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## Incidence of West Nile Virus in House Sparrow Nestlings in Relation to Parental Effort

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I am a graduating senior, majoring in Biology with an Environmental Studies minor. While at the University of Kentucky, I was a runner-up in the Biological Sciences portion of the Oswald Research and Creativity Program. I am also a member of Beta Beta Beta biological honors society and Omicron Delta Kappa leadership honors society.



In September of 2004, I am leaving to serve in the U.S. Peace Corps in The Gambia. The animal behavior analyses and bird identification skills I learned while completing this project will help me greatly in my position as an environmental extension agent, instructing native people in the importance of preserving a sustainable environment.

After the Peace Corps, I plan to return to the United States and apply to graduate schools in ecology. The experience I gained in the field and in the lab have helped to prepare me for a future in ecological research. The help and attention I received from both Dr. Westneat and Dr. Stewart truly showed me the amount of work and dedication it takes to start a new research project and see it through to the completion of a final paper.

My personal interests are wide and varied. As a student at the University of Kentucky, I have enjoyed starting up two women's rugby teams (one at the University of Kentucky, and a Lexington women's team) and serving as their captain. I also play soccer on two recreational teams, and snowboard, when time allows. I spend spare time hiking, camping, and writing poetry.

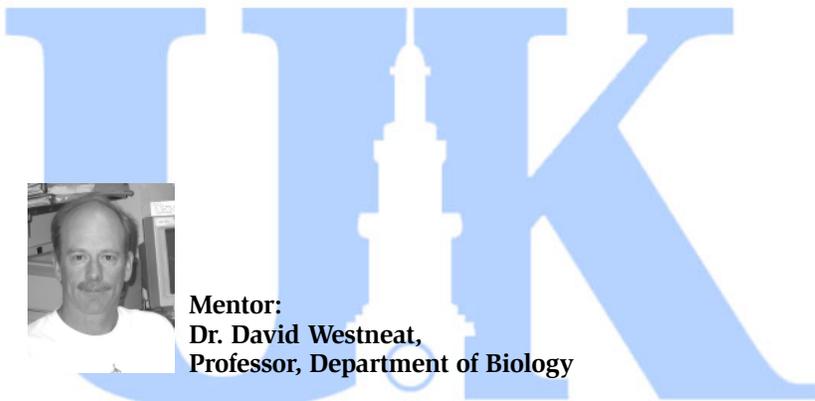
## Incidence of West Nile Virus in House Sparrow Nestlings in Relation to Parental Effort

### Abstract

The house sparrow, *Passer domesticus*, is known to be an important host of West Nile (WN) virus and may contribute to the transmission of WN virus to humans. However, little is known about the factors that influence the occurrence of WN virus in birds. I conducted a study to determine if parental care was related to the rates of WN virus infections among nestling birds. I observed house sparrow nests for one hour periods noting the time the male and female parent spent *on* the nest box, *in* the nest box, and the number of food trips, as measures of parental care. Each nestling was then tested for WN virus by PCR and gel electrophoresis. I found that, as the summer progressed, WN virus infections increased and each measure of parental care decreased. Although a number of factors may have contributed to lower parental care, my data shows that WN virus infections increased as parental effort decreased.

### Introduction

The West Nile (WN) virus began to make headlines in the summer of 1999 when it appeared in many birds and some humans in the New York City Area. Over the next 3 years, the number of human infections increased dramatically, hundreds of deaths resulted, and the virus was found in 45 states. WN virus is an Old World flavivirus, related to St. Louis encephalitis virus, and known in



**Mentor:**  
Dr. David Westneat,  
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Emerging infectious diseases, such as West Nile Virus (WNV) and SARS, seem to be on the increase. Most of the research on them is either in the laboratory or clinical setting (or, in the case of WNV, at public health labs). But the appearance and spread of a disease is inherently an ecological process that demands more focused research on the natural events that lead to contracting the virus. In the case of WNV, what affects which birds acquire the virus, and then what happens to those individuals? Natalie's project, part of the Independent Research course offered in Biology to majors (Biology 395) takes a much closer look at how the activities of individuals might affect WNV. Natalie sat for long hours watching parent birds feed their nestling, collected blood samples, used some tricky molecular techniques to identify those infected with WNV, and then put the field data together with the lab results. She had to learn a diverse array of skills in doing this, from behavioral to molecular. Her finding that nestlings contract the virus at fairly high rates is important because such birds are at a critical stage in their lives. Her other results, such as the effects of parents, are more ambiguous (as is often the case in research, particularly in natural systems). Natalie presents both types of findings here, and her paper is a great example of the ways doing research can lead students to advance technically and intellectually. Her study also makes a wonderful case for the value of mixing field and lab research.



**House Sparrows on Nest Box**

North Africa and the Middle East since the 1930s. Birds of many species appear to be the major introductory or amplifying hosts, with mosquitoes the primary vector between hosts. (Rappole et al., 2000) Indeed, the location and timing of human infections was found to be closely related to the appearance of bird deaths. (Guptill et al., 2003)

Avian exposure to WN virus is thus a critical element in the spread of this disease, but we know remarkably little about what affects exposure. For example, we know that many different species can carry the virus, but we do not know which individuals are more susceptible and why. For example, nestlings rather than adults may be a preferable host for mosquitoes. Nestlings are relatively immobile, warm, and bare; adult house sparrows are very mobile and catch insects as a food source for nestlings. Nestlings feed almost entirely on insects. (Clement, 1993) An unguarded nestling serves as an ideal host for a mosquito, which increases the risk for WN virus. I wondered if the frequency of infections in nestlings might be influenced by the parental care provided.

Parents may be able to affect the chance that young birds are exposed to mosquitoes and hence WN virus. A parent sitting on a nest protects the young by either catching the mosquito for food or receiving the bite in lieu of the nestlings. WN virus can be fatal to birds, so increased parental care should result in greater offspring survival and reduced WN virus infections. Parental investment (via high quality food) improved the immunocompetence of barn swallow offspring. (Saino et al., 1997) Providing high quality food for offspring may increase their ability to resist WN virus. The numbers of trips to bring food to a nest as well as the amount of time spent on the nest (which

can prevent mosquitoes on nestlings) are indicators of parental care.

“House sparrows in particular served as hosts for most avian WN virus infections in the bird populations we sampled...” (Komar et al., 2001, p. 622) Dispersal of house sparrow (*Passer domesticus*) fledglings might be a significant cause in the rapid westward spread of the WN virus. This along with the relative abundance of House Sparrows paired with being a common host for the WN virus makes it an ideal study subject for monitoring the WN virus. House sparrows are common reservoirs for WN Virus and might aid in spreading the virus to migratory birds.

By studying the relationship between West Nile infections in house sparrow nestlings and parental investment, I predicted that nestlings receiving more food and time from parents on the nest will have lower incidences of WN virus infection. I also predicted that August and late July should have the highest rates of parental care because that is the predominant season of WN virus infections.

### **Methodology**

House sparrows were studied on the Maine Chance Farm within the University of Kentucky’s Agricultural Experiment Station, north of Lexington, KY. The site included 6 different barns, relatively close in location, with nest boxes lining the sides. Adults had been marked earlier by trapping and banding with 3 colored leg bands and a numbered aluminum leg band. Nest boxes were checked every 3 days for eggs and then monitored until hatching.

At each nest containing nestlings 4-8 days of age, I observed the parental care for one hour. This timing is essential because parental care is the highest at this age, and nestlings were banded and tested for West Nile at 10 days old. This schedule also allowed for a suitable incubation period so that any virus infection during the peak of parental care could accumulate by the time the blood was drawn from the nestlings. These observations were taken between June 13 and August 22, 2003, between the hours of 10:30 am and 8:00 pm. Preference was given to observing in the evening hours when mosquitoes were likely to be out and an active threat to nestlings.

During each observation, I noted which parent (male or female) visited the nest, when it arrived and departed, whether it brought food for nestlings, and the time each parent (male or female) spent on top of the nest box. The data were recorded on preformed sheets and then transcribed into data files for analysis.

Blood samples were collected from all nestlings at 10 days of age. Nestlings were removed from the nest, banded, measured, and blood taken by pricking

**Table 1.**  
Parental Care, Average Clutch Size, and Rates of WNV Infections

	Avg time <i>in</i> nest (seconds)	Avg time <i>on</i> nest (seconds)	Avg no. of food trips	Avg clutch size	% nestlings with WNV
June	1668.6	302.1	14.7	3.14	22.7
July	1381.4	229.3	13.3	3.08	37.5
August	714.0	71.6	11.9	3.00	100.0

the exposed brachial vein in the wing with a needle. Blood was stored in centrifuge tubes and the nestling was returned to the nest. In the lab, I tested the blood samples for the WN virus, using a reverse transcription-nested polymerase chain reaction (PCR). (Johnson et al., 2001)

**Results**

I found that house sparrow nestlings do act as hosts for the WN virus, because roughly one-third of nestlings tested positive (36.05%) for the WN virus. Thirty-one of the 86 nestlings born to parents I observed were positive for WN virus.

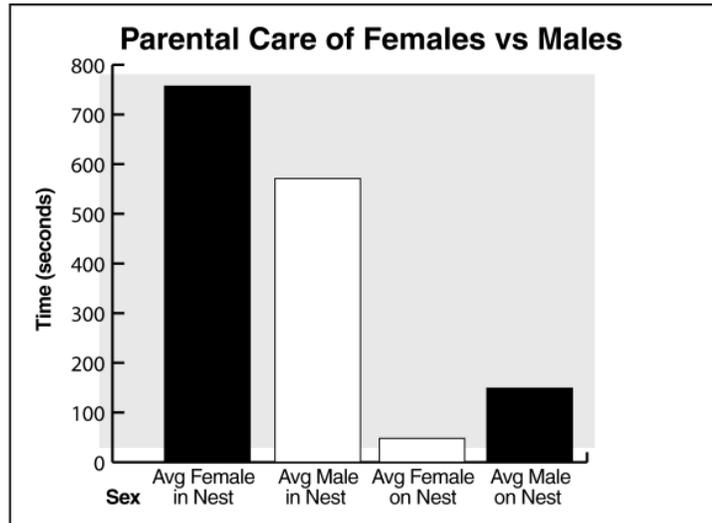
The rates of West Nile in the offspring varied across nests and months. For example, there were five nest boxes in which all nestlings tested positive for WN virus, whereas in seven other nest boxes all nestlings were negative. In June, five nestlings out of 22 tested (22.73%) were positive for WN virus. For the month of July, 12 out of 32 nestlings (37.5%) were infected. In the month of August, all 14 tested nestlings (100%) were infected with WN virus. There is an evident trend of more WN virus infections in the later summer months.

I observed a total of 28 nests, noting parental care for exactly one hour over the months of June, July, and August. The parents cared for a total of 86 nestlings, ranging from 1-5 nestlings per nest box, with an average of slightly over 3 nestlings per parental pair (Table 1). The complete data calculated from the field observations and the laboratory results is found in Appendix 1, available in the on-line version of this report at [www.uky.edu/Kaleidoscope/fall2004](http://www.uky.edu/Kaleidoscope/fall2004).

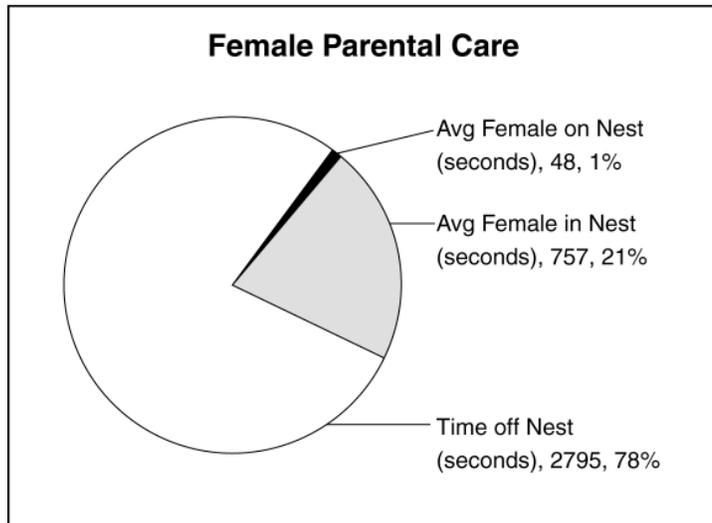
Males and females spent most of their time foraging rather than in or on the nest. Table 1 shows the number of food trips made by both males and females. Females, on average, made more food trips at 7.36 (206 food trips total) in one hour than the average number of male food trips of 5.86 (164 food trips total).

I found that females showed higher levels of parental care. The measures of time spent *in* and *on* nest are based on the one hour (3,600 second) observation period. Females spent an average of less time

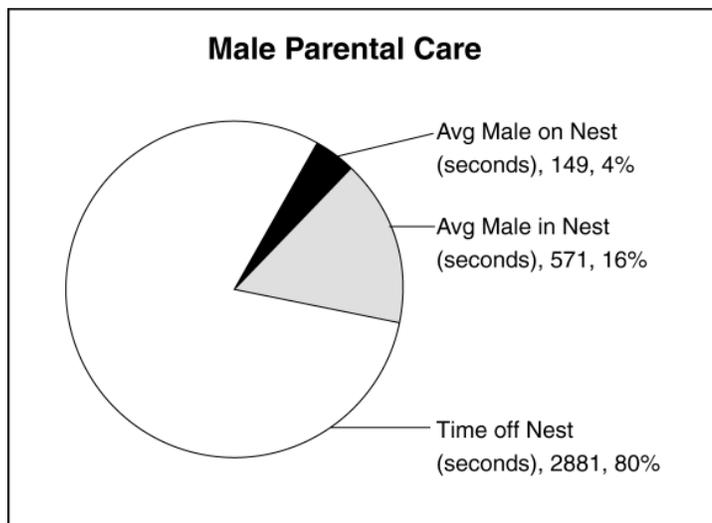
**Figure 1.**



**Figure 2.**



**Figure 3.**



on the nest (48 seconds) and more time *in* the nest (756.75 seconds) than the males. Males spent less time caring for the nestlings, spending 570.61 seconds *in* the nest, but more time than the females *on* the nest (148.82 seconds). Figure 1 shows the average time females and males spent *in* the nests and *on* the nests. Figure 2 and Figure 3 illustrate the parental care rates of females and males, respectively as a proportion of time observed. As a percentage of total time, females spent 5% more of their time *in* the nests than males (21% vs. 16%).

Nests where the male provided higher care than the female failed to show any different rates in nestling WN virus infections. This led me to conclude that male care is perhaps of equal quality to female care.

There was a decreasing trend in parental care as it got later in the season. From June to August, the total amount of time spent *on* each nest (per nest), *in* each nest (per nest), and the average number of food trips per nest decreased, as illustrated in Table 1. To reduce the effect of brood size, all values were divided by the number of nestlings hatched in that month. The greatest drop in parental care occurred from July to August and it occurred drastically in every category used to measure parental care. There was also a decrease in clutch size as it got later in the season.

There were five nest boxes in which all nestlings tested positive for WN virus, and seven nest boxes in which all nestlings tested negative. When comparing the average times *in* the nest box, *on* the nest box, and the number of food trips of the nests with WN virus positive versus negative, there was no significant difference. The standard deviation for both groups was very large, so important differences between these groups could exist and not be detected. The parents of nestlings that had WN virus even made more food trips (12.4 vs. 9.9) than parents of healthy nestlings. There was great variation in both groups for time spent *on* and *in* the nests and food trips.

## Discussion

My study shows clearly that house sparrows are exposed to WN virus at an early age, while still in the nest. This exposure increases as the season progresses. I tested for a correlation between parental behavior and WN virus infections in nestlings. I expected to see nests that experienced higher levels of parental care to have lower rates of WN virus in the nestlings. A direct comparison of nests with and without WN virus revealed no difference in parental care, but the number of nests involved was small.

However, both parental care and WN virus

changed as the season progressed. It is possible that the change in parental care may help explain the change in WN infection, although other explanations are also possible.

Food trips and time spent on the nest may have decreased over the summer months because parents became infected with WN virus. House Sparrows are common reservoirs for the WN virus and most infected birds do not die as a result of the infection. (Henderson, 2000) Parents living with the WN virus will have lower fitness due to immune response use of energy. It has been found that the large energy expenditures associated with immune response cause an overall decrease in fitness, which can result in lower brood sizes. (Martin et al., 2003) This is consistent with the lower parental care and smaller brood sizes through the high West Nile season. Unfortunately, I could not sample most of the parents, and so cannot test this hypothesis.

There is also the possibility that parents gave less care to the second or third broods of the season. This could be tested by testing the parents for WN Virus around the time of my observations. I could have also monitored whether it was the first, second, or third brood for the specific parents to evaluate this.

An unrelated but interesting finding from my study was the difference in care by parent sparrows. Females were found to spend more time on the nest and make more food trips for various reasons. Females put a greater amount of energy into reproduction due to egg production, egg laying, and spending more time incubating the eggs. This is consistent with past findings. Females primarily brood the nestlings, although males help. (The Birdhouse Network, 2003)

There could be a variety of other factors that explain lower male care. Males may have spent less time *in* the nest because they traveled farther to get food, getting more food, or catching higher quality food. Males also might be *on* the nest box to search for food, because insects are the primary food source for nestlings.

Although sparrows are considered monogamous, extrapair copulation is observed in many cases. (Rising, 1996) Paternity is not always clear, but the female can be certain that the eggs she laid will contain half of her genes. Males need to be territorial, not only to protect the nestlings, but to prevent other males from mating with the female. This may also explain why males spent more time on top of the nest box. The defended nesting territory is immediately around the nest site. (Rising, 1996) Sitting on top of the nest box is the best site to observe the males' nesting territory. Females might spend less time on the nests because they are less territorial. There is also a chance

that lower quality males did not find mates until later in the season, and this could account for lower care.

WN virus is an important disease both in its effects on wildlife and on humans or livestock. My study found that nestling birds may often be exposed to WN virus at a very young age. This new finding affects our understanding of the spread of this disease, because nestlings soon leave the nest and wander, potentially spreading the disease to other locations. Given that the nest site may be a prime location for mosquitoes to find hosts, I attempted to uncover factors, such as parental behavior, that might affect exposure to WN virus. I found few such factors, but did note that WN virus increased through the season while parental care decreased. My data are not sufficient to conclude whether it was the higher probability of being infected with WN virus later in the season or the lower parental care that caused the increasing rates of infection. However, this research opens interesting new questions about how parental care of house sparrow nestlings might relate to the spread of infectious disease in other species.

#### Acknowledgements

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*Male House Sparrow*

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