large range over Europe and Western Siberia and winters in Western Africa. *Ficedula hypoleuca* is single-brooded and the modal clutch size is 5–6 eggs. It is an abundant species that easily accepts artificial nest boxes, and thus it is a common model species in studies of avian ecology and evolution.

Nest boxes were checked at least once a week to collect basic breeding data, including hatching date, hatchling numbers and fledgling numbers. If the nest was not visited at the day of hatching the date was estimated with the wing length of small nestlings by comparing it with the growth curve drawn from the nests with exact hatching dates (TE: unpublished data). Unlike some other bird species the pied flycatchers do not seem to actively search and remove nest parasites from the nest material (own observation, based on 148 h follow-up of the parents feeding 6–11-day-old chicks; Eeva *et al*. 2005). To reduce the among-nest box variation in parasite numbers at the start of each breeding season due to overwintering parasites (especially fleas) we removed old nest material from all the nest boxes after fledging. Nest material was spread on the forest floor near the nest box.

Sampling of parasitic fly pupae

Numbers of fly pupae were counted from 639 nests of *F. hypoleuca* collected 0–30 days after fledging in summers 2006–2012 (mean ± S.D.: 91 ± 16·3 nests per summer). Nests were collected randomly except that we tried to collect samples from all study sites and at different parts of each site to spread the spatial distribution of samples as much as possible. All nest material in the nest box was carefully collected and preserved in plastic bags at −20 °C until thoroughly searched for pupae of parasitic flies in the laboratory. Although some pupae were hatched before the nest collection the empty fly cocoons could be found in the nest material and were included in the total numbers. We counted the numbers of pupae in nest material for two ectoparasitic fly genera, a calliphorid fly *Protocalliphora* sp. and a hippoboscid fly *Ornithomyia* sp. (Fig. 1). Note that our data only include the nests that produced at least one fledgling, so the possible effects on nesting failure cannot be estimated. Comparing only successful nests, however, was reasonable because early failed nests may not be parasitized and later failed nests would vary in periods where infection by flies would be possible, and this would again affect the number of fly pupae in the nest.

The calliphorid pupae most likely consist of one species, *Protocalliphora azurea* (Fallén), which is the most common and widely distributed *Protocalliphora* (Diptera, Calliphoridae) species in Fennoscandia (Rognes, 1991). In a sample of pupae grown to adulthood (n = 107 individuals), collected from our
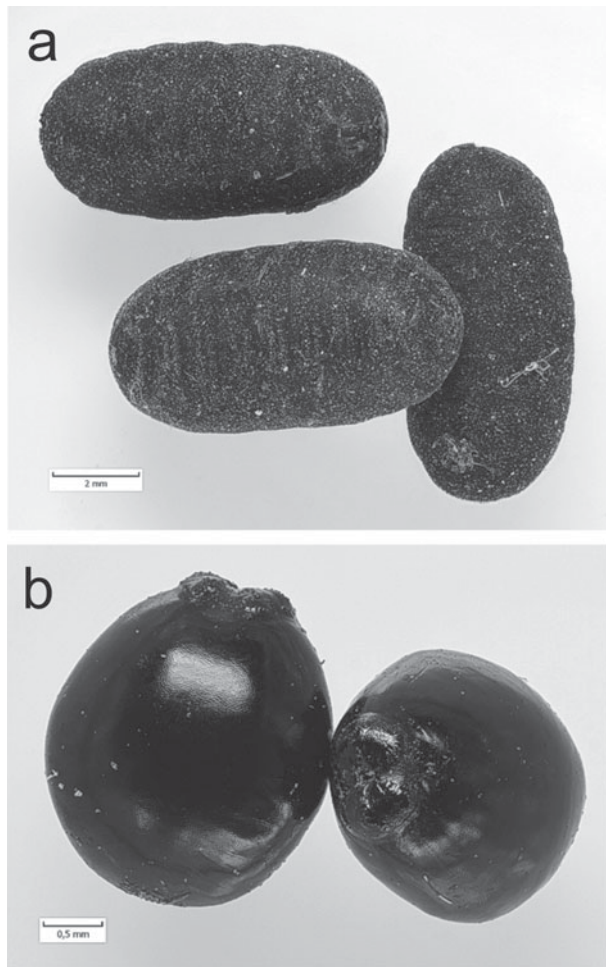
Fig. 1. Examples of pupae of *Protocalliphora* sp. (a) and *Ornithomyia* sp. (b) collected from the nests of *Ficedula hypoleuca*.

study sites in the beginning of the 1990s, no other species were found (Eeva *et al*. 1994). Adult flies are not parasitic but feed on flowers (Bennett and Whitworth, 1991). They lay their eggs in bird nests and the larvae are nest-dwelling ectoparasites that intermittently feed on nestling blood before burrowing into the nest material to pupate (Sabrosky *et al*. 1989; Bennett and Whitworth, 1991). The adult flies eclose some weeks later and they overwinter (Bennett and Whitworth, 1991; Rognes, 1991).

We could not identify the hippoboscid species, but *Ornithomyia fringillina* (Curtis) and *Ornithomyia chloropus* (Bergroth) remain as most likely species on the basis of their size, distribution and ecology (Maa, 1969; Grunin, 1989). See Petersen *et al*. (2007) for their taxonomic status. In *Ornithomyia* the larval development takes place within the mother's body (vivipary). The females feed on bird blood and deposit single, fully grown and immobile larvae, which pupate immediately and overwinter as the pupal stage (Corbet, 1956). Larvae are deposited on the bodies of nestlings and parent birds, where they fall on the nest (from nestlings and parents) or elsewhere outside the nest (from parents) (Corbet, 1956).

Habitat and forest floor vegetation

The forests in the area are dominated by Scots pine *Pinus sylvestris* L., which forms mixed stands with Norway spruce *Picea abies* (L.) Karsten and birches (*Betula* spp.). To avoid extra variation in the data due to varying habitat quality, we selected study sites of the same habitat type, i.e. relatively barren, pine-dominated forests typical of the study area. Shrubs like *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. dominate in the field layer, but in the polluted area ground-layer vegetation is more scanty due to long-term pollution effects (Salemaa *et al*. 2001). Since earlier studies have found associations between forest floor vegetation type and occurrence of *P. azurea* (Eeva *et al*. 1994), we classified all *F. hypoleuca* territories (in 50 m radius from the nest box) as barren or luxuriant by following the forest type classification of Kalliola (1973) that is based on indicator plant species. In short, territories characterized by *Cladonia* sp., *Empetrum* sp., *Calluna* sp. and *V. vitis-idaea* were classified as barren and those characterized by *V. myrtillus, Oxalis* sp., *Maianthemum* sp. and *Ledum* sp. were classified as luxuriant. Territories with no or scanty forest floor vegetation were included in the barren category. Therefore, it needs to be recalled that variation in habitat quality partly reflects the effects of long-term pollution.

Heavy metal analyses

In 2008 we collected fecal samples from some *F. hypoleuca* broods (n = 48) to assess the levels of metal pollution in birds. Fecal samples have been proven to be a good tool to monitor environmental metal levels in birds (Dauwe *et al*. 2000; Berglund *et al*. 2012). Fresh feces were collected from defecating nestlings at the age of 7 days directly to plastic Eppendorf tubes and dried in the laboratory at 50 °C for 72 h. Metal levels (As, Cd, Cu, Ni and Pb) were determined with ICP-MS (Elan 6100 DRC+ from PerkinElmer-Sciex) together with certified reference material (mussel tissue ERM-CE278). A more detailed description of the method and quality control is given in Eeva *et al*. (2012*b*). To provide a single measure describing the variation in pollution levels, we calculated the first principal component (PC1) of the concentrations of the five heavy metals. The PC1 explained 73% of the variation in data (eigenvalue: 3·66), describing well the general level of heavy metal exposure. PC1 correlated strongly negatively with logarithmic distance to the copper smelter ($r = -0.81$, $P < 0.0001$, n = 48) and was used to explain ectoparasite prevalence in further analyses.

Statistics

All analyses were performed with SAS statistical software (SAS, 2008). We first analysed the annual

and pollution-related variation in prevalence (0 = not parasitized, 1 = parasitized) and intensity (numbers/infected nests) with generalized linear models (GLM), by using year, zone and their interaction as fixed effects. In these models we used binomial error distribution for prevalence and negative binomial distribution for intensity. Second, we wanted to find out which factors could explain the prevalence of flies in *F. hypoleuca* nests. In these generalized linear mixed models (GLMM) with binomial error distributions we used yearly standardized hatching date (yearly mean = 0 and standard deviation = 1; separately for the two zones), brood size (at hatching), average daily temperature during the nestling period (14 day period, including the hatching day), field layer vegetation type (barren vs. luxuriant), and the relative numbers of breeding *F. hypoleuca* pairs (% nest boxes occupied by *F. hypoleuca* at each site) as explanatory factors. Standardization of hatching date makes the years more comparable and reduces collinearity between hatching date and temperature. Since the distances of nest boxes are relatively constant in our study sites (mean ± S.D.: 34 ± 5 m) the occupation rates correlate strongly with actual breeding density per unit area (T. Eeva, unpublished) and we use here occupation rates as a surrogate of population density. Year was included in the model as a random factor to control for the non-independence of the observations within years. Missing values in some of the explanatory variables decrease the sample size, depending on the model. Finally, we analysed the possible effects of parasite numbers (including nests with no parasites) on nestling survival with a binomial model where we used events/trials type dependent variable, events denoting fledgling number and trials denoting hatchling numbers. Year was used here as a random factor as well. Non-significant terms were dropped from all the models one-by-one, starting from interactions. They were added again in the final model one-by-one and kept if significant. For the models containing random factors the denominator degrees of freedom were calculated with Kenward–Rogers method. The significance level was set at $P < 0.05$ in all analyses.

RESULTS

Prevalence and intensity

The sample sizes, annual prevalences (% infested) and intensities (numbers of fly pupae/infested nests) are shown for both ectoparasites and study zones in Fig. 2. The mean (with 95% CLs) prevalence of *Protocalliphora* was smaller in the polluted zone (21·4%; 16·7–26·9) than in the unpolluted zone (29·1%; 24·6–34·1) (Table 1). The prevalence of *Protocalliphora* pupae also varied among years (range 18–43%), but there was no interaction between

zone and year (Table 1). The mean prevalence of *Ornithomyia* did not differ between the polluted (30·8%; 24·8–37·5) and unpolluted (32·0%; 26·7–37·8) zone (Table 1). Their prevalence still varied considerably among years (range 7·6–61%), again with no interaction between zone and year (Table 1; Fig. 2).

The intensity of *Protocalliphora* infestation was slightly lower in the polluted (3·7 pupae/nests; 2·8–5·0) than in the unpolluted (5·1 pupae/nests; 4·2–6·2) zone, but this difference was not statistically significant (Table 1). *Protocalliphora* intensity varied among years (range 3·2–10·4 pupae/nests), with no interaction between zone and year (Table 1; Fig. 2). The intensity of *Ornithomyia* varied annually as well but their intensity further showed an annually varying pattern between the two study zones, being significantly higher in the unpolluted area in 2010 but not in the other years (Table 1; Fig. 2). The mean *Ornithomyia* intensity in our study area was 2·5 pupae/nest (2·3–2·8).

Factors explaining prevalence

After including the other explanatory factors (standardized hatching date, temperature during nestling period, field layer vegetation and population density of *F. hypoleuca*) in the model the *Protocalliphora* prevalence indicated a pollution-related density effect, prevalence decreasing with decreasing host population density in the polluted area while such an effect was not found in the unpolluted area (Table 2; Fig. 3a). The prevalence of *Ornithomyia* decreased along with decreasing host density, independent of pollution level (Table 2; Fig. 3a). Late *F. hypoleuca* nests (relative to the yearly mean of the population) showed much higher prevalence of *Ornithomyia* than the early ones (probabilities ranging from 8% to 71%) while *Protocalliphora* prevalence was not dependent on hatching date (Table 2). There were no significant associations between brood size and prevalence, though *Ornithomyia* showed a weak positive association with hatchling numbers (Table 2). Instead, the prevalence of both parasites was positively related to nestling time temperature, the probability of occurrence increasing from 16% to 42% in *Protocalliphora* and from 15% to 65% in *Ornithomyia* in the range of mean temperatures from 12 to 18 °C (Table 2; Fig. 3b). In *Ornithomyia* this association was further pollution-dependent, temperature increasing prevalence more in the polluted area (Table 2; Fig. 3b). The prevalence of *Protocalliphora* was also associated with the quality of field layer vegetation, the probability of occurrence being significantly higher in territories with luxuriant vegetation (33%; 23·9–44·2) than in less productive territories (21%; 14·3–29·2) (Table 2). This effect was not found for *Ornithomyia*.
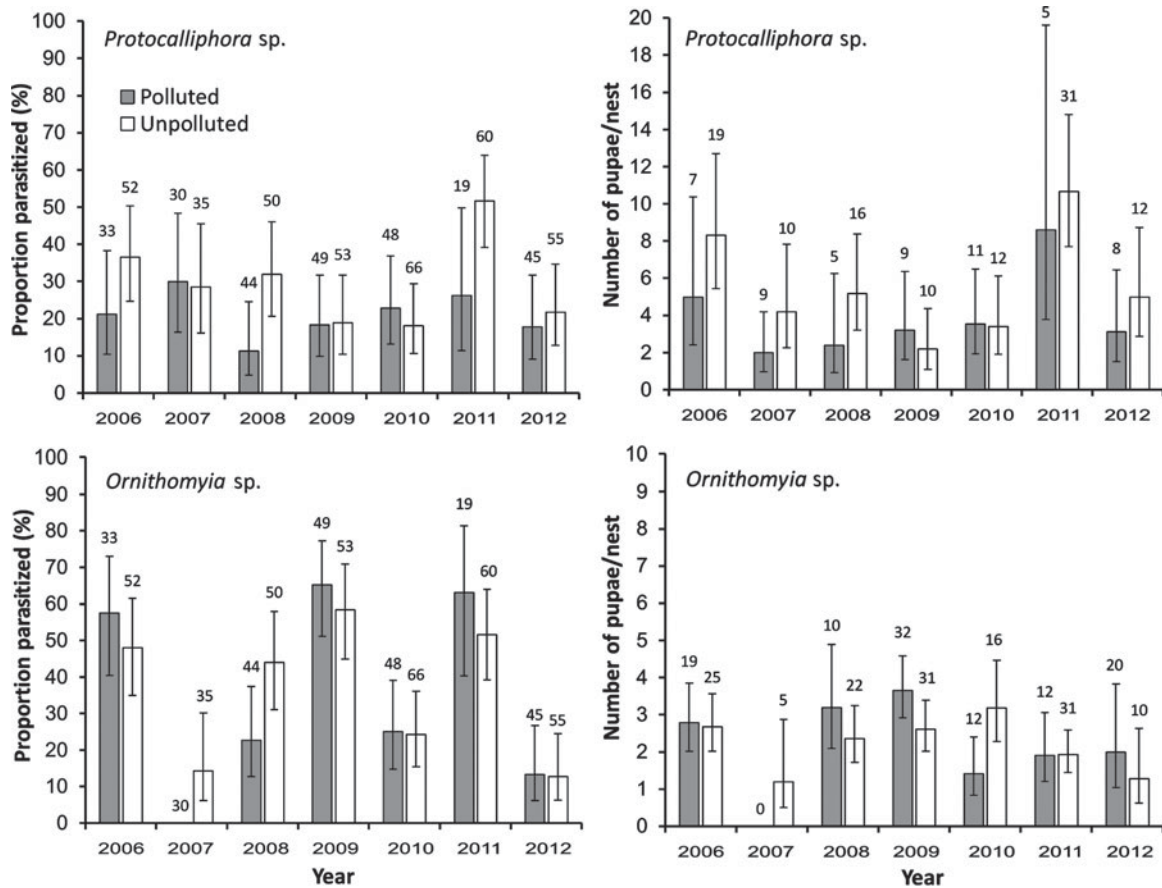
Fig. 2. Annual prevalences (proportion of nests parasitized ± 95% CL) and intensities (numbers/infected nests; means ± 95% CL) of pupae of ectoparasitic flies, *Protocalliphora* and *Ornithomyia* in the nests of a passerine bird, *Ficedula hypoleuca*, breeding in metal-polluted (near copper smelter) and unpolluted sites. Confidence limits come from the full factorial GLM models (including year and zone). The numbers above the bars indicate sample sizes.

Prevalence and heavy metal levels

In the subsample of nests from 2008, we found no association between fecal metal levels (PC1) of 7-day-old *F. hypoleuca* nestlings and prevalence of *Protocalliphora* sp. ($F_{1,46} = 0.06$, $P = 0.81$) or *Ornithomyia* sp. ($F_{1,46} = 1.24$, $P = 0.27$).

Effects on breeding success

Nestling survival was slightly higher in the unpolluted zone (94%; 87·8–97·1) as compared with the polluted one (91%; 81·7–95·5) (Table 3). The number of *Ornithomyia* pupae had a significant, albeit weak negative effect on nestling survival, the probability of surviving decreasing 5·6% in the range of infestation from 0 to 16 pupae/nest (Table 3; Fig. 4). This association was marginally stronger in the polluted zone (Table 3). However, the variation in the numbers of *Protocalliphora* pupae was not associated with nestling survival (Table 3), despite a much higher range in intensity, the numbers varying from 0 to 50 pupae/nest. Mean temperature during nestling period or yearly standardized hatching dates did not significantly explain nestling survival, though the

latter showed a marginally non-significant negative association with nestling survival (Table 3).

DISCUSSION

The prevalence and intensity of both ectoparasites varied among years, the annual variation in prevalence being more prominent in the hippoboscid fly, *Ornithomyia*. The prevalence of the calliphorid fly, *Protocalliphora*, was further dependent on location, the nests in the polluted zone being less frequently parasitized. A similar pattern was noted for *Protocalliphora* in the same study area in the beginning of the 1990s (Eeva *et al*. 1994). Although emission levels of metals and sulphuric oxides have been considerably reduced during the past decades (Berglund *et al*. 2012), the difference in prevalence seems to persist, aside of yearly variation. This is not surprising because the *Protocalliphora* prevalence seems to be rather associated with habitat disruption than the metal levels *per se*. More luxuriant vegetation seems to support more ample fly populations (this study; Eeva and Lehikoinen, 1994), but despite reduced emissions the recovery of forest floor vegetation

Table 1. Generalized linear models (GLM) for the prevalence (% nests infested) and intensity (numbers/infected nests) of pupae of two parasitic fly species in the nests of *F. hypoleuca* during 2006–2012. Terms left in the final model are shown in bold

	Protocalliphora sp. prevalence[a] n = 639		*Ornithomyia* sp. prevalence[a] n = 639		*Protocalliphora* sp. intensity[b] n = 164		*Ornithomyia* sp. intensity[b] n = 228	
	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P
Year	**$3.39_{6,631}$**	**0.0027**	**$14.6_{6,632}$**	**<0.0001**	**$6.52_{6,157}$**	**<0.0001**	**$2.83_{6,215}$**	**0.011**
Pollution zone	**$4.49_{1,631}$**	**0.035**	$0.09_{1,631}$	0.76	$2.89_{1,156}$	0.091	$0.16_{1,215}$	0.69
Year × zone	$1.33_{6,625}$	0.24	$1.09_{6,625}$	0.36	$0.76_{6,150}$	0.60	**$2.27_{5,215}$**	**0.048**

[a] GLM with binomial error distribution and logit link function.
[b] GLM with negative binomial error distribution and log link function.

Table 2. Generalized linear mixed models (GLMM)[a] for explaining the prevalence of pupae of two parasitic flies, *Protocalliphora* sp. and *Ornithomyia* sp. in the nests of *Ficedula hypoleuca* (n = 634) during 2006–2012. Terms left in the final model are shown in bold

	Protocalliphora sp.		*Ornithomyia* sp.	
	F_{df}	P	F_{df}	P
Pollution zone	**$4.46_{1,628}$**	**0.035**	**$3.69_{1,628}$**	**0.055**
Standardized hatching date	$0.21_{1,627}$	0.64	**$13.0_{1,628}$**	**0.0003**
Brood size	$0.09_{1,626}$	0.76	$3.59_{1,627}$	0.059
Temperature	**$6.19_{1,34.3}$**	**0.018**	**$4.72_{1,310}$**	**0.031**
Forest floor vegetation	**$6.00_{1,628}$**	**0.015**	$0.75_{1,626}$	0.39
Occupation of nest boxes	$2.87_{1,628}$	0.091	**$12.6_{1,628}$**	**0.0004**
Zone × hatching date	$0.43_{1,625}$	0.51	$0.53_{1,622}$	0.47
Zone × brood size	$0.16_{1,624}$	0.69	$0.46_{1,623}$	0.50
Zone × temperature	$<0.01_{1,622}$	0.97	**$4.37_{1,628}$**	**0.037**
Zone × vegetation	$<0.01_{1,623}$	0.95	$0.89_{1,624}$	0.35
Zone × occupation	**$4.51_{1,628}$**	**0.034**	$1.29_{1,625}$	0.26

[a] GLMM with binomial error distribution and logit link function. Year was included in the model as a random factor.

is a slow process (Salemaa *et al*. 2001). Our study therefore suggests that lower *Protocalliphora* prevalence in the polluted environment is not likely due to flies suffering from metal toxicity or due to birds suffering from metal-related deterioration of parasite defence but rather because of the suboptimal habitat for the parasite.

Adult *Protocalliphora* are known to feed on flowers of *Vaccinium* species (Bennett and Whitworth, 1991; Rognes, 1991). The abundance of some common dwarf shrubs in our study area, such as *V. vitis-idaea*, decrease towards the copper smelter together with a general decrease in vegetation coverage and number of plant species (Salemaa *et al*. 2001). Less abundant food sources might therefore decrease *Protocalliphora* abundance in the polluted environment. Instead, adult *Ornithomyia* only feed on blood (Corbet, 1956; Grunin, 1989) and therefore do not show a corresponding association to forest floor vegetation quality. Our result on smaller prevalence of *Protocalliphora* in the polluted area is an opposite to

the one observed around a Russian copper smelter (Revda), where proportion of infested *F. hypoleuca* chicks and intensity of infestation were higher in the polluted areas than in the unpolluted reference areas (Belskii *et al*. 2005). Belskii *et al*. (2005), however, report that the fly larvae were found under the skin of nestlings, which is not typical for *Protocalliphora*, and their result likely applies to a closely related fly, *Trypocalliphora* sp., as was later identified from a sample collected from *F. hypoleuca* nestling at their study site (E. Belskii, personal communication). Different parasite species show variable trends in abundance also within our study area, since while *Protocalliphora* decreased and *Ornithomyia* generally showed no change, the numbers of ectoparasitic fleas (*Ceratophyllus gallinae*) increased in *F. hypoleuca* nests towards the pollution source (Eeva *et al*. 1994). Taken together, pollution-related trends are species-specific and there seems to be no general abundance pattern among avian ectoparasites in relation to environmental pollution.
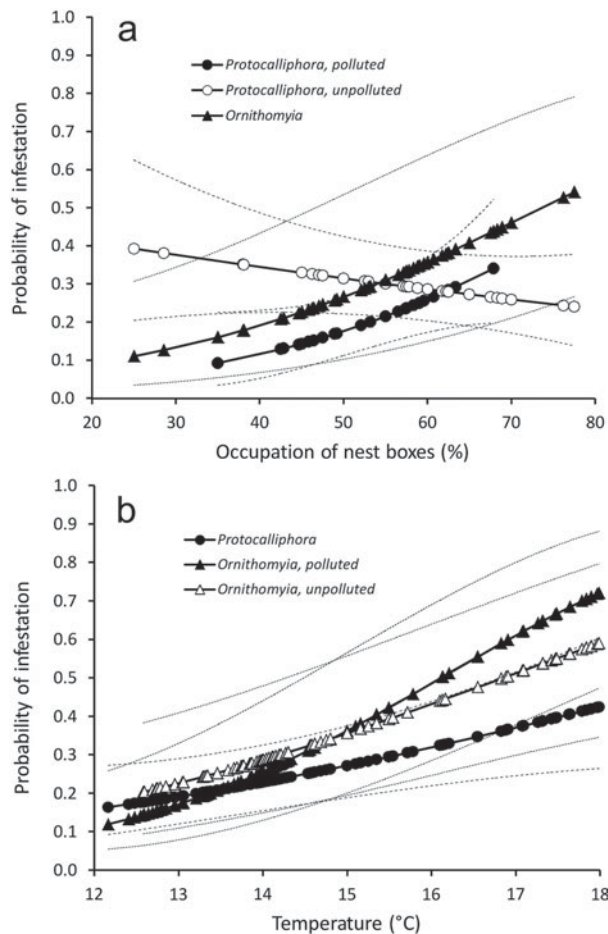
Fig. 3. The prevalence (probability ± 95% CL) of parasitic fly pupae in the nests of *Ficedula hypoleuca* (n = 634) as a function of (a) host density (occupation rate of nest boxes) and (b) nestling time mean temperatures. Circles: *Protocalliphora*; triangles: *Ornithomyia*. Data points are predicted values from logistic regression. For the sake of clarity the other explanatory factors (see Table 2) were omitted from the logistic regression model to plot the predicted prevalence values against density or temperature.

Table 3. Generalized linear mixed model (GLMM)[a] for explaining the breeding success (fledglings/hatchlings) of *Ficedula hypoleuca* (n = 619) during 2006–2012. Terms left in the final model are shown in bold

	Breeding success	
	F_{df}	P
Pollution zone	**$15 \cdot 7_{1,616}$**	**< 0·0001**
Standardized hatching date	$3 \cdot 45_{1,613}$	0·064
Temperature	$1 \cdot 72_{1,614}$	0·19
Protocalliphora pupae	$1 \cdot 39_{1,612}$	0·24
***Ornithomyia* pupae**	**$5 \cdot 66_{1,616}$**	**0·018**
Zone × Protocalliphora	$0 \cdot 81_{1,611}$	0·37
Zone × Ornithomyia	$2 \cdot 97_{1,615}$	0·086

[a] GLMM with binomial error distribution and logit link function, with events/trials type dependent variable (events = fledgling number; trials = hatchling numbers). Year was included in the model as a random factor.
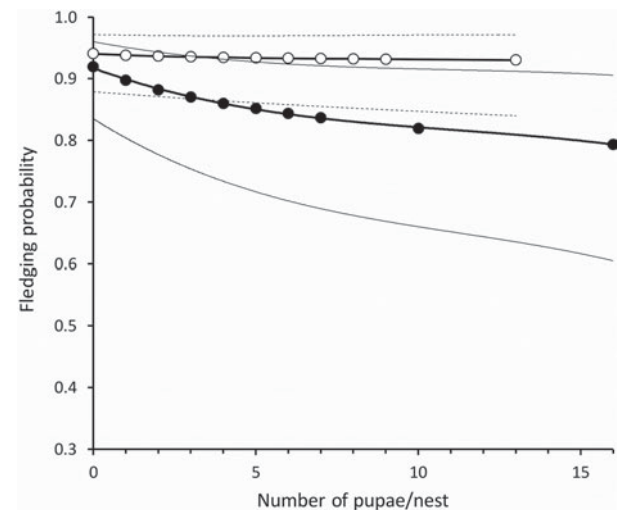

Fig. 4. The association between fledging probability (± 95% CL) and number of *Ornithomyia* pupae in the nests of *Ficedula hypoleuca* (n = 619). Open circles: unpolluted zone; filled circles: polluted zone. Probabilities are based on generalized linear model with zone, number of pupae and zone × *n* of pupae as explanatory factors (year is included in the model as a random factor, but common predicted values and confidence limits are presented for all years).

Both ectoparasitic flies showed higher prevalence when nestling time temperatures were high. Being poikilothermic animals the emergence time and flying activity of these insects can be expected to be temperature-related. Senar *et al.* (1994) showed that prevalence of bird louse flies (*Ornithoica* sp. and *Ornithomyia* sp.) in adult serins (*Serinus* sp.) was positively correlated with preceding temperatures and they considered weather variables (temperature and rainfall) as important determinants of annual variations in prevalence. For a moose-dwelling hippoboscid species, *Lipoptena cervi*, it was found that low summer temperatures prolonged the developmental periods and led to reduced emergence probabilities and reduced host search times (Härkönen *et al.* 2010). Although bird-dwelling hippoboscids, such as *Ornithomyia*, supposedly experience relatively steady ambient temperatures during their on-host period we may expect that cold and perhaps rainy periods reduce their flight activity and dispersal, and may produce such seasonal and yearly variation in their prevalence that was apparent in our data. The response of *Ornithomyia* number to temperature was further slightly stronger in the polluted area. This may be because of slightly higher daily temperatures at polluted sites (daily maximum in June being 1·3 °C higher; T. Eeva, unpublished data) due to the relatively sparse forests enabling the ground to be more exposed to sunlight. The same explanation might apply to *Protocalliphora*, though

for them the relationship between prevalence and temperature was much weaker (see also Rogers *et al*. 1991; Dawson *et al*. 2005). Temperature-dependent annual prevalence of *Protocalliphora* was also noted in a *F. hypoleuca* population by Merino and Potti (1996). A positive association between prevalence and summer temperature was further observed for another parasitic calliphorid, *Trypocalliphora braueri* (Pavel *et al*. 2008). Temperature dependence of ectoparasite prevalence may change host–parasite relationships along changing climate. Advanced phenology and increased prevalence and intensity of *Ornithomyia* were indeed recently reported in a long-term study on barn swallows (*Hirundo rustica*) (Møller, 2010).

The prevalence of *Ornithomyia* further increased in the course of the breeding season, late broods getting parasitized more heavily. This association might partly be explained by increasing average temperatures in the course of the breeding season. However, in our data there was a great deal of independent variation between breeding times and temperatures and we believe that both factors are involved. Late summer peak prevalence seems to be typical of *Ornithomyia* and some other hippoboscids (Corbet, 1956; McClure, 1984; Senar *et al*. 1994; but see Walker and Rotherham, 2010*a*) and higher prevalence among late breeding birds may just reflect the seasonal phenology and abundance pattern of flies but phenology of the birds may also play a role. *Ornithomyia* are not host specific but thrive on many bird species and also change host species (Corbet, 1956). For example, they parasitize the Parids (Ash and Monk, 1959; Trilar and Krčmar, 2005; own observations) that frequently breed at our nest box sites (e.g. *Parus major* and *Cyanistes caeruleus*). Nestlings of the Parids fledge *c*. 2 weeks earlier than those of *F. hypoleuca*, and while many of the Parid broods are still in their nests during the early *F. hypoleuca* broods, very few remain during the late *F. hypoleuca* broods. Since outside the nest birds can more easily remove ectoparasites from their plumage we consider it possible that a part of the *Ornithomyia* flies leave their original host bird after fledging and search for other broods still in the nestling phase, such as those of later breeding *F. hypoleuca*. This would increase prevalence in late broods. Late broods might also show lower immune defence against parasites and consequently suffer from higher parasite load (Dubiec and Cichon, 2005).

We found that the *Ornithomyia* prevalence was higher when *F. hypoleuca* breeding density was high. When parasite transmission is a function of direct contacts, the prevalence is expected to increase with population density (Arneberg, 2001; McCallum *et al*. 2001). The host switch behaviour of *Ornithomyia* is poorly known and we are not aware of any other studies relating host population density and *Ornithomyia* prevalence. We, however,

believe that dense *F. hypoleuca* populations could promote the transmission of flies between individuals. *Ficedula hypoleuca* males typically check and visit several closely locating nest boxes, a part of which are occupied by the same or different bird species. Such behaviour could increase parasite transmission in a density-dependent manner. Instead, *Protocalliphora* flies are not carried by birds and we did not find a general association between their prevalence and host density but the association differed between polluted and unpolluted sites, numbers decreasing with decreasing bird population density only in the polluted area. This might be due to the fact that the sites where long-term pollution has resulted in most scanty ground layer vegetation and relatively low *Protocalliphora* populations also tend to be less attractive for *F. hypoleuca* and many other forest passerines (Eeva *et al*. 2012*a*). The lack of direct association between *Protocalliphora* prevalence and host density was apparent also among colonial and more dispersed populations of tree swallows, *Tachycineta bicolor* (Rogers *et al*. 1991).

Breeding success was slightly higher in those *F. hypoleuca* nests that were not parasitized by *Ornithomyia* or only contained a few pupae. However, the numbers of pupae in infested broods were generally low in our study population, only 9% of nests having more than five pupae (or more than one pupa/nestling), though one should recall that some pupae may have been laid outside the nest. Several studies have reported negative effects of *Protocalliphora* infestation on blood parameters (reduced haematocrit and haemoglobin levels, increased leukocyte concentration) but generally they increased nestling mortality only when occurring in very high numbers (Pinkowski, 1977; Gold and Dahlsten, 1983; Sabrosky *et al*. 1989; Whitworth and Bennett, 1992; Merino and Potti, 1995; Saino *et al*. 1998; Puchala, 2004; Hannam, 2006). Although *Protocalliphora* larvae are larger than those of *Ornithomyia*, acute detrimental effects have been observed only when their numbers have been much higher than in our study. Therefore we consider it unlikely that relatively low numbers of smaller *Ornithomyia* larvae could have had direct effects on nestling mortality via the amount of blood consumed. Instead, the association between *Ornithomyia* intensity and breeding success might be due to broods in poor condition being parasitized more heavily. Negative correlation between parasite numbers and offspring survival might also arise if lower-quality parents (e.g. in terms of parental care) are more heavily parasitized than good-quality parents (Gallizzi *et al*. 2008; Heylen *et al*. 2009). Correlational and experimental studies have not found any association between the reproductive success of swifts (*Apus* sp.) and the numbers of parasitic hippoboscid flies, *Crataerina* sp., suggesting generally low virulence (Lee and Clayton, 1995; Tompkins *et al*. 1996;

Walker and Rotherham, 2010*b*). Hippoboscids, however, serve as intermediate hosts for some avian blood protozoans (e.g. *Haemoproteus* sp. and *Trypanosoma* sp.), and this way they could have indirect/delayed effects on nestling condition or mortality (Baker, 1967; Martinez-De La Puente *et al.* 2010).

In conclusion, our study shows that environmental pollution decreased the prevalence of ectoparasitic *Protocalliphora* via changed habitat quality but did not affect the prevalence of another ectoparasitic fly, *Ornithomyia*. Both ectoparasites were more common in warm breeding seasons, emphasizing the potential of climate change to modify host–parasite relationships. The prevalence of *Ornithomyia* was further dependent on host population density and showed a marked seasonal abundance pattern, the prevalence being highest in dense *F. hypoleuca* populations and late broods. We found a negative relationship between *Ornithomyia* number and nestling survival and this association was somewhat stronger in the polluted area, though the correlative nature of our study does not allow confirming causality of this association. In general, the numbers of ectoparasitic flies in our study population are rather low. However, despite overall weak effect of parasites on survival, the possible delayed and/or sublethal effects of these ectoparasites call for further studies. Our results suggest that pollution-related effects on avian ecto-parasite numbers are species-specific and reflect habitat changes rather than direct toxic effects of heavy metals.

REFERENCES

Arneberg, P. (2001). An ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. *Ecography* **24**, 352–358.

Ash, J. S. and Monk, J. F. (1959). A collection of *Ornithomyia* spp. (Dipt., Hippoboscidae) from Oxfordshire. *Entomologists' Monthly Magazine* **95**, 80–81.

Baker, J. R. (1967). A review of the role played by the Hippoboscidae (Diptera) as vectors of endoparasites. *Journal of Parasitology* **53**, 412–418.

Belskii, E. A., Lugas'kova, N. V. and Karfidova, A. A. (2005). Reproductive parameters of adult birds and morphophysiological characteristics of chicks in the pied flycatcher (*Ficedula hypoleuca* Pall.) in technogenically polluted habitats. *Russian Journal of Ecology* **36**, 329–335.

Bennett, G. F. and Whitworth, T. L. (1991). Studies on the life history of some species of *Protocalliphora* (Diptera: Calliphoridae). *Canadian Journal of Zoology* **69**, 2048–2058.

Berglund, Å. M. M., Ingvarsson, P. K., Danielsson, H. and Nyholm, N. E. I. (2010). Lead exposure and biological effects in pied flycatchers (*Ficedula hypoleuca*) before and after the closure of a lead mine in northern Sweden. *Environmental Pollution* **158**, 1368–1375.

Berglund, Å. M. M., Koivula, M. J. and Eeva, T. (2011). Species- and age-related variation in metal exposure and accumulation of two passerine bird species. *Environmental Pollution* **159**, 2368–2374.

Berglund, Å. M. M., Rainio, M. and Eeva, T. (2012). Decreased metal accumulation in passerines as a result of reduced emissions. *Environmental Toxicology and Chemistry* **31**, 1317–1323.

Blanar, C. A., Munkittrick, K. R., Houlahan, J., MacLatchy, D. L. and Marcogliese, D. J. (2009). Pollution and parasitism in aquatic animals: a meta-analysis of effect size. *Aquatic Toxicology* **93**, 18–28.

Brotons, L., Magrans, M., Ferrus, L. and Nadal, J. (1998). Direct and indirect effects of pollution on the foraging behaviour of forest passerines during the breeding season. *Canadian Journal of Zoology* **76**, 556–565.

Clayton, D. H. and Moore, J. (1997). *Host–Parasite Evolution, General Principles and Avian Models*. Oxford University Press, New York, USA.

Corbet, G. B. (1956). The life-history and host-relations of a Hippoboscid fly *Ornithomyia fringillina* Curtis. *Journal of Animal Ecology* **25**, 403–420.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R. and Eens, M. (2000). Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? *Archives of Environmental Contamination and Toxicology* **39**, 541–546.

Dawson, R. D., Hillen, K. K. and Whitworth, T. L. (2005). Effects of experimental variation in temperature on larval densities of parasitic *Protocalliphora* (Diptera: calliphoridae) in nests of tree swallows (Passeriformes: Hirundinidae). *Environmental Entomology* **34**, 563–568.

Dubiec, A. and Cichon, M. (2005). Seasonal decline in nestling cellular immunocompetence results from environmental factors – an experimental study. *Canadian Journal of Zoology–Revue Canadienne de Zoologie* **83**, 920–925.

Eeva, T. and Lehikoinen, E. (1994). Effects of air pollution on the breeding of pied flycatcher *Ficedula hypoleuca* and great tit *Parus major*. *Journal für Ornithologie* **135**, 236.

Eeva, T., Lehikoinen, E. and Nurmi, J. (1994). Effects of ectoparasites on breeding success of great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) in an air pollution gradient. *Canadian Journal of Zoology* **72**, 624–635.

Eeva, T., Lehikoinen, E. and Pohjalainen, T. (1997). Pollution-related variation in food supply and breeding success in two hole-nesting passerines. *Ecology* **78**, 1120–1131.

Eeva, T., Ryömä, M. and Riihimäki, J. (2005). Pollution-related changes in diets of two insectivorous passerines. *Oecologia* **145**, 629–639.

Eeva, T., Belskii, E., Gilyazov, A. S. and Kozlov, M. V. (2012*a*). Pollution impacts on bird population density and species diversity at four non-ferrous smelter sites. *Biological Conservation* **150**, 33–41.

Eeva, T., Rainio, M., Kanerva, M. and Salminen, J.-P. (2012*b*). Plasma carotenoid levels are not directly related to heavy metal exposure or reproductive success in three insectivorous passerines. *Environmental Toxicology and Chemistry* **31**, 1363–1369.

Flückiger, W., Braun, S. and Hiltbrunner, E. (2002). Effects of air pollutants on biotic stress. In *Air Pollution and Plant Life* (ed. Bell, J. N. B. and Treshow, M.), pp. 379–406. John Wiley & Sons, Chichester, UK.

Gallizzi, K., Alloitteau, O., Harrang, E. and Richner, H. (2008). Fleas, parental care, and transgenerational effects on tick load in the great tit. *Behavioral Ecology* **19**, 1225–1234.

Gentes, M. L., Whitworth, T. L., Waldner, C., Fenton, H. and Smits, J. E. (2007). Tree swallows (*Tachycineta bicolor*) nesting on wetlands impacted by oil sands mining are highly parasitized by the bird blow fly *Protocalliphora* spp. *Journal of Wildlife Diseases* **43**, 167–178.

Gold, C. S. and Dahlsten, D. L. (1983). Effects of parasitic flies (*Protocalliphora* spp.) on nestling mountain and chestnut-backed chickadees. *Wilson Bulletin* **95**, 560–572.

Grunin, K. Ya. (1989). Family Hippoboscidae. In *Keys to the Insects of the European Part of the USSR*, Volume V. Diptera and Siphonaptera. Part II (ed. Bei-Bienko, G. Ya. and Steyskal, G. C.), pp. 979–986. E.J. Brill, Leiden, the Netherlands.

Hannam, K. (2006). Ectoparasitic blow flies (*Protocalliphora* sp.) and nestling Eastern Bluebirds (*Sialia sialis*): direct effects and compensatory strategies. *Canadian Journal of Zoology–Revue Canadienne de Zoologie* **84**, 921–930.

Härkönen, L., Härkönen, S., Kaitala, A., Kaunisto, S., Kortet, R., Laaksonen, S. and Ylönen, H. (2010). Predicting range expansion of an ectoparasite – the effect of spring and summer temperatures on deer ked *Lipoptena cervi* (Diptera: Hippoboscidae) performance along a latitudinal gradient. *Ecography* **33**, 906–912.

Heylen, D., Adriaensen, F., Dauwe, T., Eens, M. and Matthysen, E. (2009). Offspring quality and tick infestation load in brood rearing great tits *Parus major*. *Oikos* **118**, 1499–1506.

Janssens, E., Dauwe, T., Pinxten, R. and Eens, M. (2003). Breeding performance of great tits (*Parus major*) along a gradient of heavy metal pollution. *Environmental Toxicology and Chemistry* **22**, 1140–1145.

Kalliola, R. (1973). Suomen kasvimaantiede. WSOY.

Khan, R. A. and Thulin, J. (1991). Influence of pollution on parasites of aquatic animals. *Advances in Parasitology* **30**, 201–238.

Kiikkilä, O. (2003). Heavy-metal pollution and remediation of forest soil around the Harjavalta Cu-Ni smelter, in SW Finland. *Silva Fennica* **37**, 399–415.

Kozlov, M., Zvereva, E. L. and Zverev, V. (2009). *Impacts of Point Polluters on Terrestrial Biota*. Springer, Dordrecht, the Netherlands.

Lambrechts, M. M., Adriaensen, F., Ardia, D. R., Artemyev, A. V., Atiénzar, F., Banbura, J., Barba, E., Bouvier, J.-C., Camprodon, J., Cooper, C. B. et al. (2010). The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. *Acta Ornithologica* **45**, 1–26.

Lee, P. L. M. and Clayton, D. H. (1995). Population biology of swift (*Apus apus*) ectoparasites in relation to host reproductive success. *Ecological Entomology* **20**, 43–50.

Loye, J. E. and Zuk, M. (1991). *Bird–Parasite Interactions: Ecology, Evolution and Behaviour*. Oxford University Press, Oxford, UK.

Lundberg, A. and Alatalo, R. V. (1992). *The Pied Flycatcher*. T. & A. D. Poyser, London, UK.

Lürling, M. and Scheffer, M. (2007). Info-disruption: pollution and the transfer of chemical information between organisms. *Trends in Ecology and Evolution* **22**, 374–379.

Maa, T. C. (1969). A revised checklist and concise host index of Hippoboscidae (Diptera). *Pacific Insects Monographs (Honolulu: Bishop Museum, Honolulu, Hawaii)* **20**, 261–299.

Martinez-De La Puente, J., Merino, S., Tomas, G., Moreno, J., Morales, J., Lobato, E., Garcia-Fraile, S. and Belda, E. J. (2010). The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. *Biology Letters* **6**, 663–665.

McCallum, H., Barlow, N. and Hone, J. (2001). How should pathogen transmission be modelled? *Trends in Ecology and Evolution* **16**, 295–300.

McClure, H. E. (1984). The occurrence of Hippoboscid flies on some species of birds in Southern California. *Journal of Field Ornithology* **55**, 230–240.

Merino, S. and Potti, J. (1995). Mites and blowflies decrease growth and survival in nestling pied flycatchers. *Oikos* **73**, 95–103.

Merino, S. and Potti, J. (1996). Weather dependent effects of nest ectoparasites on their bird hosts. *Ecography* **19**, 107–113.

Møller, A. P. (2010). Host–parasite interactions and vectors in the barn swallow in relation to climate change. *Global Change Biology* **16**, 1158–1170.

Pavel, V., Chutný, B., Petrusková, T. and Petrusek, A. (2008). Blow fly *Trypocalliphora braueri* parasitism on Meadow Pipit and Bluethroat nestlings in Central Europe. *Journal of Ornithology* **149**, 193–197.

Petersen, F. T., Damgaard, J. and Meier, R. (2007). DNA Taxonomy: how many DNA sequences are needed for solving a taxonomic problem?

The case of two parapatric species of louse flies (Diptera: Hippoboscidae: *Ornithomya* Latreille, 1802). *Arthropod Systematics and Phylogeny* **62**, 119–125.

Pinkowski, B. C. (1977). Blowfly parasitism of eastern bluebirds in natural and artificial nest sites. *Journal of Wildlife Management* **41**, 272–276.

Puchala, P. (2004). Detrimental effects of larval blow flies (*Protocalliphora azurea*) on nestlings and breeding success of tree sparrows (*Passer montanus*). *Canadian Journal of Zoology–Revue Canadienne de Zoologie* **82**, 1285–1290.

Rogers, C. A., Robertson, R. J. and Stutchbury, B. J. (1991). Patterns and effects of parasitism by *Protocalliphora sialia* on tree swallow nestlings. In *Bird-Parasite Interactions – Ecology, Evolution and Behaviour* (ed. Loye, J. E.), pp. 123–139. Oxford University Press, Oxford, UK.

Rognes, K. (1991). *Blowflies (Diptera, Calliphoridae) of Fennoscandia and Denmark*. E. J. Brill/Scandinavian Science Press, Leiden, The Netherlands.

Sabrosky, C. W., Bennett, G. F. and Whitworth, T. L. (1989). *Bird Blow Flies (Protocalliphora) in North America (Diptera: Calliphoridae), with Notes on the Palearctic Species*. Smithsonian Institution Press, Washington, DC, USA.

Saino, N., Calza, S. and Møller, A. P. (1998). Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow, *Hirundo rustica*, nestlings. *Oikos* **81**, 217–228.

Salemaa, M., Vanha-Majamaa, I. and Derome, J. (2001). Understorey vegetation along a heavy-metal pollution gradient in SW Finland. *Environmental Pollution* **112**, 339–350.

SAS (2008). *SAS/STAT ® 9.2 User's Guide*. SAS Institute Inc., Cary, NC.

Senar, J. C., Copete, J. L., Domenech, J. and Vonwalter, G. (1994). Prevalence of louse-flies Diptera, Hippoboscidae parasiting a cardueline finch and its effect on body condition. *Ardea* **82**, 157–160.

Tompkins, D. M., Jones, T. and Clayton, D. H. (1996). Effect of vertically transmitted ectoparasites on the reproductive success of swifts (*Apus apus*). *Functional Ecology* **10**, 733–740.

Trilar, T. and Krčmar, S. (2005). Contribution to the knowledge of louse flies of Croatia (Diptera: Hippoboscidae). *Natura Croatica* **14**, 131–140.

Walker, M. D. and Rotherham, I. D. (2010*a*). Characteristics of *Crataerina pallida* (Diptera: Hippoboscidae) populations; a nest ecto-parasite of the common swift, *Apus apus* (Ayes: Apodidae). *Experimental Parasitology* **126**, 451–455.

Walker, M. D. and Rotherham, I. D. (2010*b*). The breeding success of common swifts *Apus apus* is not correlated with the abundance of their louse fly *Crataerina pallida* parasites. *Bird Study* **57**, 504–508.

Weis, J. S., Bergey, L., Reichmuth, J. and Candelmo, A. (2011). Living in a contaminated estuary: behavioral changes and ecological consequences for five species. *BioScience* **61**, 375–385.

Whitworth, T. L. and Bennett, G. F. (1992). Pathogenicity of larval *Proto-calliphora* (Diptera: Calliphoridae) parasitizing nestling birds. *Canadian Journal of Zoology* **70**, 2184–2191.

Williams, H. H. and Mackenzie, K. (2003). Marine parasites as pollution indicators: an update. *Parasitology* **126**, S27–S41.