

12-2014

## Effect of Predator Type, Season, Brood Size, and West Nile Virus Infection on the Nest Defense Behavior of Male and Female Eastern Bluebirds (*Sialia Sialis*)

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### Recommended Citation

Gilbert, K. L., & Ritchison, G. L. (2015). Effect of Predator Type, Season, Brood Size, and West Nile Virus Infection on the Nest Defense Behavior of Male and Female Eastern Bluebirds (*Sialia Sialis*). *The Kentucky Warber*, 91, 39-47.

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EFFECT OF PREDATOR TYPE, SEASON, BROOD SIZE, AND WEST  
NILE VIRUS INFECTION ON THE NEST DEFENSE BEHAVIOR OF  
MALE AND FEMALE EASTERN BLUEBIRDS (*SIALIA SIALIS*)

Kayde L. Gilbert and Gary Ritchison

INTRODUCTION

Parent birds may benefit by defending nests from potential predators if such behavior increases the likelihood that their young will survive. However, such behavior may also be costly, with some risk of injury or even death. Thus, because birds seek to maximize lifetime reproductive success rather than current reproductive success, parents must assess the danger posed by a potential nest predator and then choose appropriate responses (Radford and Blakey 2000).

The intensity of nest defense by birds can be influenced by many factors. For example, females defend nests more vigorously than males in some species (Weatherhead 1989), males more vigorously than females in other species (Winkler 1992), and males and females with equal vigor in still other species (Nealen and Breitwisch 1997). Similarly, the intensity of nest defense has been found to increase with brood size in some species (Radford and Blakey 2000), but not others (Halupka 1999), and intensity varies with stage of the breeding season in some species (Redmond et al. 2009), but not others (Hobson et al. 1988).

Another factor that can influence the nest defense behavior of birds is the type of predator. For example, Tree Swallows (*Tachycineta bicolor*) defended nest sites more vigorously against a ferret (*Mustela putorius*) than a black rat snake (*Pantherophis obsoleta*) (Winkler 1992), and Brunton (1990) found that Killdeer (*Charadrius vociferous*) defended nests more intensely against ground-based predators than aerial predators. Such differences in response might be influenced by the relative risk posed by a predator to both parents and offspring (Brunton 1990).

Another factor that may affect nest defense behavior is a bird's physical condition and, specifically, whether a bird has been exposed to a pathogen and is immune-challenged. Previous studies suggest that birds responding to induced immune challenges (i.e., caused by injecting non-pathogenic antigens) may increase reproductive investment (Bonneaud et al. 2004). Exposure to a pathogen could potentially influence nest defense behavior because birds and other animals invest more in current reproductive effort if the chance of surviving to reproduce again is low, i.e., the terminal investment hypothesis (Clutton-Brock 1984).

Because previous studies have produced conflicting results concerning the effects of factors such as sex, brood size, stage of the breeding season, and predator type on the nest defense behavior of birds, additional studies are needed to better clarify how and why such factors influence behavior. In addition, although investigators have induced immune challenges to study how such challenges might influence reproductive investment (e.g., clutch sizes and likelihood of re-nesting), no one to date has examined the possible effect of a viral infection on avian nest defense behavior. Thus, our objectives were to determine the effect of adult sex, brood size, stage of the breeding season, predator type, and infection with West Nile Virus on the nest defense behavior of male and female Eastern Bluebirds (*Sialia sialis*).

METHODS

Eastern Bluebirds were studied from March through August 2003 at the Blue Grass Army Depot (BGAD), located in Madison County, Kentucky. The BGAD consists of 5907 ha (14,597 ac) of open grassland and scattered woodlots. Lab work was conducted at the University of Kentucky from August 2003 through December 2004.

Prior to nest building and territory establishment by male Eastern Bluebirds, we placed

nest boxes ( $n = 100$ ) in open habitats on the BGAD. Once territories had been established and nest boxes occupied, we captured bluebirds by using mist nets. Bluebirds were lured into nets either by using playback of the songs of Eastern Bluebirds or by placing nets near occupied boxes. A blood sample (40–70  $\mu$ l) was collected from each captured bird and bluebirds were banded with a U. S. Geological Survey aluminum band plus a unique combination of three colored plastic bands.

Nest boxes were monitored every two to three days to determine their status. Blood samples were also collected from nestlings when 7–12 days old. For each nest, we determined the number of nestlings. All blood samples were analyzed to determine the presence of West Nile Virus (WNV). Blood samples were analyzed for the virus by using a reverse transcription-nested polymerase chain reaction (RT-nPCR) assay. This assay had been used previously to detect WNV in several species, including birds (Lanciotti et. al. 2000, Johnson et. al. 2001). A blood sample testing positive with the RT-nPCR indicated that the bird had WNV.

Nest defense behavior of adult bluebirds was examined when nestlings were 15–18 days old. Pairs of bluebirds were presented with two predators. During separate trials, at least 24 hrs apart, a human and a live, non-releasable Eastern Screech-Owl (*Megascops asio*) were presented at each nest. Predators moved to or were placed 0.5 m (1.6 ft) in front of nest boxes when the adults were not present. Trials began when at least one adult came within 30 m (98 ft) of the nest. Nest defense behavior was recorded for 3 min. Behaviors recorded for each bluebird included (1) the closest distance of approach to the predator ( $\pm$  0.5 m [1.6 ft]), (2) mean distance from the predator (with distances recorded every 30 sec), (3) number of songs, (4) number of alarm calls ('chit' calls; Gowaty and Plissner 1998), (5) number of flights (number of times each adult flew within 3 to 30 m (1.6 to 98 ft) of the predator), (6) number of flybys (number of flights within 1 to 2 m (1.6 to 3.2 ft) of the predator), (7) number of attacks (number of flights within 1 m (1.6 ft) of the predator), and (8) number of hits (number of times a bluebird struck the predator). For variables 1 and 2, we assumed larger numbers (i.e., staying farther from the predator) indicated a weaker response, whereas, for variables 3 through 8, we assumed higher numbers indicated a stronger response. We also assumed that attacks and hits represented the strongest response and bluebirds engaging in such behavior, by approaching a potential predator so closely, were taking the greatest risk. As such, for analysis, we used this formula:

$$\text{Nest defense intensity (NDI)} = (\text{number of songs} + \text{number of calls} + \text{number of flights} + \text{number of flybys} + \text{number of attacks} \times 2 + \text{number of attacks} \times 3) - (\text{closest distance} + \text{mean distance}),$$

to generate a single variable that quantified the intensity of nest defense by male and female Eastern Bluebirds.

We examined the possible effect of predator type (human vs. screech-owl), month (May, June, July, and August), and brood size (3, 4, or 5 young) on the nest defense behavior of Eastern Bluebirds. In addition, to examine possible effects of WNV infection on bluebird behavior, we compared the intensity of nest defense of (1) bluebirds infected with WNV to that of bluebirds not infected, and (2) bluebirds with at least one nestling infected with WNV to that of bluebirds with no nestlings infected. All analyses were conducted using analysis of variance, and all analyses were conducted using the Statistical Analysis System (SAS Institute 1989). Significance was accepted at  $P < 0.05$ , and values are presented as means  $\pm$  SE.

## RESULTS

We conducted predator trials with 65 pairs of Eastern Bluebirds. For all variables examined, analysis revealed no differences in the responses of bluebirds with different-aged nestlings (all  $P \geq 0.08$ ; all trials were conducted with pairs that had 15–18 day-old nestlings) so nestling age was not included in subsequent analyses. The intensity of nest defense



differed with predator type, with bluebirds responding more aggressively to an Eastern Screech-Owl (mean NDI =  $35.9 \pm 3.5$ ) than a human (mean NDI =  $0.1 \pm 2.8$ ; Tables 1 and 2). Male bluebirds defended with greater intensity (mean NDI =  $23.1 \pm 3.3$ ) than females (mean NDI =  $13.2 \pm 3.8$ ; Table 1), but further analysis revealed that the only significant difference between males and females was the number of songs (Table 3). The number of young (3, 4 or 5) did not influence the intensity of nest defense (Table 1). Similarly, the intensity of nest defense did not vary among months (May, June, July, and August), and no interactions were significant (Table 1).

Table 1. Effects of sex, month, predator type, and number of young on the intensity of nest defense by male and female Eastern Bluebirds defending nestlings near fledging age (15-18 days old).

Variable	SS	df	MS	F	P
Sex	4844.9	1	4844.9	5.0	0.03
Month	6047.5	3	2015.8	2.1	0.11
Predator	51908.8	1	51908.8	53.1	<0.01
Number of yg.	707.9	2	354.0	0.1	0.70
Month x number of yg.	3459.3	4	864.8	0.9	0.47
Month x predator	1695.3	3	565.1	0.5	0.65
Number of yg. x predator	3083.2	2	1541.6	1.6	0.21
Sex x predator	612.8	1	612.8	0.6	0.43
Sex x month	5590.6	3	1863.5	1.9	0.13
Sex x number of yg.	503.7	2	251.9	0.3	0.77
Sex x predator x month	1962.5	6	327.1	0.3	0.92
Sex x number of yg. x month	487.2	4	121.8	0.1	0.97
Month x number of yg. x predator	1003.5	6	250.9	0.2	0.91
Error	256021.5	187			

Of the 130 adult bluebirds, 10 were infected with WNV (7.7%; 5 males and 5 females, with the other member of the pair not infected in all cases). In addition, at least one nestling was infected with WNV in nine of the 65 nests (14%). We found no difference in the intensity of nest defense (NDI) between pairs where one adult was infected with WNV and pairs where neither adult was infected ( $F_{1, 212} = 1.4$ ,  $P = 0.24$ ). Similarly, for pairs where one adult was infected and the other was not, we found no difference between them in the intensity of nest defense ( $F_{1, 27} = 0.1$ ,  $P = 0.72$ ). Finally, the intensity of nest defense did not differ between pairs with no infected nestlings and pairs with at least one infected nestling ( $F_{1, 212} = 0.1$ ,  $P = 0.86$ ).

## DISCUSSION

### Adult sex

Our results indicate that male and female Eastern Bluebirds defended nests with equal intensity. Although males uttered more songs than females during nest defense trials, that difference may have been more the result of the tendency of males to sing more than females (Gowaty and Plissner 1998) than a different response to nest predators. Previous studies have provided conflicting results concerning the relative intensity of nest defense by males and females. As with Eastern Bluebirds in our study, male and female Northern Cardinals (*Cardinalis cardinalis*; Nealen and Breitwisch 1997) and male and female Northern Flickers (*Colaptes auratus*; Fisher and Wiebe 2006) defended nests with equal intensity. In contrast, studies of a number of other species have revealed that males defend nests more vigorously than females (Hogstad 2005, Gibson and Moehrensclager 2008, Redmond et al. 2009, Kryštofková et al. 2011). In yet other species, females defend nests more vigorously than males (Weatherhead 1989).

Table 2. Responses of Eastern Bluebirds (males and females combined) to two potential nest predators (a human and an Eastern Screech-Owl).

Variable	Responses to Eastern Screech-Owl		Responses to human	
	Mean	SE	Mean	SE
Closest approach (m)***	4.0	0.5	12.1	0.9
Mean distance (m)***	8.8	1.1	16.5	0.9
Number of songs**	5.4	0.6	3.3	0.5
Number of calls**	22.8	0.8	19.9	1.0
Number of flights**	1.8	0.2	3.3	0.3
Number of flybys***	2.9	0.4	0.5	0.2
Number of attacks***	7.4	1.0	0.2	0.2
Number of hits*	0.1	0.1	0.0	—

Key to probabilities: \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.0001$

Males in some species may defend nests more vigorously and take more risks than females because only females incubate eggs and brood young and, as a result, are essential for nest success (Redmond et al. 2009, Kryštofková et al. 2011). Being injured would be maladaptive for both sexes (Montgomerie and Weatherhead 1988), but, if a female is severely injured or killed, nest failure would be inevitable. However, the likelihood of nest failure if a female is injured or killed varies with nest stage. During incubation and early in the nestling period when young must be brooded, the loss of a female in species where only females incubate eggs and brood young would almost certainly mean the loss of the nest. However, we conducted trials when young bluebirds were near fledging (15-18 days post-hatching). For young near the age of fledging and for species like Eastern Bluebirds where both adults provision young and fledglings (Gowaty and Plissner 1998), injury or death of the adult female would likely not result in nest failure. In such cases, females may defend nests as vigorously as males.

Table 3. Comparison of the responses of male and female Eastern Bluebirds to potential nest predators (a human and an Eastern Screech-Owl).

Variable	Males		Females	
	Mean	SE	Mean	SE
Closest approach (m)	7.3	0.8	8.7	0.9
Mean distance (m)	11.7	0.9	13.9	1.3
Number of songs*	5.4	0.6	2.6	0.5
Number of calls	19.9	1.1	17.9	1.1
Number of flights	2.2	0.2	2.1	0.3
Number of flybys	1.7	0.4	1.2	0.3
Number of attacks	3.2	0.7	3.0	0.7
Number of hits	0.05	0.04	— <sup>a</sup>	—

Key to Probability: \* $P < 0.0001$

<sup>a</sup> One female hit a screech-owl during one trial (out of 99 trials)

#### Predator type

Eastern Bluebirds in our study defended nests more vigorously in response to an Eastern Screech-Owl than a human. Other species of birds also respond differently to different potential nest predators (Veen 1977, Kleindorfer et al. 2005, Morrison et al. 2006). For example, Tree Swallows defending nest sites responded more vigorously to a ferret (*Mustela putorius*) than a black rat snake (*Elaphe obsoleta*; Winkler 1992). Brunton (1990) reported that Killdeer defending nests responded more intensely to ground-based predators than aerial predators.



Several factors may influence how birds respond to different potential nest predators, including nest stage (eggs vs. nestlings), the likelihood that predator defense can be effective, and the degree of threat a potential predator poses to adults. Eastern Bluebirds in our study may have responded more aggressively to an Eastern Screech-Owl than to a human because an avian (aerial) predator represents a threat not only to nestlings, but to adults as well. Similarly, the intensity of nest defense by Black-billed Magpies (*Pica pica*) was also found to vary with type of predator, with the most vigorous defense directed toward raptors regardless of nest stage (Buitron 1983). Such aggression toward raptors may be beneficial because they represent a threat to adult magpies as well as nestlings (Buitron 1983). In addition, raptors would also represent a greater threat to young birds than terrestrial predators, like humans, after they fledge. For cavity-nesting species like Eastern Bluebirds, a raptor like an Eastern Screech-Owl may not represent a serious threat to nestlings because they would likely be too large to enter most bluebird nest cavities. However, we examined the behavior of Eastern Bluebirds defending nests with young near fledging age and an Eastern Screech-Owl would pose a threat to young bluebirds after they leave nests. Thus, if an aggressive response by Eastern Bluebirds toward an Eastern Screech-Owl causes the owl to leave the area (move-on hypothesis; Curio 1978) then the risk of predation for both adults and fledglings might be reduced.

One possible explanation for the reduced response of bluebirds to a human in our study is that nest boxes were checked every two to three days once brooding began. As a result, bluebirds were exposed to a human approaching nests and checking nest contents as many as five or six times before predator trials were conducted. Bluebirds, therefore, may have habituated to human presence and activity and perceived a human as a less threatening predator. Similarly, Lord et al. (2001) found that New Zealand Dotterels (*Charadrius obscurus aquilonius*) nesting on beaches with more human activity exhibited a decreased intensity of response to a human approaching nests than did dotterels at more remote beaches. In contrast, Knight and Temple (1986) suggested that repeated visits by humans to bird nests can result in an increased intensity of response, i.e., after repeated visits by a human where no adults or nestlings are harmed, adults can 'lose fear of the predator' and, as a result, increase the intensity of their responses. Other investigators, however, have reported that repeated visits by humans to nests do not affect the intensity of responses by adult birds (e.g., Weatherhead 1989, Winkler 1992). Given the conflicting results of previous studies, the possible effect of our repeated visits to nests on the responses of adult Eastern Bluebirds during nest defense trials remains unclear.

Another possible explanation for the less vigorous response by Eastern Bluebirds to a human near their nests is that bluebirds may make judgments concerning their ability to successfully drive different predators away from nests and respond accordingly. Thus, because a large predator like a human is unlikely to be driven from nests, bluebirds may exhibit a less vigorous response. Similarly Patterson et al. (1980) suggested that the responses of White-crowned Sparrows (*Zonotrichia leucophrys*) to potential nest predators varied with their ability to drive them away. For example, adult White-crowned Sparrows exhibit reduced responses to snakes, possibly because sparrows are unable to drive snakes away from nests (Patterson et al. 1980).

#### Brood size

We found that brood size did not influence the intensity of nest defense by Eastern Bluebirds. Similar results have been reported for several other species of birds (Winkler 1992, Sandercock 1994, Halupka 1999, Tryjanowski and Golawski 2004). In other species of birds, the intensity of nest defense has been found to increase with increasing brood size (Wallin 1987, Wiklund 1990, Radford and Blakey 2000). Montgomerie and Weatherhead (1988) suggested that the intensity of nest defense should increase with increasing brood size because the benefits of deterring a predator increase with the number of young. At least

two factors may contribute to differences among species in the effect of brood size on the intensity of nest defense. First, the value of a given number of young may vary with the reproductive potential of parents (Montgomerie and Weatherhead 1988) and, therefore, parents capable of raising fewer young might be expected to defend their smaller brood as intensively as parents with greater reproductive potential defend their larger brood. As a result, the results of studies where only natural variation in brood size is considered may not reveal any differences in the intensity of nest defense among pairs with different brood sizes (Montgomerie and Weatherhead 1988).

A second factor that might explain differences among studies in the effect of brood size on nest defense behavior is the type of predator used in experiments. As noted previously, some predators represent a threat to both adults and young (e.g., Eastern Screech-Owl in our study) and, because inducing such predators to leave the area is beneficial to adults, responses to such predators may be similar regardless of brood size. In contrast, given that the benefits of deterring a predator increase with the number of young, the intensity of nest defense by adults may be more likely to vary with brood size when responding to predators that only threaten young.

#### Stage of breeding season

We found no seasonal (monthly; May–August) variation in the intensity of nest defense by Eastern Bluebirds. Similar results have been reported for Redwings (*Turdus iliacus*; Bjerke et al. 1985) and Yellow Warblers (*Dendroica petechia*; Hobson et al. 1988). However, previous studies have revealed a decline in the intensity of nest defense as the breeding season progresses for some species of birds (Weatherhead 1989, Halupka and Halupka 1997, Redmond et al. 2009), whereas others have reported an increase in intensity as the breeding season progresses (Regelmann and Curio 1983). A possible explanation for a decline in intensity of nest defense later in the breeding season is the declining value of nestlings later in the season (in terms of adult fitness) because of the reduced likelihood of successful recruitment of such nestlings into the breeding population late in the season (Montgomerie and Weatherhead 1988). In contrast, an increase in the intensity of nest defense as the breeding season progresses may occur because of a decline in re-nesting potential, i.e., with a reduced likelihood of being able to re-nest later in the season, adults should be willing to take greater risks to defend current nests (Montgomerie and Weatherhead 1988). For species like Eastern Bluebirds and others where the intensity of nest defense remains constant throughout the breeding season, Weatherhead (1989) proposed that the declining value of offspring as the season progresses may be balanced by the effect of declining re-nesting potential. However, another possible explanation is that, as with responses by parents with different-sized broods described previously, responses to predators like Eastern Screech-Owls that threaten both adults and young may remain constant throughout the breeding season because, regardless of time of year, inducing such predators to leave the area is always beneficial for adults as well as offspring.

#### Effect of WNV infection

The nest defense behavior of Eastern Bluebirds infected with WNV did not differ from that of non-infected bluebirds and, in addition, the behavior of adult bluebirds with an infected nestling did not differ from that of adults with no infected nestlings. Previous studies suggest that birds responding to induced immune challenges (i.e., caused by injecting non-pathogenic antigens) may increase reproductive investment (e.g., Bonneaud et al. 2004, Hanssen 2006, Velando et al. 2006, Bowers et al. 2012). Because increased effort in current reproduction can negatively impact future reproduction, animals should generally restrict current efforts to maximize lifetime reproductive success (Curio 1983). However, Clutton-Brock (1984) suggested that animals should invest more in current reproductive effort if the chance of surviving to reproduce again is low, i.e., the terminal investment



hypothesis. Our results suggest that WNV infection, at least during the viremic stage, did not affect the physical condition of Eastern Bluebirds enough to affect their nest defense behavior. Similarly, Hill et al. (2010) found that being seropositive for WNV had no negative effects on the reproduction or survival of Eastern Bluebirds in Alabama.

#### ACKNOWLEDGMENTS

We thank David Westneat and Ian Stewart for their hard work and support in the lab, Janet Gorrell for assistance in the field, and Eileen Wicker (Raptor Rehabilitation of Kentucky, Inc.) for the loan of a non-releasable Eastern Screech-Owl. Financial assistance was provided by the ECU Research Committee.

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### WINTER 2014-2015 SEASON

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Climatic conditions during winter 2014-2015 definitely affected the avifauna in a significant way! Precipitation and temperature were normal to slightly above normal statewide during December, but during the first week of January, a shift in the “North Polar Vortex” ushered in extremely cold air that resulted in temperatures across the region that were lower than had been experienced in at least a decade. The colder-than-normal temperatures persisted well into February, resulting in mean average monthly temperatures of 4-7°F below normal at most recording stations. Precipitation during January was below normal at most stations, so although not much snow fell during the period, what did accumulate persisted on the ground for several weeks. February precipitation returned to above-normal across central Kentucky, resulting in a relatively brief appearance of the transient lakes from Warren County west to Trigg County. The persistence of cold and snow/ice cover apparently resulted in the mortality in some species, particularly Eastern Bluebirds.

Rarity highlights included Cinnamon Teal, Red-necked Grebe, Pomarine Jaeger, multiple Iceland and Great Black-backed Gulls, Snowy Owl, several continuing Rufous Hummingbirds, Prairie Falcon, and Yellow-headed Blackbird. The period of extremely cold weather resulted in the disappearance of all of the hummingbirds that were still present. However, it also was responsible for the appearance of Snow Buntings at several locales, as well as an unprecedented mid-winter flight of waterbirds off the Great Lakes that included White-winged Scoters and Long-tailed Ducks, as well as several interesting Larids. This flight continued into March and also will be reported on in the spring 2014 summary.

Publication of any unusual sightings in the seasonal report does not imply that these reports have been accepted for inclusion in the official checklist of Kentucky birds. Reports of out-of-season birds and rarities should be accompanied by written and/or photographic documentation. This documentation is reviewed by the Kentucky Bird Records Committee (KBRC). Decisions regarding the official Kentucky list are made by the KBRC and are reported periodically in *The Kentucky Warbler*.

**Abbreviations** – County names appear in *italics*; when used to separate dates, the “/” symbol is used in place of “and”; “ba” next to an observer’s initials indicates that the bird was banded; “ph.” next to an observer’s initials indicates that the observation was documented with photograph(s); “vr.” next to an observer’s initials indicates that the observation was documented with recordings of call notes; “†” next to an observer’s initials indicates that written details were submitted with the report; **Place names:** Ballard WMA, Ballard; Barklev Dam, Lyon (unless otherwise noted); Barren River Lake, Allen/Barren (unless otherwise noted); Blood River = Blood River embayment, Ky Lake, Calloway; Calvert City, Marshall; Cave Run Lake, Bath/Rowan; Cecilia, Hardin; Cedar Creek Lake, Lincoln; Chamberlain Lane, ne. Jefferson; CVG = Cincinnati/Northern Kentucky airport, Boone; Falls of the Ohio, Jefferson; Freeman Lake, Hardin; Gibraltar = former Gibraltar Mine, e. Muhlenberg; Green River Lake, Adair/Taylor (unless otherwise noted); Jacobson Park, Fayette; Jonathan Creek = Jonathan Creek embayment, Ky Lake, Marshall; Ken Unit, Peabody WMA, Ohio; Ky Dam = Kentucky Dam, Livingston/Marshall; Kentucky Dam Village SRP, Marshall; KDFWR = Ky. Dept. of Fish and Wildlife Resources; Ky Lake = Kentucky Lake, Calloway/Marshall/Trigg; Lake Linville, Rockcastle; Lake Peewee,