

## The Use of Nest Boxes to Sample Arboreal Vertebrates

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**Abstract** - Tree cavities are rarely incorporated into surveys of forest ecosystem biodiversity, due to difficulty in their systematic sampling. We examined the feasibility of using southern flying squirrel (*Glaucomys volans* Thomas) nest boxes for monitoring arboreal vertebrates at 11 sites within the Savannah River Site, SC. We recorded 3130 vertebrates of 11 species (3 mammals, 3 birds, 5 reptiles) using nest boxes for nesting, roosting, and foraging. *G. volans* represented the majority of these with 3019 individuals, but flying squirrel occupancy did not affect occupancy of boxes by other species. Upland hardwood forests had the most species that used boxes; however, due to uneven sampling, nest boxes placed in dense-canopy plantations detected the most species per box. We conclude that nest boxes are a useful means of surveying for cavity-dwelling species. We recommend a protocol that uses different size nest boxes at varying heights to accurately survey a traditionally under-sampled component of forest ecosystems, those species using tree cavities.

### Introduction

Tree cavities in forest ecosystems are a vital resource, providing sites for nesting, roosting, and foraging for many species. Despite their importance, surveys of biodiversity rarely include tree cavities as a component of forest ecosystems. This in part, results from difficulties of systematically sampling and capturing animals from inside tree cavities. Studies of cavity-using birds and mammals often use artificial cavities as a means of promoting nesting and allowing easy access for monitoring breeding. However, non-focal species that use nest boxes are often not mentioned by researchers (but see: Caster et al. 1994, Heidt 1977, McComb and Noble 1981, Miller 2002, Poysa et al. 2001). The difficulty of systematically sampling arboreal cavity-dwelling species can potentially be addressed using artificial cavities, which can act as monitoring units, much like artificial cover objects (ACOs) used for herpetofaunal surveys.

Interactions between southern flying squirrels (*Glaucomys volans* Thomas) and cavity-nesting birds have been documented including: Red-cockaded Woodpeckers (*Picoides borealis* Vieillot; Conner et al. 1996, Harlow and Lennartz 1983, Jackson 1978, Laves and Loeb 1999, Loeb 1993), Eastern Bluebirds (*Sialia sialis* Linnaeus; Goertz et al. 1975), Hairy Woodpeckers (*Picoides villosus* L.; Kilham 1968), Red-headed Woodpeckers (*Melanerpes erythrocephalus* L.; Stabb et al. 1989), European Starlings (*Sturnus vulgaris* L.; Stabb et al. 1989), Black-capped Chickadees (*Poecile atricapilla* Oberholser; Stabb et al. 1989) and Red-bellied

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Woodpecker (*Melanerpes carolinus* L.; Stickle 1963). In addition, competition between *G. volans* and other tree squirrel species, such as the gray squirrel (*Sciurus carolinensis* Gmelin; Stone et al. 1996), and northern flying squirrel (*Glaucomys sabrinus* Shaw; Weigl 1978) have been investigated. Yet non-focal species are typically not reported, so studies that quantify the interactions of a suite of cavity-nesting species are lacking.

We reported mammals, birds, and reptiles associated with nest boxes, installed in a variety of forested habitats as part of a large study of southern flying squirrels. Our aim was to evaluate their usefulness in sampling a broad range of vertebrates, to provide an index of the arboreal cavity-using community. We examined the potential impacts of southern flying squirrel occupancy of nest boxes on arboreal vertebrate occupancy, the significance of species-specific behaviors, and the nature of interactions among the various species.

### Study Area

Our study was conducted at the Savannah River Site (SRS), on the upper coastal plain, near Aiken, SC. The area is bordered on the southwest by the Savannah River. Habitats included four forest types: bottomland hardwood composed of yellow poplar (*Liriodendron tulipifera* L.), swamp gum (*Nyssa sylvatica* Marsh), and dog hobble (*Leucothoe axillaris* Lamarck) (Jones et al. 1981); upland hardwood dominated by water oak (*Quercus nigra* L.) and pignut hickory (*Carya glabra* Miller); and both dense-canopy and open-canopy pine forest plantations, consisting of slash pine (*Pinus elliottii* Engelman), loblolly pine (*P. taeda* L.), and longleaf pine (*P. palustris* Mill.) (Workman and McLeod 1990).

### Methods

We used a nest box design similar to that of Sonenshine et al. (1973), and nest boxes were constructed out of pine or redwood. The entrance hole was 4 cm in diameter, which is sufficiently large for access by southern flying squirrels, but small enough to exclude gray squirrels. The hole was located on the side of the box, thus providing easy access from the tree trunk. A total of 993 nest boxes were placed in grid-like arrangements, following the protocol in Brady et al. (2000). Briefly, we placed nest boxes at grid points

Table 1. Captures of southern flying squirrels and other vertebrates (mammalian, avian, and reptilian) for 11 locations at the Savannah River Site, SC, from August 1992 to May 1998.

	1	2	3	4	5	6	7	8	9	10	11
# of boxes	99	100	100	100	68	100	60	41	101	100	75
# of southern flying squirrels	326	138	460	458	302	247	74	107	244	249	154
# of other vertebrates	7	10	32	16	11	2	2	6	9	4	6
# of species	3	5	9	4	4	2	2	2	3	2	4
Duration of operation (days)	1184	1617	1951	1954	1590	1746	826	801	1547	908	1381
Days checked	33	38	91	80	57	23	15	18	57	20	40

with each point 40 m from adjacent points (boxes per site: range 41–101; Table 1). Nest boxes were placed 4 to 5 m high on suitable trees (i.e., large enough to accommodate the box and provide easy access). We did not place initial nesting material in the boxes, and material was allowed to accumulate until the end of the season (mid-summer) when boxes were no longer occupied, after which it was removed. This decreased likelihood of insect infestations, particularly ants.

Eleven grids were established throughout the SRS at differing periods between 6 August 1992 and 1 December 1995. Nest boxes were placed in each of the four habitat types. A single grid may contain one or all of these habitats. We obtained GPS locations of each nest box and these points were converted to 1 m radius polygons overlaid on a detailed vegetation land cover data set for the SRS, using ESRI ArcMAP 8.3<sup>®</sup> and ArcView GIS 3.2<sup>®</sup> software. We modified the habitat categories into the four broad habitat types (upland and bottomland hardwood, dense and open canopy pine plantation) for analysis.

Boxes were checked until June 1998. Since grids vary in their date of set-up, there is an uneven duration of operation among grids, ranging from 801 to 1954 days (Table 1). Boxes were checked on average every 7–14 days, between 15 September and 15 June, and then every 28–35 days for the rest of the year. Checks were made during the day in all but the most severe weather conditions.

We calculated southern flying squirrel occupancy (occupied by at least one squirrel) as a percentage of available nest boxes for each of 11 sites. We recorded presence of all vertebrate species including location, number of individuals within the box, and behavior associated with box use (e.g., nesting, foraging, etc.). We calculated the percentage occupancy of nest boxes by vertebrates, other than *G. volans*. All proportion data was arcsine square root transformed to satisfy assumptions of normality. We marked all snakes captured after 25 March 1996 with Passive Integrated Transponder (PIT) tags to allow us to identify individuals and measured snout-vent length (SVL), tail length, and mass during each capture.

## Results

We observed a total of 3130 vertebrates using nest boxes. Southern flying squirrels accounted for the majority of observations (3019), while 111 observations were made of 10 other species of vertebrates using nest boxes during the study period (Table 2). Our total includes only adults and not the number of nestlings or eggs found within bird nests. A total of 35 nestlings and 125 eggs of three bird species were observed (Great Crested Flycatchers, *Myiarchus crinitus* L.; Eastern Screech-Owls, *Otus asio* L.; and Carolina Wrens, *Thryothorus ludovicianus* Latham). Comparisons among sites revealed a significant positive correlation ( $r = 0.96$ ,  $p < 0.001$ ) between the number of observations of other vertebrates and the number of species recorded per site. Flying squirrel occupancy did not affect the number of other vertebrates recorded using boxes ( $r = 0.39$ ,  $p > 0.20$ ).

The highest nest box use was in April for both southern flying squirrels and other vertebrates (Fig. 1). Lowest nest box occupancy was in September and November, for *G. volans* and other species, respectively. Nest boxes were used more when placed in hardwood forests as opposed to pine plantations (Table 3). More observations of other vertebrates were made in upland hardwoods than in other habitats, however upland hardwoods also contained more boxes (Table 3). When considered in proportion to the

Table 2. Reptile, bird, and mammal species recorded using artificial cavities from 1993 to 1997 at the Savannah River Site, SC.

Species	1993	1994	1995	1996	1997
<b>Reptiles</b>					
Carolina Anole	0	1	1	2	0
Broad-headed Skink	1	2	0	6	0
Eastern Kingsnake	0	0	0	2	0
Corn Snake	0	1	0	1	0
Black Rat Snake	1	2	1	16	5
<b>Birds</b>					
Carolina Wren	0	1	1	0	0
Great Crested Flycatcher	0	4	9	22	7
Eastern Screech-Owl	0	0	2	6	1
<b>Mammals</b>					
Eastern gray squirrel	5	2	2	1	0
Cotton mouse	0	0	1	5	0
Southern flying squirrel	487	503	750	810	469
<b>Total</b>	<b>494</b>	<b>516</b>	<b>767</b>	<b>871</b>	<b>482</b>

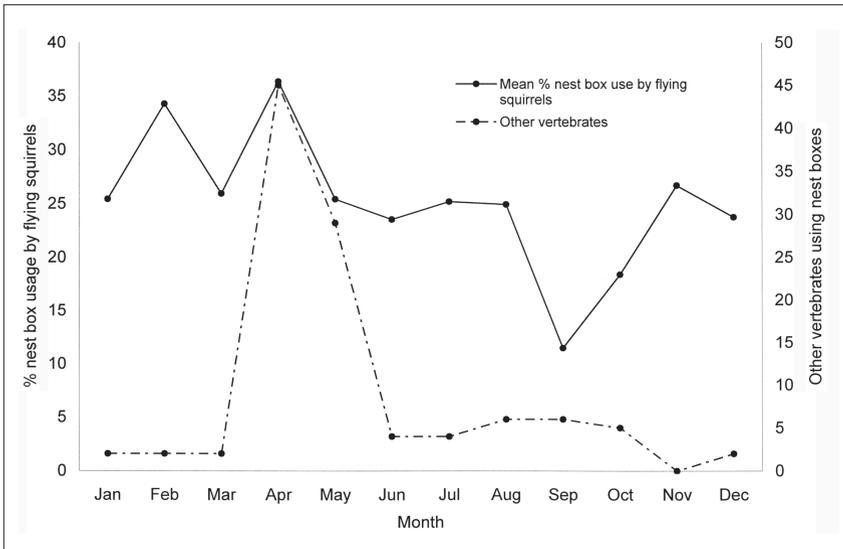


Figure 1. Mean monthly comparison of % occupancy of nest boxes by southern flying squirrels (*Glaucomys volans*) and other vertebrates (mammals, birds, and reptiles) from 11 locations at the Savannah River Site, SC, from August 1992 to May 1998.

number boxes available, pine plantations with dense-canopy structure yielded the highest number of species per box.

With the exception of southern flying squirrels, the most abundant box residents were secondary cavity-nesting birds, with Great Crested Flycatchers being the most common occupant. Of 35 Great Crested Flycatcher nests found, 11 contained snake skins, owl feathers, and/or gray squirrel fur, which were not incorporated into the infrastructure of the nest, but rather were placed on top of the nest rim. Although, Great Crested Flycatchers were recorded in all four habitat types, upland hardwoods and open-canopy pine plantations were most widely used.

Two other bird species, Eastern Screech-Owls and Carolina Wrens, were found using nest boxes, and both species were able to produce successful nests (i.e., fledge young). The owls occupied nest boxes whose entrance holes were previously enlarged by gray squirrels. Birds were found to begin using nest boxes from mid-April to mid-July, coinciding with the breeding season for these species. Eastern Screech-Owls were reported on 11 December 1996, thus these cavities are also used for roosting. Carolina Wrens were only observed in upland habitats.

We recorded 44 observations of five reptile species in nest boxes (two lizards and three snakes); 27 of these were Black Rat Snakes (*Pantherophis obsoleta* Say). Notably, a Black Rat Snake was observed on 3 December 1994 swallowing a marked southern flying squirrel. Black Rat Snakes were observed preying on southern flying squirrels on five separate occasions, and in four cases the flying squirrels were marked with PIT tags that could be read from within the snake's body. Most Black Rat Snakes captured in

Table 3. Sampling effort and vertebrates observed by habitat type on the Savannah River Site, SC.

Habitat	No. sample points	% sample points	Total species	Reptiles	Birds	Mammals	Total captures
Hardwood forest							
Bottomland	112	11.3	4	3	4	0	7
Upland	461	46.4	10	24	26	12	62
Pine plantation							
Dense-canopy	112	11.3	5	4	4	2	10
Open-canopy	308	31.0	5	11	15	2	28

Table 4. Morphometric measurements for three snake species taken from artificial cavities at the Savannah River Site, SC.

Species	N	Sex	Snout-vent length (mm)	Tail length (mm)	Body mass (g)
<i>Lampropeltis getula</i>	1	Female	346	53	18
<i>Pantherophis guttata</i>	1	Male	698	122	80
<i>Pantherophis obsoleta</i>	7*	Female	1010	205	293
	10*	Male	1083	234	290
	17*	Total	1053	222	291

\*Denotes that measurements are reported as means.

boxes were large (> 850 mm; Table 4), suggesting larger snakes may preferentially use cavities.

We also observed Corn Snakes (*Pantherophis guttata* L.) on two separate occasions and on 16 May 1996, two Eastern Kingsnakes (*Lampropeltis getula* L.) in the same nest box placed in upland hardwood forest site. Two lizard species were frequently seen within nest boxes: Carolina Anoles (*Anolis carolinensis* Voigt) and Broad-headed Skinks (*Eumeces laticeps* Schneider). Both species are highly arboreal and are very common on the SRS. *A. carolinensis* was observed only in hardwood forest, whereas *E. laticeps* was found in upland hardwoods and open-canopy plantations.

Including *G. volans*, 3 species of mammals were found in nest boxes: the eastern gray squirrel (*Sciurus carolinensis*) and the cotton mouse (*Peromyscus gossypinus* Le Conte). We captured 10 adult gray squirrels and four litters (of 2–3 young) in nest boxes in all habitat types except bottom-land hardwood forests. Dolan and Carter (1977) report that *G. volans* prefer to nest in natural cavities with entrance holes measuring 4 to 5 cm in diameter, which allows them to enter but excludes larger tree squirrels. Nest boxes used in this study had entrance holes of 4 cm and *S. carolinensis* often enlarged entrance holes to obtain access. By measuring 12 boxes with entrance holes enlarged by *S. carolinensis*, we determined they increase the entrance diameter by 3 cm, to about 7 cm. Enlarged entrances were repaired at the end of the season, when boxes were empty. Seven cotton mice (*P. gossypinus*) used nest boxes, but none of our observations were of a litter. We only observed *P. gossypinus* in upland hardwood forests.

## Discussion

### Advantages of nest box surveys

Total arboreal vertebrate richness was 11 species (including *G. volans*) for 11 sites, located throughout the SRS. Although the overall number of observations was relatively low when compared to the duration of the study, nest boxes allowed species to have been recorded and captured that would have otherwise have been difficult to locate in natural cavities, such as Eastern Screech-Owls or cotton mice. Unlike the use of live traps, sampling using nest boxes can be opportunistic without risk of mortality from the failure to check frequently. Also, they do not impede the movement of animals. Nest boxes are not baited and thus provide an index of arboreal diversity and relative abundance that is not potentially biased by differing bait preference among species. Repeated searches of natural cavities may be intrusive and may result in altered or even damaged habitat. This risk is greatly lessened by using nest boxes. Nest boxes can be checked repeatedly and easily over long periods of time, being repaired or replaced when needed. Thus, rarer species or those with strictly nocturnal habits can eventually be detected.

The likelihood of detecting rarer species will increase with the frequency of checks. Despite this, a number of mammal species with arboreal

habits that are considered common on the SRS were not recorded using the artificial cavities in our study, such as golden mice (*Ochrotomys nuttalli* Harlan), silver-haired bat (*Lasionycteris noctivagans* Le Con.), eastern wood rat (*Neotoma floridana* Ord), and fox squirrel (*Sciurus niger* L.) (Cothran et al. 1991). These species may under-use cavities, despite their arboreal habits. Surprisingly, no Gray Tree Frogs (*Hyla versicolor* Le Con.) were observed in our study, although they commonly occupy nest boxes (Caster et al. 1994, Heidt 1977). Alternatively, our flying squirrel box design may be inadequate for sampling these species. Nest boxes can also document arboreality of species that are considered primarily terrestrial, such as *L. getula* in our study.

### Sampling considerations

A clear prediction of sampling arboreal habitats with nest boxes is that boxes will be most effective at attracting cavity-dwelling species in habitats where nesting holes are limited. Consistent with this prediction is the higher percentage of vertebrate detections we made in upland hardwood forests than in pine plantations due to our emphasis in sampling hardwood, even though snags are thought to be more limiting in managed pine stands (Moorman et al. 1999). In this study, boxes in dense-canopy plantations were the most effective at detecting vertebrate use, however the number of species and individuals detected was strongly associated with the number of boxes in the given habitat.

The size of the entrance hole influences which species can access the nest box (Stone et al. 1996), although in some instances, such as with gray squirrels, animals may alter the entrance to allow access. Several bird species show nest site selection related to specific cavity orientations such as: American Kestrels (*Falco sparverius* L.; Brauning 1983, Toland and Elder 1987); Red-napped Sapsucker (*Sphyrapicus nuchalis* Baird), Northern Flicker (*Colaptes auratus* L.), Tree Swallow (*Tachycineta bicolor* Vie.), House Wren (*Troglodytes aedon* Vie.), Mountain Bluebird (*Sialia currucoides* Bechstein), and European Starling (*Sturnus vulgaris* L.) (Dobkin et al. 1995); and Eastern Screech-Owl (McComb and Noble 1981). These species primarily prefer easterly or secondarily southerly cavity orientations, thought to decrease illumination, and hence prevent temperature increases (reviewed by Walsberg 1985). Excluding the Eastern Screech-Owl, these species nest in open-country and/or edge habitats, where direct illumination on cavity interiors can play a major role in the nest. In contrast, illumination in canopy covered forests may be less important in nesting.

The height at which nest boxes are placed can affect species occupancy. Risch and Brady (1996) found height of traps strongly effected capture success of arboreal mammals. Our study was conducted as a portion of a larger study of *G. volans*, and thus nest boxes were aimed to maximize *G. volans* occupancy. However, a study of arboreal cavity

using species would require consideration of variable heights to accurately assess diversity in an area. Another consideration is that a nest box may only be occupied by one species or even one individual at a time. This may potentially underestimate inferences of relative abundance and population density of these arboreal communities, which are typically under-sampled (Risch and Brady 1996).

The addition of nest boxes often increases population density, since cavity availability is usually a limiting resource (Newton 1994). Nest box addition has been demonstrated to increase populations of Common Goldeneye (*Bucephala clangula* L.; Poysa and Poysa 2002), Bewick's Wrens (*Thryomanes bewickii* Audubon; Taylor 2003), Mountain Chickadee (*Parus gambeli* Ridgway), Pygmy Nuthatch (*Sitta pygmaea* Vigors), and House Wren (*Troglodytes aedon* Vieillot) (Bock and Fleck 1995). In contrast, similar studies conducted on mammals, showed den site availability does not limit populations of southern flying squirrels *G. volans* (Brady et al. 2000), northern flying squirrels *G. sabrinus* (Carey 2002, Ransome and Sullivan 2004), and Douglas' squirrel (*Tamiasiurus douglasii* Bachman; Ransome and Sullivan 2004).

In secondary cavity-nesting birds, reproductive success is often higher in nest boxes than in natural cavities, due to decreased predation rates (Miller 2002, Mitrus 2003, Nilsson 1984, Rendell and Robertson 1993) or increased reproductive output (Evans et al. 2002, Miller 2002, Rendell and Robertson 1993).

Miller (2002) compared nesting success in the Great Crested Flycatcher breeding in natural and artificial cavities and found higher nest success in nest boxes in the first year of the study, but lower in the second year of the study. Decreased nesting success in nest boxes was attributed to increased nest predation (primarily from Corn Snakes and southern flying squirrels). It has been suggested that nest predators can develop search images and associate nest boxes as a prey resource (Poysa et al. 2001). In our study, Great Crested Flycatchers added artifacts (i.e., snake sheds, owl feathers, and gray squirrel fur) at the nest box. We suggest this may act to discourage predation by southern flying squirrels that are known to depredate bird nests (Dolan and Carter 1977, Stabb et al. 1989).

### **Designing a protocol**

The specific sampling protocol required would obviously depend on the study objectives. We strongly recommend preliminary research to address concerns of nest box placement and sampling design (grids, transects, random points, etc.), particularly in relation to the habitats being sampled. Special attention should be paid to the potential biases in sampling associated with placement near edge habitats. To consider effects of entrance size and height of nest box placement, we suggest the use of three box sizes, with varying size entrance holes placed at alternating heights. Three sizes — small (10 x 10 cm floor with 3.5 cm entrance), for species such as Prothonotary

Warbler (*Protonotaria citrea* Boddaert) or White-breasted Nuthatch (*Sitta carolinensis* Lath.); medium (15 x 15 cm floor with 4 cm entrance), for species such as southern flying squirrel or Great-crested Flycatcher; and large (20 x 20 cm floor with 8 cm entrance), for species such as Eastern Screech-Owl or gray squirrel—should be placed per sampling point. Each box size is placed at one of three heights, low (2 m), middle (5 m), and high (10 m), per point, and the arrangement is altered at each point. Thus, three points in a row will have one of each box size at each of the three heights. Nest boxes within a sampling point should be about 10 m apart on different trees to avoid predator presence or monopolization effects on other cavity users. We suggest distance between sampling points should be no less than 40 m, as our data shows adjacent boxes are often occupied. An important consideration is that the sample size required to address your study objectives would have to increase 3-fold as related to the number of boxes placed out. Obviously, this design can be simplified by using only two sizes of nest box, however this may compromise detectability of certain species. Studies have demonstrated that artificial cavities containing old nesting material are frequently avoided as nesting sites and refugia (Rendell and Verbeek 1996, Utsey and Hepp 1997), and thus regular removal of old nesting material would allow continued unbiased sampling. Similarly, to prevent enlargement of nest holes by certain species (such as *S. carolinensis*), metal guards could be put into place around the entrance.

The use of multiple box types at varying heights provides a reliable and relatively inexpensive means of documenting the arboreal vertebrate fauna of an area, over a long period of time that is free of trapping mortality.

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