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Predation of Rio Grande Wild Turkey Nests on the Edwards Plateau, Texas

Justin Z. Dreibelbis,[1] Kyle B. Melton,[2] Ray Aguirre,[3] Bret A. Collier,[1,5] Jason Hardin,[4] Nova J. Silvy,[1] and Markus J. Peterson[1]

ABSTRACT.—We followed the fate of nests of Rio Grande Wild Turkeys (*Meleagris gallopavo intermedia*) on the Edwards Plateau of Texas during 2006 and 2007 using motion-activated digital cameras on a subset of nests to evaluate the frequency of nest predation and to identify nest predators. Predation was the primary cause of loss for nests with cameras, accounting for 57 and 65% in 2006 and 2007, respectively. Predation for nests without cameras also was high (69 and 65% for 2006 and 2007, respectively) suggesting the cameras did not increase the probability of nest failure. We documented partial-and multiple-predator events that could result in misidentification of nest predators. Our results provide insight into nest predator communities and confirm that multiple predator events occur with regularity in the wild. *Received 3 December 2007. Accepted 29 April 2008.*

Natality is one of the primary biological processes influencing dynamics of wildlife populations (Everett et al. 1980). Understanding which factors cause changes in individual and group natality is important for managing bird populations. Methods to estimate and understand components of nest survival have received recent attention, particularly for species of ground nesting birds (Dinsmore et al. 2002, Shaffer 2004, Grant et al. 2005). A variety of factors can influence nest survival, but for ground nesting birds, nest predation appears most influential (Ricklefs 1969, Farnsworth and Simons 2000, Rollins and Carroll 2001, Stephens et al. 2005). Given the vulnerability of ground nesting species, predation will affect nest survival and population productivity (Baker 1978, Rollins and Carroll 2001).

Accurate identification of nest predators of ground nesting birds is important in understanding effects of predation on population parameters (Lariviere 1999, Rader et al. 2007). Nest predation studies often rely on physical evidence at the nest, such as tracks, hair, and eggshell fragments to identify predators (Major 1991, Lariviere 1999). Use of physical evidence can be highly subjective (Trevor et al. 1991, Lariviere 1999), and may fail to account for multiple-predator and partial-predation events (Leimgruber et al. 1994). Predation events may be difficult to identify if eggshells are removed by the incubating hen following partial nest predation (Lariviere and Walton 1998), or if predation is by reptilian or avian species, as snakes consume whole eggs in the nest (Staller 2001) and avian species often remove eggs from the nest before consumption (Montevecchi 1976).

Abundance of Rio Grande Wild Turkeys (*Meleagris gallopavo intermedia*) on the southeastern Edwards Plateau, Texas has declined since the late 1970s (Randel et al. 2005, Collier et al. 2007a). Recent work has focused on evaluating factors contributing to this decline (Collier et al. 2007b), including variation in reproductive potential and nest survival (Melton 2007). Predation is the primary cause of nest failure in the region (Cook 1972, Melton 2007), and nest loss can adversely influence Wild Turkey populations (Davis 1959, Baker 1978). Our objectives were to: (1) identify predators of Rio Grande Wild Turkey nests and (2) examine the frequency of total nest loss, partial predation events, and multiple-predator predation events.

METHODS

Study Area.—Our study area was within the Edwards Plateau region of Texas; we studied

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[1] Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA.

[2] Texas Parks and Wildlife Department, Waco, TX 76706, USA.

[3] Texas Parks and Wildlife Department, Comfort, TX 78013, USA.

[4] Texas Parks and Wildlife Department, Palestine, TX 75803, USA.

[5] Corresponding author; e-mail: bret@tamu.edu

Wild Turkey nesting from January through July 2006 and 2007 on four sites in Kerr, Real, Bandera, and Medina counties. All study sites were rangelands with flat to rolling divides, shallow soils, and limestone bedrock (Gould 1975), and included private ranches and the Kerr Wildlife Management Area (Texas Parks and Wildlife Department). Study sites ranged in size from 984 to 8,858 ha and all were managed for hunting of native and exotic wildlife; livestock grazing occurred on three of the sites (Kerr, Medina, and Bandera counties).

Field Procedures.—We trapped Wild Turkey hens during January–March, 2006 and 2007. We attached radio transmitters (69.0–95.0 g; Advanced Telemetry Systems, Isanti, MN, USA) to 39 and 22 hens in 2006 and 2007. We located individual hens three times weekly (White and Garrott 1990) during the breeding season until behaviors indicated a hen had initiated a nest (Ransom et al. 1987). We located nests within 1 day after we suspected hens had begun incubation. Once located, we ascertained initiation date, clutch size, and approximate age for each nest. We estimated nest age and initiation date by backdating from the day we found the nest to the day we first located the hen in the nest area. We defined the active nesting period as 39 days; the sum of the average number of eggs in a clutch (11) and a 28-day incubation period (Bailey and Rinnell 1967, Melton 2007). We floated eggs to estimate age of nests found during incubation (Westerskov 1950), and monitored nests three times weekly from a distance of ≥100 m to prevent further disturbance to the hen. We assumed the nest was active if hen locations remained constant. One week before estimated hatch date, we visited nests daily to ensure accurate identification of hatch date.

We used motion-activated trail cameras (Game Spy 100 and Outfitter Cam, Moultrie Feeders, Alabaster, AL, USA) at a sample of nests. Each camera was equipped with 16 MB of internal memory (we added a 256 MB memory card to each camera in 2007), a 10.2 mm lens, and a 9.14 m flash. We learned through a pilot study in 2005 that cameras set within 5 m of a nest require flash reduction, otherwise night photographs were over-exposed. To reduce flash, we covered 100% of the flash surface with one to three layers of masking tape, dependent upon nest distance (most often one layer/m from the nest under 5 m). We attached the camera, based on vegetation surrounding the nest area, to a tree near the nest or to a post. We programmed cameras to take two pictures ~5 sec apart, followed by a 5 or 10-min delay. After the delay period, the next event in the nest area would trigger the camera. We checked cameras after initial setup, only when the bird was located out of the nesting area for more than 1 day. Nests receiving camera surveillance were chosen randomly across study sites depending on camera availability and nest initiation timing.

TABLE 1. Nest predators documented via remotely–triggered cameras at active Rio Grande Wild Turkey nests in the Edwards Plateau, Texas, 2006–2007 (*n* = number of nests with photographed predation events).

Species	2006 (*n* = 7 nests)	2007 (*n* = 11 nests)
Nine-banded armadillo (*Dasypus novemcinctus*)	0	1
Bobcat (*Lynx rufus*)	0	1
Feral hog (*Sus scrofa*)	2	1
Gray fox (*Urocyon cinereoargenteus*)	4	2
Common raccoon (*Procyon lotor*)	2	7
Common Raven (*Corvus corax*)	0	3
Striped skunk (*Mephitis mephitis*)	2	0
Texas rat snake (*Elaphe obsolete lindheimeri*)	1	0
Total multiple predator events	3	4

RESULTS

We placed cameras at 21 of 47 active turkey nests in 2006, with 12 (57%) nests depredated and eight (38%) nests abandoned. These rates are comparable to 69% depredation and 15% abandonment for those nests in our study without cameras. Three of 12 depredated nests with cameras involved more than one predator, four involved a single predator, and five had no photographs of the nest predator (Table 1). We placed cameras at 31 of 71 active nests in 2007. Twenty of 31 (65%) nests with cameras were depredated and 6 of 31 (19%) were abandoned. Four of the depredated nests involved more than one predator, seven involved a single predator, and nine had no predator photographs. We observed 68% (27/40) predation and 18% (7/40) abandonment at nests without cameras in 2007. Nests survived on average 12.5 and 13.0 days with and without cameras in 2006, and 18.4 and 18.7 days with and without cameras in 2007.

We were able to examine timing of predation events in greater detail on approximately half the nests with cameras. For example, a multiple predator event occurred at a nest of a yearling hen found incubating her first nest containing 11 eggs on 17 May 2006. We flushed the hen, estimated nest age at 6 days of incubation, and placed a camera at the nest. We recorded a remarkable series of predation events on 19 May at this nest. At 1818 hrs, a common raccoon (scientific names of predator species are in Table 1) was recorded leaving the nest area and subsequent photographs showed a raccoon consuming an egg ~2 m from the nest. Later that evening (2212 hrs), two photographs (<10 sec apart) were taken of a raccoon predating the nest. Shortly thereafter (2242 hrs), a gray fox visited the nest. Less than 1 hr later (2328 hrs), a striped skunk depredated the nest followed by a gray fox that visited the nest at 2344 hrs. We documented additional predator visits on subsequent days. Raccoons were observed at the nest on 20 May at both 0111 and 0705 hrs as well as on 22 May at 0005 hrs. We photographed feral hogs at the nest on 22 May at 0534 hrs and 2315 hrs, removing the remaining shell fragments from earlier predation events. The last recorded nest visitor was a raccoon on 24 May at 0409 hrs. The hen remained in the general vicinity of the nest until 24 May when we examined the nest site at 1126 hrs, finding no eggshell remains and little disturbance to the leaf litter. Given there was no evidence (egg shells, tracks, scat, hair, etc.) at the nest site when researchers arrived, we initially believed a reptilian or avian predator was responsible.

We also documented an instance of partial nest predation. We located the nest on 17 April 2006, during incubation by an adult hen of her first nest of the season, which contained 16 eggs. We monitored the nest for 28 days, which was successful, and located the hen with eight poults on 14 May. When we returned to the nest area to collect eggshells, we found remnants of only nine hatched eggs. Upon checking the photographs, we found the nest had been partially depredated by a Texas rat snake 11 days earlier. On 3 May at 2118 hrs, we photographed the snake in the nest. The hen hatched the remaining nine eggs on 13 May 2006. There was no physical evidence at the nest, and we initially believed the nest was predated by either a reptilian or avian predator.

DISCUSSION

Our observations indicate that nest predation was the proximate factor affecting overall nest survival of Rio Grande Wild Turkeys during our study, although our sample of nests was fairly small. Additionally, our results suggest that nest predation events involving multiple predators were common. There is a diverse predator community on the Edwards Plateau (Davis and Schmidly 1994) and key predators can change from year to year. The method of depredation used and the evidence left at the nest site after depredation events (e.g., eggshell fragments) may overlap among species. Gray fox were documented in 2006 at 57% of the predation events but were photographed at only 2 (18%) predation events in 2007 (both of which involved multiple predators). Three of 11 (27%) camera nests in 2007 identified Common Ravens removing eggs; however, no ravens were photographed in 2006. Nests depredated by ravens were similar to those depredated by snakes as they contained no shell fragments and had little disturbance around the nest.

Staller (2001) correctly identified 61% of predators at Northern Bobwhite (*Colinus virginianus*) nests using physical evidence at the nest site as compared to data from miniature video cameras; however, diversity of predators on his study area was small. Only 12% of predation events from Staller (2001) involved multiple predators compared to Leimgruber et al. (1994) who observed multiple predator visits (2–5 species) in 43% of predation events, a rate similar to ours. Hernandez et al. (1997) attempted to construct a dichotomous key for identification of ground-nest predators in west Texas but were not successful because of insufficient physical evidence and overlap of nest predation habits among species. Incubating Blue-winged Teal (*Anas discors*) and Mallard (*A. platyrhynchos*) hens are known to remove damaged eggs and shell fragments from the nest area following partial predation events by striped skunks (Lariviere and Walton 1998).

The relationship between ground nesting birds and nest predators is complicated, and we caution researchers to understand the limitations of using physical evidence to predict nest predator species. Our results provide insight into nest predator communities and confirm that multiple predator events are frequent (39% of the predation events recorded with cameras in our study) in the wild. Multiple predation events can greatly alter physical evidence left at the depredated nest site; thus, it is crucial that researchers test and apply any method which is used to assess nest predator communities before mitigation strategies are developed.

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No Evidence for Spring Re-introduction of an Arbovirus by Cliff Swallows

Valerie A. O'Brien,[1] Amy T. Moore,[1] Kathryn P. Huyvaert,[2] and Charles R. Brown[1,3]

ABSTRACT.—We sampled 100 Cliff Swallows (*Petrochelidon pyrrhonota*), just after arrival in Nebraska breeding areas, to ascertain if migrating birds re-introduce Buggy Creek virus (BCRV; Togaviridae) to north-temperate localities in spring. Most birds sampled were previously banded and were known to have used parasite-free nesting colonies in past summers and/or were seronegative to BCRV; thus, they were unlikely to have been previously exposed to the virus in their breeding areas. None of the birds had evidence of viral RNA in blood, as measured by RT-PCR. These results are consistent with other studies that have shown little evidence that migratory birds re-introduce arboviruses to temperate localities between years. *Received 14 February 2008. Accepted 5 May 2008.*

Whether arthropod-borne viruses (arboviruses) are re-introduced in spring by migratory birds in temperate latitudes is a major question in the study of bird-associated viruses (Reeves 1974, Scott and Weaver 1989, Crans et al. 1994). Arboviruses are rarely found in over-wintering insect vectors such as mosquitoes (Rosen 1987, Reeves 1990, Day 2001), and the conventional wisdom is that infected birds from the tropics—that are fed upon by insect vectors (e.g., mosquitoes) after arrival in breeding areas—may provide a mechanism for annual recurrence of virus in temperate latitudes of central and northern North America (Cilnis et al. 1996, Unnasch et al. 2006). Empirical evidence for this scenario is limited, however, and consists mostly of a few records of birds (bound for unknown destinations) with eastern equine encephalomyelitis virus when captured in spring after crossing the Gulf of Mexico (Calisher et al. 1971). Demonstrating virus re-introduction requires sampling birds upon their arrival at breeding sites sufficiently early in the nesting season that re-infection by local vectors can be excluded if positive birds are found. No studies have systematically surveyed newly arrived migratory birds for arboviruses.

Buggy Creek virus (Togaviridae, *Alphavirus*) is an unusual arbovirus vectored primarily by the swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*), an ectoparasite of the

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[1] Department of Biological Sciences, University of Tulsa, Tulsa, OK 74104 USA.

[2] Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, CO 80523 USA.

[3] Corresponding author; e-mail: charles-brown@utulsa.edu

colonially nesting Cliff Swallow (*Petrochelidon pyrrhonota*). Vertebrate hosts for Buggy Creek virus (BCRV) are Cliff Swallows and House Sparrows (*Passer domesticus*) that occupy nests in swallow colonies (Hayes et al. 1977, Scott et al. 1984). A related alphavirus, Fort Morgan virus, is a strain of BCRV (Pfeffer et al. 2006). BCRV, although not documented to affect humans, is phylogenetically and serologically related to western equine encephalomyelitis virus (WEEV) (Calisher et al. 1988, Powers et al. 2001), and WEEV affects both people and livestock (Reisen and Monath 1989). Birds have been suggested to move WEEV between North and South America (Weaver et al. 1997).

We sampled Cliff Swallows for virus immediately after the birds' arrival at breeding sites in southwestern Nebraska, USA as part of our efforts to understand the population dynamics of BCRV and its association with Cliff Swallows (Brown et al. 2001; Moore et al. 2007; Brown et al. 2007, 2008). Our objective was to examine whether these birds were infected with BCRV upon their return and could potentially re-introduce the virus to their breeding areas.

Cliff Swallows breed throughout much of North America, nesting in large colonies underneath cliff overhangs and bridges, and winter in southern Brazil, Uruguay, and northern Argentina (Brown and Brown 1995). BCRV occurs annually in our Nebraska study area and is commonly isolated from the insect vectors (Brown et al. 2001, 2007; Moore et al. 2007). Its predictable annual occurrence suggests the virus either over-winters in swallow bugs and/or in resident House Sparrows, or is re-introduced each season by Cliff Swallows when they return from their winter range in South America.

METHODS

Long-term work in our study area (in Keith, Garden, Lincoln, and Morrill counties, Nebraska) indicates the first Cliff Swallows appear on about 18 April each year with numbers slowly increasing during the following 10 days (Brown and Brown 1996: 443). The first arrivals tend to concentrate at the same 2–3 colony sites in the study area (C. R. Brown, pers. obs.). We mist-netted Cliff Swallows between 23 and 29 April 2006 and 2007 at two colony sites (41° 15′ N, 101° 37′ W; 41° 13′ N, 101° 37′ W) that contained most birds present in the study area at that time. The early sampling dates ensured that birds at both sites were newly arrived. Both colony sites sampled had been fumigated multiple times per summer during the previous 10 seasons to remove swallow bugs, suggesting that few bugs were present in April and the likelihood of any bird being infected by a bug after arrival and before sampling was low. The insecticide used is highly effective against swallow bugs (Brown and Brown 2004). Fumigation procedures are described by Brown and Brown (1996).

Birds caught were bled by jugular or brachial venipuncture, in which 0.1 ml of blood was collected and placed in 0.4 ml of BA-1 diluent (Moore et al. 2007). Samples were centrifuged and 25 μl of supernatant was added to 100 μl of a guanidine thiocyanate-based lysis buffer. RNA was extracted after the addition of 100 μl of 100% ethanol using the QIAmp Viral RNA Mini Kit (Qiagen), following the manufacturer's protocol. A positive BCRV control was included in each extraction.

Reverse-transcription PCR (RT-PCR) was performed using the OneStep RT-PCR Kit (Qiagen) following the manufacturer's protocol. We used BCRV-specific primers that yielded a 208-bp fragment from the E2 region of the viral genome. Primer sequences and thermocycler conditions are described in Moore et al. (2007). Product (6.5 μl) was electrophoresed on a 4% Nusieve/agarose gel to identify any positive pools, using at least one BCRV positive control on each gel and a 100-bp ladder. This protocol was used for detecting BCRV in both swallow bug pools and sera of nestling House Sparrows, which are commonly infected (about 25% of bug pools and >20% of nestling sparrows; Moore et al. 2007; V. A. O'Brien and C. R. Brown, unpubl. data). Our RT-PCR methods have also detected BCRV in sera of Cliff Swallows during the summer nesting season, including samples confirmed by both RT-PCR and plaque assay on Vero cells (V. A. O'Brien and C. R. Brown, unpubl.data).

RESULTS

We captured 100 Cliff Swallows during the sampling periods in the 2 years. None of the 100 birds had evidence of circulating BCRV

RNA in blood, as judged from RT-PCR. All but 14 birds had been banded in the study area in a previous breeding season. Eighty-one were at least 2 years of age and their history of breeding-colony use was known for at least one previous year. Five had been banded the previous year as recently fledged juveniles. Fifty-nine of the 86 birds with past histories were known to have been resident at only fumigated colonies in the past (the same sites sampled in this study), 13 had used only non-fumigated sites in the past, and 9 had used both fumigated and non-fumigated sites in previous seasons. The 5 juveniles had been captured at fumigated colonies a few days after fledging.

DISCUSSION

Birds that had used parasite-free sites in past seasons were unlikely to have been exposed to BCRV in a previous summer and therefore not likely to show latent, chronic infections (as seen for some arboviruses; Reisen et al. 2003). The 47 birds sampled in 2007 were tested for BCRV-specific antibodies using a plaque reduction neutralization test (Huyavert et al. 2008); none of these birds was seropositive (G. R. Young and N. Komar, pers. comm.). Thus, the individuals sampled in this study were well suited to studying whether virus could be introduced by migrants that were infected prior to arrival in breeding areas.

Hayes et al. (1977) sampled 52 adult Cliff Swallows for the Fort Morgan strain of BCRV on 30 May 1974 in northeastern Colorado, ~215 km from our study area. That study used plaque assay and found no evidence of BCRV in swallows. Hayes et al. (1977) concluded that no evidence existed for spring re-introduction of virus by returning birds, although their samples were taken sufficiently late in the season that birds had begun egg-laying at time of sampling. Using a more sensitive assay (RT-PCR), we also found no evidence of circulating BCRV (i.e., viral RNA) in blood of adult Cliff Swallows, and our birds had arrived at most only a few days before sampling.

Most birds we sampled had probably not been exposed to BCRV in breeding areas, by virtue of their use of fumigated colony sites in past years (and, for some, their lack of antibodies to BCRV). Thus, they were prime candidates for transporting virus from wintering areas or from stopover sites en route. Surveys for BCRV have not been conducted in South America, and whether it occurs in wintering areas is unknown. BCRV is found at Cliff Swallow colony sites south of our study area, for example in west central Oklahoma, about 750 km from the Nebraska study area (Hopla et al. 1993; C. R. Brown, V. A. O'Brien, and A. T. Moore, unpubl. data). Migrating Cliff Swallows conceivably could be infected there and move the virus north to Nebraska.

Our results are consistent with the absence of direct evidence that migrating birds, re-introduce arboviruses to temperate localities. It is more likely these viruses persist annually by over-wintering in insect vectors or alternative resident hosts. Over-wintering of BCRV in swallow bugs is known to occur (Hayes et al. 1977, Rush et al. 1980, Strickler 2006). House Sparrows may be more suitable hosts for BCRV than Cliff Swallows, at least in summer (V. A. O'Brien and C. R. Brown, unpubl. data), and may provide another mechanism for annual persistence of virus. This is especially true if BCRV is maintained via latent, chronic infections in vertebrate tissue over long periods of time (Huyvaert et al. 2008). The role of migratory birds in re-introducing arboviruses to temperate latitudes in spring is unclear, and we urge all studies finding even negative evidence for re-introduction be reported.

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