

**NOTE****Ectoparasites of nestling European starlings (*Sturnus vulgaris*) from a nest box colony in Nova Scotia, Canada**

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Birds are infested by a variety of ectoparasites that can have detrimental effects on host fitness (reviewed in Loye and Zuk 1991; Clayton and Moore 1997; Hamstra and Badyaev 2009; Wolfs et al. 2012). The European starling (*Sturnus vulgaris* Linnaeus) is a cavity-nesting passerine native to much of Eurasia and introduced to several other geographic areas, including North America (Cabe 1993). This species, like most, is infested by many ectoparasites including lice, mites, fleas, flies, and ticks (Boyd 1951; Clayton et al. 2010; Wolfs et al. 2012; Hornsby et al. 2013). In North America, European starlings can harbor both New and Old World ectoparasites (Boyd 1951). However, the ectoparasite community of this host has not been well documented for much of its North American range. Despite being present in Nova Scotia, Canada since the 1930s (Cabe 1993), the ectoparasite faunal information is limited to one study wherein *Carnus hemapterus* Nitzsch (Diptera: Carnidae), and unidentified species of lice, fleas, and mites infested nestlings (Hornsby et al. 2013). Here, we provide more detailed information on the ectoparasites infesting both nestling European starlings and their nests from the same population studied by Hornsby et al. (2013).

Forty-five nest boxes on the campus of Saint Mary's University in Halifax, Nova Scotia, Canada (44°39'N, 63°34'W) were available for breeding European starlings (see Hornsby et al. 2013). European starlings often have two broods per breeding season and may change nest location and/or genetic mate for the second brood (Cabe 1993). Data were collected during the breeding seasons of 2009 and 2010 and we removed nesting material after each brood fledged; nest removal does not appear to influence renesting probability (C.A. Barber, St. Mary's University, personal observation).

Nestlings were dust-ruffled to remove ectoparasites (N = 53 nestlings from 18 broods in 2009, N = 89 nestlings from 27 broods in 2010) 11 or 12 days post-hatch (day 0 is hatch day). Nestlings are partially feathered at this age; thus, ectoparasites that require feathers for food or substrate, e.g. lice (Lee and Clayton 1995), as well as those that do not, may be present at this time. Dust-ruffling removes a portion of ectoparasites from the host and produces a reliable, relative measure of ectoparasite abundance on avian hosts (Walther and Clayton 1997; Clayton and Drown 2001), including European starlings (Koop and Clayton 2013). With the nestling held over a plastic collecting container, commercial insecticide powder (Scott's EcoSense[®] powder, active ingredients: pyrethrins and piperonyl butoxide) was applied throughout the plumage for 2 minutes; thereafter, the plumage was ruffled manually for 6 minutes in 2009, and 4 minutes in 2010, to dislodge ectoparasites. We decreased ruffling time in 2010 to reduce subtle handling stress (e.g., Muller et al. 2006) that was noted during 2009, but it may have resulted in relatively lower total ectoparasite counts in 2010. The time spent on ruffling in other studies varied from 2–6 min (Poiani et al. 2000; Shutler et al.

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2004; Koop and Clayton 2013). Ruffling for longer than 6 min may not yield proportionate gains in ectoparasitic counts. Contents of the collecting container (i.e., insecticide powder, ectoparasites) were then transferred to individually-labelled plastic bags. Ectoparasites were counted with the aid of a dissecting microscope. Mites were numerous (i.e., dozens of individuals) when present; thus, we scored mites as present or absent and only retained a small subsample from each mite-infested nestling. Mites removed from the insecticide powder in both years were pooled and a small subsample of the pooled collection (N = 6) was identified by Dr. Heather Proctor at the University of Alberta. All other ectoparasites removed by dust-ruffling, with the exception of a small number of lice and fleas that were damaged, were identified to species.

In 2009, dust-ruffling of nestlings yielded chewing lice (*Menacanthus eurysternus* (Burmeister) (Phthiraptera: Menoponidae) (male N = 1, female N = 2), *Brueelia nebulosa* (Burmeister) (Phthiraptera: Philopteridae) (male N = 1, female N = 4), one damaged), fleas (hen flea, *Ceratophyllus gallinae* (Schrank) (Siphonaptera: Ceratophyllidae) (male N = 7, female N = 12)), *Carnus hemapterus* (male N = 53, female N = 60)), and mites. In 2010, dust-ruffling also yielded chewing lice (*Menacanthus eurysternus* (female N = 3, nymph N = 1), *Brueelia nebulosa* (male N = 3, female N = 3, nymph N = 1), three damaged), fleas (*Ceratophyllus gallinae* (male N = 1 male, female N = 1), one damaged), and carnid flies (*Carnus hemapterus* (male N = 72, female N = 67)). Mites from the 2009 and 2010 pooled sample were identified as northern fowl mites (*Ornithonyssus sylviarum* (Canestrini and Fanzago) (Mesostigmata: Macronyssidae)) (Table 1).

In 2009, 11.1% of broods yielded no parasites, 38.9% had one parasite order, 44.0% had two parasite orders, 5.6% had three parasite orders, while no broods contained all four parasite orders. The median number of parasite orders was 1.5 (limits of interquartile range (IQR), i.e., the difference between the upper and lower quartiles = 1.0-2.0). In 2010, 3.7% of broods yielded no parasites, 59.3% had one parasite order, 33.3% had two parasite orders, 3.7% had three parasite orders, and again, no broods contained all four parasite orders. The median number of parasite orders per brood was 1.0 (limits of IQR=1.0-2.0).

Bird fleas often spend the majority of their time in the nest material (Marshall 1981; Lehane 1991); thus, to provide more information about flea infestation, all nest material was removed from a subsample of nests (N = 11) in June and July of 2009, ≤ 5 days after nestlings fledged. All sampled nests were the first nests of the year from

Table 1. Descriptive statistics for ectoparasites collected by dust-ruffling nestling European starlings from nest boxes on the campus of Saint Mary's University in Halifax, Nova Scotia, Canada. Nestling prevalence refers to the proportion of nestlings from which at least one parasite was collected; brood prevalence refers to the proportion of broods where at least one parasite was collected, and intensity refers to the number of parasites collected from an infested individual. **NOTE:** Fifty-three nestlings from 18 broods in 2009; 89 nestlings from 27 broods in 2010; IQR = interquartile range.

	Prevalence			Intensity		
	Total collected	Nestling (%)	Brood (%)	Range	Median	Limits of IQR
2009						
Lice	9	13.2	27.8	1-2	1	1-1.5
Fleas	19	15.1	38.9	1-10	1	1-2
<i>Carnus hemapterus</i>	113	56.6	72.2	1-9	3	2-6
Mites	-	17.0	16.7	-	-	-
2010						
Lice	13	7.9	18.5	1-6	1	1-1
Fleas	3	3.4	11.1	1	-	-
<i>Carnus hemapterus</i>	139	53.9	85.2	1-13	2	1-3
Mites	-	10.1	18.5	-	-	-

a given nest box. Nest material was placed in labelled plastic bags and stored at -20 °C until processed (i.e., as soon as 1 month, but no later than 1 year, afterward). To remove fleas from the nest material, we used a method adapted from Stephenson et al. (2009). Nest material was placed in water in a plastic container (35 cm x 35 cm x 80 cm) and thoroughly stirred with a wooden rod. The contents were then poured through a wire screen placed on top of a fine sieve (250 μ m). Material collected on the sieve was carefully examined for ectoparasites and then placed back into the plastic container. The container was again filled with water and the process repeated. The resulting material was examined under a dissecting microscope and fleas removed. Voucher specimens were deposited in the J.B. Wallis/R.E. Roughley Museum of Entomology (University of Manitoba, Winnipeg, MB).

Fleas were found within 10 of 11 (90.9%) nests processed in 2009. We quantified the number of flea pupae (N = 370) and adults (including teneral) (N = 285) that were collected from the 10 infested nests. Larval fleas were also observed but were not present in large numbers and were easily damaged during processing; thus, they were not quantified. Adult fleas were all *Ceratophyllus gallinae* (male N = 89, female N = 195). The number of flea pupae per nest ranged from 0-67 (median = 37.0, lower limit of IQR = 15.5, upper limit of IQR = 48.5), the number of adult fleas in a nest ranged from 0-63 (median = 20.0,

lower limit of IQR = 14.5, upper limit of IQR = 31.5), and the number of pupal and adult fleas combined ranged from 0–121 (median = 66.0, lower limit of IQR = 31.0, upper limit of IQR = 86.0). A small number of adult *Carnus hemapterus* and unidentified larval and pupal Diptera were also observed within the nest material.

The only other study of European starling ectoparasites from Nova Scotia was conducted on the same population as studied here. In that study, Hornsby et al. (2013) reported the presence of *Carnus hemapterus* (the only report of this species from any host in Nova Scotia of which we are aware) as well as unidentified species of lice, fleas and mites (Hornsby et al. 2013). The current study is the first report of *Menacanthus eurysternus* and *Brueelia nebulosa* in Nova Scotia; *Ceratophyllus gallinae* and *Ornithonyssus sylviarum* are reported for the first time on this host in Nova Scotia (see Wright 1979; Holland 1985), though *Ceratophyllus gallinae* has been collected in the nest of a European starling by J.E.H. Martin (Holland 1985).

Our results indicated that by 11–12 days post-hatch, nestlings in this population are exposed to a diversity of ectoparasites. It is not clear how early ectoparasitic infestation begins. Louse infestation is often thought to occur only after nestlings are partially feathered, as feathers are used for food and/or substrate by the lice (Lee and Clayton 1995). *Menacanthus eurysternus* feeds on blood, so it is possible chicks may be infested at an earlier age, as soon as there is a suitable environment in which these lice can live. With feathers developing as early as 5 days post-hatch, louse infestation likely began less than a week prior to dust-ruffling. However, *Ornithonyssus sylviarum*, *Carnus hemapterus*, and *Ceratophyllus gallinae* are haematophagous and could therefore have infested nestlings prior to feather growth. Indeed, *Carnus hemapterus* parasitism is often highest in younger nestlings, prior to feather development, likely since feathers and their development interfere with feeding (Liker et al. 2001). *Carnus hemapterus* were observed on nestlings 5 days post-hatch in this population (Hornsby et al. 2013), but earlier visual examination of nestlings was not performed.

The ectoparasites collected in this study have all been previously reported from European starlings in North America (Boyd 1951; Knee and Proctor 2007; Clayton et al. 2010; Hornsby et al. 2013). However, several other ectoparasite species reported from this host in North America (Boyd 1951; Clayton et al. 2010) were not collected in our study. It is possible that these species, or others, parasitized nestlings in our study population but were not identified. Considering that some ectoparasites removed

from nestlings and nest material were not identified (e.g., damaged specimens, dipterans from nest material, most mites), we may have collected species other than those reported here. Moreover, we may have missed collecting some ectoparasites due to them (1) being not easily removed by dust-ruffling (see Walther and Clayton 1997), (2) being rare (low prevalence and intensity), (3) leaving hosts rapidly when disturbed (e.g., hippoboscids), or (4) spending little time on the host or in nest material (e.g., biting flies).

Little information about the ectoparasite fauna of birds exists in Canada (Galloway and Danks 1990). Regionally-based studies such as this one represent excellent opportunities to contribute to our overall knowledge. Further work should be conducted to collect ectoparasites from European starlings (both nestlings and adults) throughout Nova Scotia, as well as in other geographic areas where ectoparasitism of this host has received little attention.

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