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OLD NESTS IN PROTHONOTARY WARBLER NEST BOXES: EFFECTS ON REPRODUCTIVE PERFORMANCE

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Abstract.—We compared reproductive performance of Prothonotary Warblers (*Protonotaria citrea*) nesting in cleaned nest boxes with that of warblers using boxes in which old nests were allowed to remain. In the present study, warblers built 207 nests in 300 boxes over a single breeding season. Mean clutch size was 5.1 ± 0.7 (SE) eggs in 118 early nests (Prothonotary Warblers are double-brooded) and 3.8 ± 0.7 eggs in 89 late nests. Presence of an old nest from the previous year did not significantly affect (1) probability of a nest box being chosen for nesting, (2) date of nest initiation, (3) clutch sizes of early or late nests, or (4) mortality rates of nestlings. Brown-headed Cowbirds (*Molothrus ater*) parasitized few nests (4%) and showed no preference for clean boxes vs those containing old nests. Nest-dwelling ectoparasites such as feather mites and bird lice were relatively rare and their numbers did not differ between warbler nests constructed in boxes containing old nests and those in empty nest boxes.

NIDOS VIEJOS EN CAJAS DE ANIDAMIENTO PARA *PROTONOTARIA CITREA*: EFECTO EN EL DESEMPEÑO REPRODUCTIVO

Sinopsis.—Comparamos el desempeño reproductivo de *Protonotaria citrea* en cajas que habían sido limpiadas y lugares en donde el nido viejo no fue removido. Se evaluaron un total de 207 nidos en 300 cajas que hubo disponible en una sola época reproductiva (1996). La camada media fue de 5.1 + 0.7 (ES) en 118 nidos de primera puesta y de 3.8 + 0.7 en 89 camadas de segunda puesta. La presencia de un nido viejo no afectó significativamente, la probabilidad de la caja ser seleccionada para anidar, la fecha de comienzo del nido, tamaño de la camada o la tasa de mortalidad de los pichones. El tordo (*Molothrus ater*) parasitó un 4% de los nidos y no tuvo preferencia por cajas limpias o aquellas que tenían nidos del año anterior. La presencia de ectoparásitos fue relativamente rara y sus números no variaron entre los diferentes tipos de cajas para anidar.

Some authors have suggested that results from studies of birds nesting in man-made boxes should be viewed cautiously because (1) they may differ from studies of birds using natural cavities (Rendell and Verbeek 1996a,b) and (2) use of nest boxes may bias results (Møller 1989, 1992). In particular, old nests in cavities may influence site selection and construction of subsequent nests (Rendell and Verbeek 1996a), phenology of nest building (Oppliger et al. 1994), reproductive success (e.g., Clark and Mason 1985), and perhaps even clutch size (Rendell and Verbeek 1996a). As a result, researchers often remove old nests from boxes between breeding seasons. Ectoparasites overwintering in old nests may increase the proportion of nesting mortality (Mappes et al. 1994), and reduction of space in the nest cavity with accumulation of nest materials may result in smaller clutch sizes (Rendell and Verbeek 1996b).

Prothonotary Warblers (*Protonotaria citrea*) nest in secondary cavities in swamps and moist woodlands throughout much of southeastern United States. They readily accept nest boxes, in which they may lay up to two clutches per breeding season (late April–July). Their clutch size decreases with date of nest initiation and varies from 2–8 eggs (Blem and Blem 1992). The present study investigates the hypotheses that removal of nests from such boxes affects cavity choice, clutch size, phenology, and/or nesting success in Prothonotary Warblers. The species has declined significantly over the past 30 yr (Sauer et al. 1997), and its future is of some concern.

METHODS AND STUDY AREA

We carried out this experiment on and near Presquile National Wildlife Refuge on the James River near Hopewell, Virginia (37°20'N, 77°15'W) in March–July, 1996. The habitat of the study area is tidal swamp. A description of the plant community and details of nest box construction are in Blem and Blem (1991, 1992). At this site in 1987 we established a 30-km transect of 300 wooden nest boxes within which Prothonotary Warblers commonly nest (Blem and Blem 1992). The boxes were marked with identifying numbers (1–300) and were placed at 100-m intervals. We have continuously monitored warbler activity in these boxes over the past 12 yr to determine proximate factors affecting year-to-year variation in clutch size (Blem and Blem 1991, 1992).

In March 1996, we removed old nests from all even-numbered boxes and 14 odd-numbered boxes ($n = 178$), and the boxes were thoroughly cleaned. Old nests were left in 136 odd-numbered boxes. Beginning with the first appearance of warblers in April, we monitored nesting activity over the breeding season by visiting individual boxes every 3–5 d. We recorded dates of first eggs, clutch size of all nests (Prothonotary Warblers typically are two-brooded), survival of nestlings, brood parasitism, and use of boxes by other species. Based on previous studies of the timing of clutch initiation (Petit 1989, Blem and Blem 1992), we categorized nests with first eggs laid on or before 20 May as early clutches. Late (second or replacement) clutches were those laid after that date. In August 1996, we collected 50 nests (25 from boxes containing old nests and 25 from boxes that were empty at the beginning of the breeding season) and extracted invertebrates with Berlese funnels. We identified and counted these under a binocular microscope (20–40×).

To determine effects of mass of nests and timing of nest building on ectoparasite populations, we measured masses of 38 single nests collected from cleaned boxes after the young had fledged. These were categorized as early ($n = 20$) or late ($n = 18$) nests. All nests were oven-dried at 65 C to constant mass and then weighed to the nearest 0.1 g on a triple-beam balance.

Analyses of frequency of box selection were performed by Chi-square analyses of 2 × 2 contingency tables of nest contents vs. presence/absence of old nests (Zar 1984). Mean clutch sizes and dates of nest initiation were compared by *t*-tests. All statistical analyses were performed by SAS (SAS Institute 1989).

TABLE 1. Clutch sizes, phenology ($\bar{x}$ ± SE) and nest success of Prothonotary Warblers in boxes that contained old nests or were empty at the beginning of the nesting season.

Measurement	Boxes containing old nests (n = 136)	Empty nest boxes (n = 164)
Clutch size: early nests (n)	5.11 ± 0.77 (56)	5.05 ± 0.53 (62)
Clutch size: late nests (n)	3.86 ± 0.72 (36)[a]	3.76 ± 0.72 (53)[b]
Julian date of first egg (n)	123.7 ± 3.8 (56)	124.3 ± 4.0 (62)
Mortality of young	7	21
Infertile eggs	37	49
Total loss of eggs/young	44 (10.4%)[c]	70 (13.7%)[c]
Brown-headed Cowbird eggs	8	4
Nests of other species[d]	6	6

[a] Twenty-four boxes had both early and late nests.
[b] Twenty-nine boxes had both early and late nests.
[c] Percent of total eggs laid.
[d] Includes four Eastern Bluebirds (*Sialia sialis*), six Carolina Chickadees (*Poecile carolinensis*), and two Carolina Wrens (*Thryothorus ludovicianus*).

RESULTS

Prothonotary Warblers built 207 nests in which clutches were completed and nested twice in 53 boxes. Eight other nests (four in empty boxes, four in boxes with old nests), abandoned for unknown reasons, were excluded from these analyses. Contents of nest boxes had no significant effect on box use by warblers. Warblers placed early clutches in 56 of 136 (41.2%) boxes containing old nests and 62 of 164 (37.8) boxes that had been cleaned (χ^2 = 0.35; P = 0.55). Late clutches were laid in 36 boxes initially having old nests and 53 without nests (χ^2 = 1.22; P = 0.27). Warblers nested twice (early and late clutches) in 53 boxes having old nests and in 36 cleaned boxes (χ^2 = 1.19; P = 0.25). The average dry mass of early nests (25.6 ± 5.8 g [SD]; n = 18) differed from the dry masses of late nests (19.5 ± 6.0 g; n = 18; t = 3.22; $P < 0.05$; variances not statistically different). Prothonotary Warblers typically collected enough material to fill each box to about the level of the entrance hole (i.e., 10–14 cm) and never removed old nest materials from boxes in preparation for renesting.

Early clutches were initiated within a narrow time frame (Table 1) and dates of first eggs did not differ significantly between box categories (t = 0.76; P = 0.44). First eggs were laid on 4 May (±3 d; [SE]). Neither early (t = 0.50; P = 0.62) nor late (t = 0.67; P = 0.51) clutch sizes differed significantly between boxes with and without old nests (Table 1). There likewise was no differences between boxes having two nests in 1996 (early clutches: t = 0.24, P = 0.80; late clutches: t = 0.52; P = 0.60).

Species other than warblers did not differ in their choice of these boxes. Both boxes with old nests and those initially cleaned were used identically by three other species (two Eastern Bluebirds [*Sialia sialis*], three Carolina Chickadees [*Poecile carolinensis*], and one Carolina Wren

TABLE 2. Percentage of Prothonotary Warbler nest boxes containing invertebrate taxa, Hopewell, Virginia (1996).

Order	Boxes with old nests (n = 25)	Boxes without old nests (n = 25)
Acarina	68	72
Feather mites	24	28
Other mites	48	52
Araenida	32	32
Diptera	32	40
Mallophaga	28	44
Thysanoptera	44	52
Collembola	8	28
Psocoptera	32	60
Protura	8	0
Hymenoptera	12	8
Orthoptera	4	0
Coleoptera	4	28
Total individuals/nest ($\bar{x} \pm$ SE)	102 ± 38	112 ± 54

[*Thryothorus ludovicianus*] in both box categories; see Table 1). Brown-headed Cowbirds (*Molothrus ater*) laid a few more eggs in boxes with old nests than in cleaned boxes (8 vs. 4), but the difference was not significant (χ^2 = 1.34; P = 0.22).

Because we almost totally eliminated predation at our study site by placing nest boxes on metal poles, all mortality of eggs or young was attributable to infertile eggs, disappearance of individual eggs or young, or death of nestlings during development (Table 1). Twenty-eight nestlings died during the study (21 in cleaned boxes, 7 in boxes containing old nests; (χ^2 = 7.00; $P < 0.5$). All of these were discovered after two periods during which ambient temperatures were above 38 C.

Prothonotary Warbler nests contained relatively few invertebrates(Table 2), and there were no significant differences in numbers of individuals for each invertebrate taxon between boxes containing nests over winter vs those cleaned in spring (all $\chi^2 < 3.84$; $P > 0.5$). The number of invertebrates found per nest was not significantly correlated with nest mass ($r = 0.11$, $P < 0.05$). We found no fleas (Siphonaptera) and no bird ticks (lxodidae) in any nest.

DISCUSSION

In the present study, old nests had no significant effect on any of several measurements of reproductive performance of Prothonotary Warblers, including probability of box use, date of nest initiation, clutch size, nestling mortality, or hatching success. We detected no difference in occurrence of ectoparasites in old nests vs. those newly made. Low rates of ectoparasitism may help to explain why Prothonotary Warblers do not discriminate against cavities containing old nests. Over 12 yr of study of warblers, we have seen no instances of nestling mortality that could be ascribed to ectoparasitism. In fact, we have handled hundreds of adults and thousands of young, and we have observed very few lice or mites and no fleas. Our Berlese funnel analyses in the present study, although far from conclusive documentation of the abundance of these invertebrates, support our observations. One hypothesis, which deserves more attention, is that the moss and/or lichens placed in nests of Prothonotary Warblers may be repellent or toxic to invertebrates (see Clark and Mason 1985).

Prothonotary Warblers collect large amounts of nest materials in a short period of time. We have observed females that completed nests and began laying in about three days. The amount of material is a function of the size of the box and late nests are smaller than first nests. Prothonotary Warbler nests at our study locality mainly were comprised of a base of mosses and liverworts, which remained green throughout the nesting season (Blem and Blem 1994), topped by a thin cup of dry materials including grasses and rootlets. The dry lining indicated the completion of the nest. In our boxes, nests typically were 7–15-cm deep. Females typically built the nests up to levels that allowed them to look directly out of the entrance hole. The use of moist moss in nests adaptively modifies nest box humidity and temperature in Prothonotary Warbler nests (Blem and Blem 1991, 1994).

Mortality of nestlings has been relatively rare over the decade of our studies. In fact, the mortality that we observed during the breeding season of the present experiment was the greatest in 11 yr and was associated with a 3-d period of inordinately high ambient temperatures (>38 C). The majority of it occurred in clean boxes.

In summary, old nest materials appear to have little or no effect on nest site selection, phenology, or nest success of Prothonotary Warblers. However, we agree with Møller (1989, 1992) that researchers should report the removal of materials from nest boxes in similar studies. We also suggest that use of nest boxes may be superior to studies of natural nests, depending upon the hypotheses being tested. Researchers seeking to design controlled studies of clutch size need to standardize effects of cavity location and structure in order to eliminate sources of variation not currently under investigation. This is a common feature of experimental research.

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