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Source: The Wilson Journal of Ornithology, 124(4):783-787. 2012.
Published By: The Wilson Ornithological Society
DOI: http://dx.doi.org/10.1676/1559-4491-124.4.783
URL: http://www.bioone.org/doi/full/10.1676/1559-4491-124.4.783

Short Communications

The Wilson Journal of Ornithology 124(4):783–787, 2012

Protocalliphora (Diptera: Calliphoridae) Infestations of Nestling Red-shouldered Hawks in Southern Ohio

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ABSTRACT.—We examined nestling Red-shouldered Hawks (*Buteo lineatus*) in 56 nests (147 nestlings) in suburban southwestern Ohio and in 25 nests (67 nestlings) in rural forested Hocking Hills in south-central Ohio, ~180 km east of southwestern Ohio. Fifteen of 25 nests in Hocking Hills had *Protocalliphora avium* larvae on one or more nestlings and/or pupae in the nest material. Nineteen nestlings had larvae in one or both ears, an additional 14 had evidence of larvae outside the ears, 32 were not visibly parasitized, and two were not examined or their status was not reported; in contrast, no nests and no nestlings were parasitized in southwestern Ohio. Reproductive rate (young fledged/nest) did not differ between southwestern Ohio and Hocking Hills (2.4 ± 0.1 young/nest at southwestern Ohio vs. 2.7 ± 0.2 at Hocking Hills; P = 0.214). Parasitized nests at Hocking Hills were no more likely to have been used in the previous breeding season than non-parasitized nests ($\chi^2 = 0.903$, $P = 0.342$, n = 22). Similarly, number of young fledged/nest at parasitized nests did not differ from that at non-parasitized nests within Hocking Hills ($U = 75.0$, $P = 1.00$, $n = 25$; mean (± SE) number of young = 2.7 ± 0.3 vs. 2.7 ± 0.3 at parasitized and non-parasitized nests, respectively). The *Protocalliphora* loads we observed did not appear to have a negative effect on the fledging rate of nestling Red-shouldered Hawks; however, we did not assess any other potential effects of parasitism. *Received 29 February 2012. Accepted 29 May 2012.*

Many raptor species are infested by one or more species of ectoparasitic *Protocalliphora* flies (Diptera: Calliphoridae), commonly called bird blow flies (Sabrosky et al. 1989, Bennett and Whitworth 1992). Adults of these Diptera lay their eggs on nestlings, typically when the nestlings are young (Tirell 1978, Sabrosky et al. 1989). The fly eggs hatch within 24–48 hrs and larval *Protocalliphora* feed on nestling blood until they mature, when they drop from the nestlings into the nest material and pupate (Sabrosky et al. 1989). The larvae are typically found in nestling ear canals, nares, axillary area, and feather sheaths in raptors (Tirrell 1978, Sabrosky et al. 1989, Philips 2007) and also in the nesting material (Sabrosky et al. 1989).

The effect of *Protocalliphora* on raptor nestling health and survival differs among species and studies. Researchers have blamed *Protocalliphora* for impaired development, weakness, and death in some cases (Philips 2007); heavily infested Red-tailed Hawk (*Buteo jamaicensis*) nestlings in North Dakota were smaller and weaker than their siblings and died as a result of siblicide; one of these nestlings had 213 larvae in multiple locations (Tirrell 1978). *Protocalliphora* likely caused the death of 26 nestling Prairie Falcons (*Falco mexicanus*) in nine eyries in Utah (White 1963). In contrast, *Protocalliphora* infestations apparently had little effect on nestling Red-shouldered Hawks (*B. lineatus*) in Wisconsin (King et al. 2010), Broad-winged Hawks (*B. platypterus*) in New York (Crocoll and Parker 1981), Red-tailed, Red-shouldered, and Cooper's hawks (*Accipiter cooperii*) in New York (Sargent 1938), and Red-tailed Hawks in Montana (Seidensticker and Reynolds 1971). Typical infestations likely produce only minor or no negative effects (Sabrosky et al. 1989).

Protocalliphora avium occurs in northern and northeastern North America and has been documented in at least 12 species (Sabrosky et al. 1989). *P. avium* is replaced in western North America by the closely related *P. asiovora* (Sabrosky et al. 1989, Bennett and Whitworth 1992). However, the distribution of *P. avium* is not well documented, and its presence and prevalence on raptor nestlings has been typically, although not exclusively, reported anecdotally (Sargent 1938, White 1963, Seidensticker and Reynolds 1971, Bohm 1978).

Our objective was to investigate the presence and prevalence of *Protocalliphora* ectoparasites

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on nestling Red-shouldered Hawks in two regions of southern Ohio: suburban southwestern Ohio (SWOH) and rural, forested Hocking Hills (HH) in south-central Ohio.

METHODS

Study Areas.—The SWOH study area is a hilly, unglaciated area in the Interior Plateau ecoregion (Omernik 1987). The hills are dissected by many small streams in ravines and two large rivers, the Great Miami River and the Little Miami River. Native forests are dominated by second-growth oak (*Quercus* spp.)-hickory (*Carya* spp.) and beech (*Fagus grandifolia*)-sugar maple (*Acer saccharum*) associations with lowland, riparian forests characterized by sycamores (*Platanus occidentalis*) and beech. Elevation ranges from ~140 to 270 m.

The SWOH study area consisted of Hamilton County, Clermont County, and southwestern Warren County, Ohio; the nests studied were in a wide band of suburban development surrounding the city of Cincinnati, Ohio. Suburban areas varied from densely-populated (residential lots ~20 × 35 m) to sparsely-populated (>2.5-ha residential lots, as well as undeveloped private land). Most residences and other buildings were surrounded by lawns and other non-native vegetation, but residences tended to be on level ground with native vegetation on steep slopes and in riparian areas.

Hocking Hills is a hilly, unglaciated region within the Western Allegheny Plateau ecoregion in southeastern Ohio (Omernik 1987), ~180 km east of SWOH. This region contains numerous small, high-gradient streams, as well as the larger Hocking River and many mid-size streams. Elevation ranges from ~200 to 310 m. The dominant forest type is oak-hickory. Drier sites include chestnut oak (*Quercus prinus*) and black oak (*Q. velutina*), and mesic slopes are characterized by tulip-tree (*Liriodendron tulipifera*). Plantations of white (*Pinus strobus*) and red (*P. resinosa*) pine are also common on public lands. Western portions of this study area also feature sandstone gorges containing northern microclimates and habitats, including eastern hemlock (*Tsuga canadensis*) and ferns. Lowland forests are characterized by sycamores, silver maple (*Acer saccharinum*), beech, and river birch (*Betula nigra*).

The HH study area consisted of Hocking County, eastern Vinton County, northern Athens County, and southern Perry County, including the Athens District of Wayne National Forest, Hocking State Forest, Zaleski State Forest, and associated private lands. Proximity to human activities varied widely with some areas containing residential development, some with recreational development such as picnic areas and trails, and some areas were fairly remote.

Location of Nests and Measurement of Nestlings.—Red-shouldered Hawk nest locations were previously known to us from an ongoing long-term study (Dykstra et al. 2000, 2004, 2009). We visited known nesting areas at least once (but typically 2–3 times) between mid-February and mid-May, 2009–2011, and viewed nests from the ground using 8 × or 10 × binoculars or a 20–60 × spotting scope. Red-shouldered Hawks do not always re-occupy the same nest in subsequent years, and it was often necessary to search for the new nest within a nesting area in the following year. We generally considered that a new nest <0.5 km from a previously active nest was within the same nesting area. A nest 0.5–0.8 km from a previously active nest was considered to be within the same nesting area if additional evidence supported that conclusion (e.g., a bird seen flying between the original and new nests, a lack of activity at the original nest in subsequent years, a temporal progression of new nests moving in that direction indicating a shift of the nesting area's boundaries). We visited some nesting areas in more than 1 year, but data from only the first year were included in this study; thus nests in this study were independent in that each represented a unique nesting area.

We included only nests where young or signs of young (excreta on ground and branches) were observed. We climbed to nests containing young in SWOH and in HH between 7 May and 15 June 2009–2011 to examine and band young when nestlings were ~2–5 weeks of age. We banded nestlings with USGS aluminum bands and weighed them using a 1,000-g spring scale to the nearest 5 g. We used a standard 33-cm ruler to measure the length of the first and second secondary to the nearest millimeter.

We examined the ears of each nestling and classified their infestation status as: (1) no larvae, (2) larvae present within ear or ears, or (3) 'larvae-evidence' outside of ear or ears, but no larvae observed. 'Larvae-evidence' was defined as the presence of black crusty material below the ears (excreta from larvae). This material was usually accompanied by enlarged ear openings. Nests were classified as 'infested' if they contained at least one nestling with larvae or larvae-evidence.

Larvae were not counted but, in some cases, samples were removed from ears for identification of the parasite. We also examined the decaying nesting material in the bottom of the nest at one nest in 2010 and six nests in 2011 in an effort to find pupal forms of the ectoparasite; the remaining nests were not examined due to time constraints. Presence or absence of pupae in the nest material was recorded and pupae were collected. Larvae and pupae were stored with a small amount of sawdust in plastic zipper bags and shipped to T. L. Whitworth of Washington State University, Pullman, for identification. Larvae and some pupae were raised until adult flies hatched; adult flies were identified by T. L. Whitworth based on (Sabrosky et al. 1989); pupae were identified based on a key to the puparia of North American *Protocalliphora* (Whitworth 2003).

Estimation of Nestling Age and Reproductive Rate.—We estimated nestling age based on first and second secondary feather lengths, using the age-feather length regression model for Red-shouldered Hawks of Penak (1982). We averaged the estimated ages of the nestlings in each nest to create a mean nest-age, as nestling age may affect the visibility and location of parasite larvae/pupae (Sabrosky et al. 1989, Bennett and Whitworth 1991).

Nestlings were counted as fledged if they were at least 3 weeks of age. At sites where nestlings were <3 weeks of age at banding, we viewed the nest from the ground when nestlings were well-grown (4.5–6 wks) and counted nestlings using a spotting scope. Reproductive rate was defined as the total number of fledged young/number of nests examined.

Nest Re-use.—Some studies suggest nest re-use can affect some parasite infestation rates (Bennett and Whitworth 1992, Rendell and Verbeek 1996), and we recorded whether each nest had been used in the previous breeding season. We used information from our historical data base for 2009 nests (JLH, unpubl. data).

Statistical Analyses.—We used Kolmogorov-Smirnov tests to compare infestation rates of nests in SWOH and HH because data were non-normally distributed. The nests were considered the unit of measurement, as it was likely that siblings' infestation status were not independent. We used a Mann-Whitney *U*-test to compare the number of young fledged per nest at SWOH with that at HH.

We used a Chi-square test for nests in the Hocking Hills region to examine whether infestation status of nests was related to nest use in the previous breeding season. Nest use in the previous season was unknown for three nests because these nesting areas were first found by us in the year that we studied *Protocalliphora* infestation there; these nests were excluded from this test. We used Mann-Whitney *U*-tests to compare mean nestling age and the number of young fledged per nest at infested nests with that at nests that were not infested.

RESULTS

We examined nestlings in 56 independent nests (147 nestlings) at SWOH and 25 independent nests (67 nestlings) at HH. Fifteen of 25 nests at Hocking Hills were infested with *Protocalliphora avium.* Nineteen nestlings were infested with larvae in one or both ears, an additional 14 had evidence of larvae, 32 were not visibly infested, and two were either not examined or their status was inadvertently not reported on the data sheets. No nests and no nestlings were infested at SWOH, which differed significantly from infestation rate of HH nests ($P < 0.001$). Reproductive rate (mean $\pm$ SE; young fledged/nest) did not differ between SWOH and Hocking Hills (2.4 ± 0.1 young/nest at SWOH vs. 2.7 ± 0.2 at HH; $U = 813.0$, $P = 0.214$, $n = 56$ and $n = 25$).

We checked nesting material for pupae at seven nests at Hocking Hills. Pupae were found in five nests, all of which also had nestlings with larvae or larvae-evidence; the other two nests had neither pupae in nesting material nor larvae in the nestlings' ears.

Infested nests at HH were no more likely to have been used in the previous breeding season than non-infested nests (Pearson's $\chi^2 = 0.903$, $P = 0.342$, $n = 22$). Mean nestling age at infested nests did not differ from that at nests that were not infested ($U = 101.50$, $P = 0.140$, $n = 25$; mean $\pm$ SE nestling age $= 22 \pm 1$ days vs. 24 ± 1 days at infested and non-infested nests, respectively). The number of young fledged/nest at infested nests did not differ from non-infested nests ($U = 75.0$, $P = 1.00$, $n = 25$; mean $\pm$ SE number of young $= 2.7 \pm 0.3$ vs. 2.7 ± 0.3 at infested and non-infested nests, respectively).

DISCUSSION

Red-shouldered Hawk nestlings in southern Ohio appeared to be unharmed by infestations of *Protocalliphora avium.* We did not count the larvae found in ear canals, but we did not observe >10 in any nestling's ear (ARW, unpubl. data). Infestations were apparently less intense than those reported by Tirrell (1978) and White (1963), in which larvae were found in multiple locations on the nestlings and nestling mortality occurred. The reproductive rate (in terms of young fledged per nest) did not differ between infested and non-infested nests at HH and did not differ from the reproductive rate at SWOH.

Sixty percent of Red-shouldered Hawk nests at HH in south-central Ohio contained the blow fly *P. avium*, but nests in SWOH, ~180 km west, were entirely free of this ectoparasite. This difference was not an artifact of the study sample size, as we have not observed *P. avium* at SWOH and we have consistently found ~50% of nests parasitized at HH in our 15-year study of Red-shouldered Hawks nesting in both study areas (CRD and JLH, unpubl. data).

One possible explanation for the presence of *P. avium* at HH but not SWOH is that the suburban landscape is somehow unsuitable for *P. avium.* It is not uncommon for *Protocalliphora* to be absent from some areas, particularly where the host population is relatively recently established or the environment is unstable (T. L. Whitworth, pers. comm.). Some parasite species are less abundant in urban areas (Marcogliese 2005) and some researchers suggest parasite communities may be appropriate indicators of environmental health (Lafferty 1997; Marcogliese 2004, 2005). However, urbanization may affect parasite populations and communities in diverse ways. Urban songbirds had fewer *Protocalliphora* (*P. sialia*; Moore 1984), fewer ticks (*Ixodes*; Gregoire et al. 2002, Evans et al. 2009) and fewer blood parasites (Fokidis et al. 2008). Prevalence of one Dipteran ectoparasite (*Philornis porteri*) on Northern Mockingbirds (*Mimus polyglottos*) was not directly related to urbanization in Florida (Le Gros et al. 2011). Ectoparasites, including *Protocalliphora* spp., may also respond to air pollution; for example, in Finland, *P. azurea* larvae were less abundant in passerine nestlings closer to a factory complex releasing sulfuric oxides and heavy metals into the atmosphere (Eeva et al. 1994).

Another possible explanation for the absence of *P. avium* at the SWOH study area is that it may be outside of *P. avium*'s distributional range, while HH is within it. The genus *Protocalliphora* is considered 'predominantly northern' (Sabrosky et al. 1989) or 'mainly boreal' (Bennett and Whitworth 1991) and the range of *P. avium* extends from Alaska and Yukon Territory to Quebec and Connecticut and south to Pennsylvania and Nebraska (Sabrosky et al. 1989). Reports of this species within the United States are from New York (Sargent 1938, Crocoll and Parker 1981), Minnesota (Bohm 1978), Wisconsin (King et al. 2010), Michigan (Hamerstrom and Hamerstrom 1954), and as far south as Pennsylvania, northern Iowa, and northern Illinois (Sabrosky et al. 1989). Our report of *P. avium* in south-central Ohio is apparently the southernmost report of this species to date. However, published reports on the presence of this species are scattered and mostly anecdotal and may not represent the full distribution range. Hocking Hills, particularly the western part of our study area, includes gorges containing northern microclimates and habitats, and is connected by a corridor of semi-wooded habitat to northeastern Ohio and western Pennsylvania. SWOH is in the Interior Plateau ecoregion and is bordered on the north by flat, primarily agricultural lands with few forests. Thus, it is possible SWOH is effectively isolated from northern species. Red-shouldered Hawks are not present or have an extremely limited distribution in the counties north of SWOH, although other hosts of *P. avium*, Red-tailed Hawks, Cooper's Hawks, and Great Horned Owls (*Bubo virginianus*), are present in those regions (Peterjohn 2001).

If HH in south-central Ohio is at the edge of the range of *P. avium*, we might expect lighter infestations and/or a lower proportion of nests infested, compared to more northern regions. We found 60% of nests infested with *P. avium* but, in northern Wisconsin, 91% of Red-shouldered Hawk nests were infested (King et al. 2010), and in central New York, nearly 100% of nests of both Red-tailed and Red-shouldered hawks were infested (Sargent 1938). We note it is possible that we underestimated the prevalence of *P. avium*, as we did not examine the nesting material at all nests, and we did not examine areas of the nestlings other than the ears; had we done so, we may have found more nests infested.

We do not know the reason for the difference in blow fly abundance between our two study sites, and hope this paper will encourage researchers to examine the distribution of ectoparasites such as *P. avium* and their relationships to their raptor hosts and to the environment. Additional information on the effects of urbanization on avian parasites may identify mechanisms by which urbanization affects survival and demographics of some raptor species.

ACKNOWLEDGMENTS

We thank Sara Johnson Miller and Sandra Stone for assistance with fieldwork, and T. L. Whitworth and Greg Dahlem for assistance with identification of blow flies. We also thank the many landowners of the Cincinnati and Hocking Hills regions who allowed us access to their private property to conduct this research. This work was supported by grants from RAPTOR Inc. and Marilyn Arn. T. L. Whitworth and two anonymous referees kindly reviewed an earlier version of this manuscript.

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