Nest design and the abundance of parasitic *Protocalliphora* blow flies in two hole-nesting passerines[1]

Vladimír REMEŠ[2], Laboratory of Ornithology, Palacky University, Tr. Svobody 26, CZ-771 46 Olomouc,
Czech Republic, e-mail: remes@prfnw.upol.cz
Miloš KRIST, Laboratory of Ornithology, Palacky University, Tr. Svobody 26, CZ-771 46 Olomouc, Czech
Republic, and Museum of Natural History, nam. Republiky 5, CZ-771 73 Olomouc, Czech Republic.

Abstract: Ectoparasites dwelling in bird nests regularly reduce reproductive success and condition of breeding birds. Thus, establishing the factors that determine the abundance of ectoparasites is important for better understanding of reproductive trade-offs and life history evolution in birds. A recent hypothesis states that interspecific differences in the abundance of ectoparasites may be caused by nest composition. For example, great tits (*Parus major*) have nests made of mosses and fur, whereas *Ficedula* flycatchers have nests made of grasses, bast, and bark, and tits are more infested by nest-dwelling ectoparasites than flycatchers. We swapped nests between pairs of great tits and collared flycatchers (*F. albicollis*) during egg-laying or early incubation and counted parasitic *Protocalliphora* blow flies at the end of breeding to test this hypothesis experimentally. We controlled statistically for habitat (oak *versus* spruce forest), brood size, season, year, and mean nestling weight before fledging. We found a significant effect of bird species (tit > flycatcher), habitat (oak > spruce), and year. There was no effect of nest type. Consequently, the hypothesis ascribing the different abundance of ectoparasites in great tits and collared flycatchers to different nest composition was not supported by our study.
Keywords: blow fly, ectoparasites, *Ficedula*, nest design, *Parus*, *Protocalliphora*.

Résumé : Les ectoparasites qui infestent les nids réduisent souvent le succès reproducteur et la condition physique des oiseaux nicheurs. Il est donc important d'identifier les facteurs qui influencent leur abondance pour mieux comprendre les caractéristiques de la reproduction et l'évolution du cycle vital chez les oiseaux. Selon une hypothèse récente, les différences interspécifiques dans l'abondance des ectoparasites pourraient être associées aux matériaux de construction des nids. Par exemple, les nids de la mésange charbonnière (*Parus major*) sont fabriqués de mousses et de poils alors que ceux des gobemouches (*Ficedula* spp.) sont faits de graminées, de liber et d'écorce. Or, on sait que les mésanges sont plus infestées par des ectoparasites que les gobemouches. Nous avons échangé les nids construits par des couples de mésanges charbonnières avec ceux construits par des gobemouches à collier (*F. albicollis*) pendant la ponte ou au début de l'incubation. Nous avons par la suite compté les mouches du genre *Protocalliphora* à la fin de la nidification pour tester l'hypothèse mentionnée plus haut. Nous avons pu contrôler de façon statistique l'habitat (forêt de chênes ou d'épicéas), la taille de la couvée, la saison, l'année et le poids moyen des jeunes avant l'envol. Nous avons trouvé un effet significatif pour l'espèce d'oiseau (mésange > gobemouche), l'habitat (chênes > épicéas) et l'année. Le type de nid n'a eu pour sa part aucun effet sur les ectoparasites. En conséquence, l'hypothèse d'un lien entre l'abondance d'ectoparasites et les matériaux de construction des nids n'est pas supporté par les résultats de cette étude.
Mots-clés : design des nids, ectoparasites, *Ficedula*, mouches, *Parus*, *Protocalliphora*.

Nomenclature: Sabrosky, Bennett & Whitworth, 1989; Cramp & Perrins, 1993.

Introduction

Ectoparasites dwelling in bird nests and feeding on the blood of nestlings and adults are an important group of parasites. In hole-nesting birds they include fleas (Siphonaptera), flies (Diptera), and mites (Acarina). Ectoparasites can cause lowered breeding performance and nest desertion in adults (Oppliger, Richner & Christe, 1994), negatively affect growth and condition of nestlings (Eeva, Lehikoinen & Nurmi, 1994; Merino & Potti, 1995; Puchala, 2004; review in Møller, Allander & Dufva, 1990), and reduce lifetime reproductive success of hosts (Fitze, Tschirren & Richner, 2004). Moreover, they also serve as vectors of internal parasites and bacterial and viral diseases (Bowman *et al.*, 1997). Thus, knowledge of the factors that the determine abundance of these ectoparasites in nests is critical for better understanding of reproductive trade-offs and life history evolution in birds (Clayton & Moore, 1997).

Abundance of nest-dwelling ectoparasites varies significantly among individuals within a given bird host species, but even bigger variation in both prevalence and parasite abundance is found between sympatric host species (Bennett & Whitworth, 1992; Whitworth & Bennett, 1992). One of the hypotheses suggested to explain interspecific differences in ectoparasite load posits that the differences are caused by differences in nest design (Bauchau, 1998), such as differences in details of nest construction and nest composition (Hansell, 2000). Fresh plant material in the

nest, for example, may have negative effects on parasite abundance (Clark & Mason, 1985; Petit *et al.,* 2002; but see Dawson, 2004). Nest design may thus affect (1) demography of ectoparasite populations within nests (larval mortality, competition, growth), because demography is driven by various parameters of the living environment (*e.g.,* humidity; Heeb, Kölliker & Richner, 2000) and the living environment depends on nest composition, and/or (2) attractiveness of the particular nest type for laying/dispersing to females of parasites, which may cue on specific features of the nest.

In this study we tested the "nest design" hypothesis on great tits (*Parus major*) and collared flycatchers (*Ficedula albicollis*). The two species build nests of very different composition: while tit nests are composed of moss and feather/hair, flycatcher nests consist of dry grass, bast, and pieces of bark (Cramp & Perrins, 1993). At the same time, the abundance of larvae of parasitic flies (*Protocalliphora* spp.) is regularly higher in nests of the great tit than in *Ficedula* flycatchers (Bauchau, 1998). We experimentally switched nests between great tit and collared flycatcher pairs and followed the effects of this treatment on the abundance of parasitic flies in nests. In this way we were able to separate independent effects of nest design and species. We also statistically controlled for a number of other factors, including habitat, year, season, brood size, and mean nestling weight before fledging.

In line with the "nest design" hypothesis we hypothesized that the lower number of ectoparasites in *Ficedula* flycatchers is caused by the composition of their nests (presence of bast and bark with toxic secondary compounds; Pearce, 1996) and, thus, predicted that there would be fewer parasitic flies in the *Ficedula* nests regardless of the host species actually dwelling in the nest.

Methods

Great tit and collared flycatcher are small, hole-nesting, insectivorous passerines breeding widely in various types of woodlands. They readily accept nest-boxes for breeding. Great tits are year-round residents, whereas collared flycatchers are long-distance migrants wintering in Africa. These two species differ in brood size [great tit: median 11 (range 7–14), collared flycatcher: 6 (4–7), timing of breeding (great tits start breeding at mid-April, collared flycatchers at the end of April), body mass (great tit: *ca* 18 g; collared flycatcher: *ca* 14 g, V. Remeš & M. Krist, unpubl. data)]. Otherwise their breeding ecology is similar.

This study was conducted in 2002-2003 in the Velký Kosíř area in the eastern Czech Republic (49° 32' N, 17° 04' E, 300-450 m asl). We studied great tits and collared flycatchers on six nest-box plots, of which three were placed in spruce (*Picea abies*) and the other three in an oak (*Quercus* spp.) forest, in both cases interspersed with birch (*Betula pendula*) and pine (*Pinus silvestris*). Each plot had 50-90 nest-boxes. In early spring (before tits started nest building), nest-boxes were checked and cleaned (old nests were removed). From mid-April to mid-June, as a part of a larger study, we followed basic breeding biology of both species.

To be able to separate independent effects of nest design and species *per se* on the abundance of nest-dwelling flies, we switched nests between great tit and collared flycatcher pairs. As a control treatment, we swapped nests between pairs of the same species. Thus, nests of all hosts were swapped. Two experimental (2 × flycatcher–tit) and two control (tit–tit, flycatcher–flycatcher) manipulations were made on the same day each time. We strove to make manipulations as early as possible in the breeding cycle. However, tits breed earlier than flycatchers. Consequently, manipulations on flycatcher nests were made during egg laying, whereas in tits we made the manipulations up to the fourth day after the clutch was complete (mean ± SD number of days from laying of the first egg to nest manipulation was 2.5 ± 2.97 in flycatchers and 12.5 ± 1.86 in tits). We increased our sample for flycatchers by also using some nests of tits in late incubation. In these experimental pairs, we followed nest-type effects only in the flycatcher, not in the great tit.

In the week following fledging, we collected nests and placed them into plastic bags that were sealed so that no fly could escape. We collected only nests from which at least one young had fledged. Within four weeks of collection, we opened the bags, took the nests to pieces, and counted the number of larvae, pupae, and adult flies (if they had emerged from pupae in the meantime). Time between fledging of young and collection of nests was not the same for all the nests. However, collection of nests within one week of fledging is a standard procedure in ectoparasite research (Eeva, Lehikoinen & Nurmi, 1994; Birdblowfly.com). More importantly, even when some flies had dispersed immediately after fledging, before a nest was collected, they could be counted by counting empty pupae, which are very conspicuous and cannot be overlooked.

Two species of parasitic flies were identified in the nests: *Protocalliphora azurea* and *P. falcozi*. Both species are regularly found in the nests of European cavity nesters (Hurtrez-Boussès *et al.*, 1997; Wesołowski, 2001). All *Protocalliphora* species overwinter as adults and do not lay eggs in host nests until young birds hatch (Gold & Dahlsten, 1989; Sabrosky, Bennett & Whitworth, 1989). Thus, our experimental procedure of switching the nests during egg laying or early incubation was sufficient to separate independent effects of nest type. The two species of blow flies were lumped together for further analyses for two reasons (see also Hurtrez-Boussès et al., 1997). First, all *Protocalliphora* flies (except *P. braueri*; Eastman, Johnson & Kermott, 1989) are intermittent feeders that feed on the blood of nestlings and in the meantime dwell in the nest substrate (Sabrosky, Bennett & Whitworth, 1989). Moreover, *P. azurea* and *P. falcozi* are of similar body size (9-11 mm and 8-10 mm, respectively; Grunin, 1970), so their effects on hosts can be expected to be similar. Second, it was not possible to identify all the flies to species because not all individuals emerged from pupae and to our best knowledge only adults can be identified in European *Protocalliphora* flies.

When analyzing the abundance of flies, we first fit a generalized linear model with Poisson error distribution and log link, which is usually suitable for count data. However, our data were strongly overdispersed (deviance/df = 23.26),

which is common in parasitology (Wilson & Grenfell, 1997). Thus, we used negative binomial error distribution and log link, which led to a reasonable dispersion index of data (deviance/df = 1.50). All these analyses were done in PROC GENMOD in SAS (SAS Institute, 2000). Initially, we fit a full model with the following explanatory effects: nest type (tit *versus* flycatcher nest), species (tit *versus* flycatcher), habitat (spruce *versus* oak forest), year (2002 *versus* 2003), brood size (number of hatched nestlings), season (Julian hatching date), and mean nestling weight before fledging (in grams, day 13 after hatching in flycatcher, day 15 in tit); we also included all two-way interactions between nest type and all other factors. The final model was selected by backward elimination of non-significant terms, except for the two main factors of interest (nest type and species), which were retained in the model regardless of their significance. Hatching date, brood size, and mean nestling weight were standardized by subtracting the value of a given nest from the mean of a given species (*i.e.,* the values were standardized within species). However, the results were the same with non-standardized values. Test statistics (χ^2-values) and *P*-values reported in Results for non-significant terms are from the backward elimination procedure just before the particular term (being the least significant) was removed from the model. Values for significant factors and/or factors of interest (*i.e.,* nest type and species) are from the final model.

The rationale for the inclusion of the above-mentioned variables was as follows. Nest type and species were the main factors of interest. Other factors were included as covariates to reduce unexplained variation and thus the power of the main test. Season and habitat could affect flying activity of the flies (through temperature, humidity, etc.). Brood size and mean nestling weight could affect survival and growth of larvae (by determining the amount of blood available for feeding). Alternatively, the latter factors could affect oviposition behaviour of fly females.

Results

In total, 13 experimental and 17 control tit pairs and 20 experimental and 19 control flycatcher pairs were available for the analyses. Sample sizes differ between experimental and control treatments because some nests were abandoned or depredated. There was a significant effect of species ($\chi^2 = 5.54$, $P = 0.019$), habitat ($\chi^2 = 9.00$, $P = 0.003$), and year ($\chi^2 = 5.99$, $P = 0.014$) on the abundance of parasitic *Protocalliphora* flies in nests (Figure 1). Neither nest type ($\chi^2 = 0.28$, $P = 0.595$, Figure 1) nor the interaction of nest type with species ($\chi^2 = 1.29$, $P = 0.256$, Figure 2) had a significant influence. Similarly, there was no significant effect of brood size ($\chi^2 = 0.01$, $P = 0.905$), mean nestling weight ($\chi^2 = 0.85$, $P = 0.358$), season ($\chi^2 = 0.94$, $P = 0.332$), or any interaction of nest type with other factors (all χ^2-values < 0.63, all *P*-values > 0.431).

Discussion

We experimentally tested the hypothesis that nest design is responsible for interspecific differences in ectoparasite infestation in two species of hole-nesting passerines,
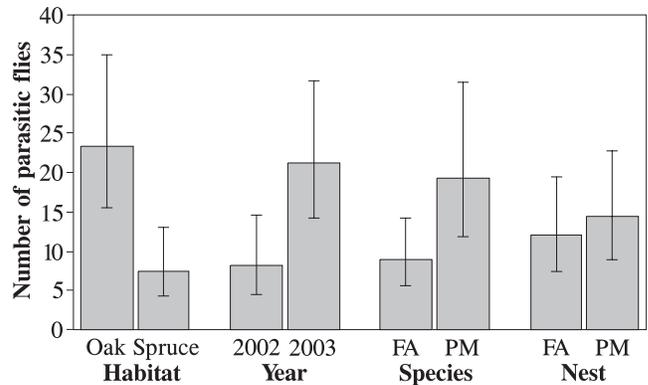

FIGURE 1. Number (least squares means ± 95% confidence limits) of parasitic *Protocalliphora* flies in the nests of great tit and collared flycatcher in relation to habitat, year, species, and nest type. FA = collared flycatcher, PM = great tit. Statistical tests are reported in Results.
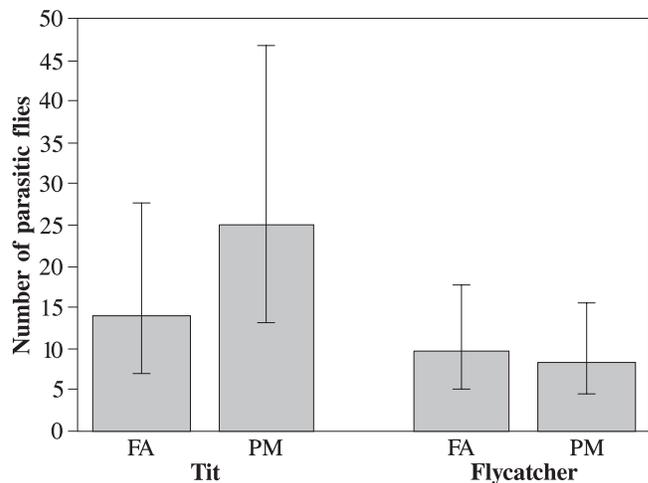

FIGURE 2. Number (least squares means ± 95% confidence limits) of parasitic *Protocalliphora* flies in the great tit (Tit) and collared flycatcher (Flycatcher) according to nest type (FA = collared flycatcher, PM = great tit). Statistical tests are reported in Results.

the great tit and the collared flycatcher (Bauchau, 1998). There was no influence of nest type on the intensity of infestation by parasitic *Protocalliphora* flies. We did, however, find a significant effect of species, habitat, and year.

Females of parasitic *Protocalliphora* flies overwinter as adults and lay their eggs in nests during the nestling phase of the host breeding cycle (Gold & Dahlsten, 1989; Sabrosky, Bennett & Whitworth, 1989). Larvae hatch within 2 d, feed on blood of nestlings while dwelling in the nest substrate, and after one to two weeks of growth pupate to complete the life cycle (Sabrosky, Bennett & Whitworth, 1989; Bennett & Whitworth, 1991). Thus, in these flies both active choice of a certain nest type by females and demography of larvae within host nests (competition, growth rate, mortality) may play a significant role in determining their abundance in relation to nest type.

Since there was no effect of nest type on fly abundance, it is likely that neither of the two possible processes played any role: fly females did not cue on nest composition when selecting their oviposition site, and demographic processes among larvae did not influence their abundance in relation

to nest type. Alternatively, these two processes may have counteracted each other in determining the abundance of larvae: flies may have selected the type of nest in which their larvae had worse performance; however, such a maladaptive habitat choice seems unlikely to evolve (but see Remeš, 2000). Nevertheless, the absence of any effect of nest type on the abundance of flies is rather puzzling. It is, for example, known that pine bark and bast contain toxic secondary compounds with a strong potential to negatively affect ectoparasites (Pearce, 1996; Bauchau, 1998). As flycatchers use this material to build their nests, this should have led to higher ectoparasite abundance in tit nests.

In contrast to nest type, there was a strong effect of species *per se* on the abundance of parasitic flies: tits were more intensely infested regardless of nest type. Tits and flycatchers differ in brood size, timing of breeding, nestling weight, and the nestling period duration (see Methods), which could in principle cause the difference between species. For example, the greater number of young in the great tit and the longer time that great tit young remain in the nest could mean that more food is available for parasitic larvae, which could lead to their higher abundance. However, those factors that we measured and included in the models had no influence on the abundance of flies within species as evidenced by their non-significance when used as standardized factors in the analysis (see Results). Thus, it seems unlikely that any of these is the causal factor behind the effect of species. This effect may have several more subtle explanations. First, adult flycatchers may be more capable of behavioural anti-parasite defences, for example in the form of nest cleaning by catching laying females and/or parasitic larvae (see also Hurtrez-Boussès *et al.,* 2000; Tripet, Glaser & Richner, 2002). Second, flycatcher nestlings may be more resistant to parasitism and fly larvae suffer greater mortality because of more effective immune defence. Third, the preference of laying females for certain bird species may significantly alter patterns of ectoparasite infestation. The preference for certain species of hosts (here great tits) may have arisen, for example, from better performance of fly larvae on their nestlings (for whatever reason, *e.g.,* different skin thickness, resistance to parasitism, length of the nestling period, etc.). Our study was not suited to revealing the proximate mechanism of the effect of species. However, given the strong effect of species *per se*, it would be interesting to find out which mechanism is responsible.

Habitat was an important determinant of the abundance of flies: they were more abundant in the oak forest than in the spruce forest. All three oak plots were situated on warmer and drier southern slopes, whereas spruce plots were situated either on the top of the hill (two of them) or on the colder and more humid northern slope (one plot). Although known effects of weather (temperature and ambient humidity) on fly abundance are in accord with this difference (Merino & Potti, 1996), many uncontrolled factors differing between the two forest types may have had an influence.

In summary, there was no effect of nest type (nest of great tit *versus* collared flycatcher) on the abundance of nest-dwelling parasitic *Protocalliphora* flies. Thus, the hypothesis ascribing different levels of ectoparasite infestation between the great tit and *Ficedula* flycatchers to nest design (Bauchau, 1998) was not supported by our experimental study.

Literature cited

Bauchau, V., 1998. Comparison of parasitism level in two sympatric passerines: The pied flycatcher and the great tit. Écoscience, 5: 164–171.

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

Bennett, G. F. & T. L. Whitworth, 1992. Host, nest, and ecological relationships of species of *Protocalliphora* (Diptera: Calliphoridae). Canadian Journal of Zoology, 70: 51-61.

Birdblowfly.com. [Online] URL: http://birdblowfly.com/, accessed 7 March 2005.

Bowman, A. S., L. B. Coons, G. R. Needham & J. R. Sauer, 1997. Tick saliva: Recent advances and implications for vector competence. Medical and Veterinary Entomology, 11: 277-285.

Clark, L. & J. R. Mason, 1985. Use of nest material as insecticidal and anti-pathogenic agents by the European starling. Oecologia, 67: 169-176.

Clayton, D. H. & J. Moore, 1997. Host-Parasite Evolution. Oxford University Press, Oxford.

Cramp, S. & C. M. Perrins, 1993. The Birds of the Western Palearctic. Volume VII. Oxford University Press, Oxford.

Dawson, R. D., 2004. Does fresh vegetation protect avian nests from ectoparasites? An experiment with tree swallows. Canadian Journal of Zoology, 82: 1005-1010.

Eastman, M. D., L. S. Johnson & L. H. Kermott, 1989. Ectoparasitism of nestling house wrens, *Troglodytes aedon*, by larvae of the blow fly *Protocalliphora braueri* (Diptera: Calliphoridae). Canadian Journal of Zoology, 67: 2358-2362.

Eeva, T., E. Lehikoinen & J. Nurmi, 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: 624-634.

Fitze, P. S., B. Tschirren & H. Richner, 2004. Life history and fitness consequences of ectoparasites. Journal of Animal Ecology, 73: 216-226.

Gold, C. S. & D. L. Dahlsten, 1989. Prevalence, habitat selection, and biology of *Protocalliphora* (Diptera: Calliphoridae) found in nests of mountain and chestnut-backed chickadees in California. Hilgardia, 57: 1-19.

Grunin, K. Y., 1970. Family Calliphoridae. Pages 607-624 *in* G. Y. Bey-Bienko (ed.). A Key to the Invertebrates of the European Part of USSR V, Part 2. Nauka, Leningrad (in Russian).

Hansell, M., 2000. Bird Nests and Construction Behaviour. Cambridge University Press, Cambridge.

Heeb, P., M. Kölliker & H. Richner, 2000. Bird-ectoparasite interactions, nest humidity, and ectoparasite community structure. Ecology, 81: 958-968.

Hurtrez-Boussès, S., P. Perret, F. Renaud & J. Blondel, 1997. High blowfly parasitic loads affect breeding success in a Mediterranean population of blue tits. Oecologia, 112: 514-517.

Hurtrez-Boussès, S., F. Renaud, J. Blondel, P. Perret & M. J. Galan, 2000. Effects of ectoparasites of young on parents' behavior in a Mediterranean population of blue tits. Journal of Avian Biology, 31: 266-269.

Merino, S. & J. Potti, 1995. Mites and blowflies decrease growth and survival in nestling pied flycatchers. Oikos, 73: 95-103.

Merino, S. & J. Potti, 1996. Weather dependent effects of nest ectoparasites on their bird hosts. Ecography, 19: 107-113.

Møller, A. P., K. Allander & R. Dufva, 1990. Fitness effects of parasites on passerine birds: A review. Pages 269-280 *in* J. Blondel, A. Gosler, J. D. Lebreton & R. H. McCleery (eds.). Population Biology of Passerine Birds: An Integrated Approach. Springer-Verlag, Berlin.

Oppliger, A., H. Richner & P. Christe, 1994. Effect of an ectoparasite on lay date, nest-site choice, and hatching success in the great tit (*Parus major*). Behavioural Ecology, 5: 130-134.

Pearce, R. B., 1996. Antimicrobial defences in the wood of living trees. New Phytologist, 132: 203-233.

Petit, C., M. Hossaert-McKay, P. Perret, J. Blondel & M. M. Lambrechts, 2002. Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. Ecology Letters, 5: 585-589.

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, 82: 1285-1290.

Remeš, V., 2000. How can maladaptive habitat choice generate source-sink population dynamics? Oikos 91: 579-582.

Sabrosky, C. W., G. F. Bennett & T. L. Whitworth, 1989. Bird blow flies (*Protocalliphora*) in North America (Diptera: Calliphoridae) with notes on the Palearctic species. Smithsonian Institution Press, Washington, DC.

SAS Institute, 2000. SAS Online Doc, version 8. SAS Institute Inc., Cary, North Carolina.

Tripet, F., M. Glaser & H. Richner, 2002. Behavioural responses to ectoparasites: Time-budget adjustments and what matters to blue tits *Parus caeruleus* infested by fleas. Ibis, 144: 461-469.

Wesołowski, T., 2001. Host-parasite interactions in natural holes: Marsh tits (*Parus palustris*) and blow flies (*Protocalliphora falcozi*). Journal of Zoology, 255: 495-503.

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

Wilson, T. & B. T. Grenfell, 1997. Generalized linear modelling for parasitologists. Parasitology Today, 13: 33-38.