



EARLY IMPACT OF WEST NILE VIRUS ON THE YELLOW-BILLED MAGPIE (*PICA NUTTALLI*)

SCOTT P. CROSBIE,¹ WALTER D. KOENIG,² WILLIAM K. REISEN,³ VICKI L. KRAMER,⁴
LAUREN MARCUS,⁴ RYAN CARNEY,⁵ EDWARD PANDOLFINO,⁶ GINGER M. BOLEN,⁷
LIZETTE R. CROSBIE,⁸ DOUGLAS A. BELL,⁹ AND HOLLY B. ERNEST^{1,10}

¹Wildlife and Ecology Unit, 216 CCAH, Veterinary Genetics Laboratory, University of California, One Shields Avenue, Davis, California 95616, USA; ²Hastings Natural History Reservation, 38601 E. Carmel Valley Road, Carmel Valley, California 93924, USA; ³Center For Vectorborne Diseases, University of California, One Shields Avenue, Davis, California 95616, USA; ⁴California Department of Health Services, Vector-Borne Disease Section, 1616 Capitol Avenue, MS 7307, P.O. Box 997413, Sacramento, California 95899, USA; ⁵California Department of Health Services, Vector-Borne Disease Section, 850 Marina Bay Parkway, Richmond, California 94804, USA; ⁶5530 Delrose Court, Carmichael, California 95608, USA; ⁷North State Resources, 5000 Bechelli Lane, Suite 203, Redding, California 96002, USA; ⁸Sacramento Area Flood Control Agency, 1007 7th Street, 7th Floor, Sacramento, California 95814, USA; ⁹East Bay Regional Park District, 2950 Peralta Oaks Court, P.O. Box 5381, Oakland, California 94605, USA; and ¹⁰Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, California 95616, USA

ABSTRACT.—Several sources of data suggest that the Yellow-billed Magpie (*Pica nuttalli*), a corvid endemic to California, is extremely susceptible to West Nile virus (WNV) and that its abundance has decreased since the establishment of WNV throughout California in 2004. From 2004 to 2006, 12,211 Yellow-billed Magpie carcasses were reported to the California Department of Health Services. Seventy-eight percent of the 1,007 Yellow-billed Magpie carcasses tested were WNV-positive, and this was the highest proportion of WNV-positive carcasses of all California bird species with reasonable sample sizes (>20). Assuming a starting population size of 180,000 in 2003, California Department of Health Services data suggest that Yellow-billed Magpie populations may have been reduced by 49% in just two years. Breeding Bird Survey (BBS) data show a 22% decline as of 2005, and Christmas Bird Count (CBC) data show a 42% decline as of 2006. Furthermore, flock size at three traditional urban communal roosts monitored in Sacramento, California, decreased dramatically after the establishment of WNV, with two becoming vacant by summer 2005 and the third declining precipitously into 2006. Of 38 serum samples obtained from 21 Yellow-billed Magpies in Davis, California, in 2006, only one individual was found to produce WNV-specific antibodies. Range-wide monitoring is warranted to detect and track population trends of this species. Population size, genetic diversity and population structure, cause-specific mortality, and population viability should be evaluated. *Received 24 February 2007, accepted 18 September 2007.*

Key words: Breeding Bird Survey, Christmas Bird Count, communal roost, Corvidae, dead-bird surveillance, *Pica nuttalli*, West Nile virus, Yellow-billed Magpie.

Impacto Temprano del Virus del Oeste del Nilo sobre *Pica nuttalli*

RESUMEN.—Varias fuentes de información sugieren que *Pica nuttalli*, un córvido endémico de California, es extremadamente susceptible al virus del oeste del Nilo (VON), y que su abundancia ha disminuido desde que el VON se estableció a través de California en 2004. Entre 2004 y 2006, 12,211 cadáveres de esta especie fueron reportados al departamento de servicios de salud de California. El 78% de los 1,007 cadáveres en los que se realizaron pruebas fueron VON-positivos, y ésta fue la mayor proporción de cadáveres VON-positivos entre todas las especies de aves de California que contaron con tamaños de muestra razonables (>20). Suponiendo que la población inicial era de 180,000 individuos en 2003, los datos del departamento de servicios de salud de California sugieren que las poblaciones de esta especie podrían haberse reducido en un 49% en sólo dos años. Los datos de los censos de aves reproductivas muestran un declive del 22% hasta 2005, y los del conteo navideño de aves un declive del 42% hasta 2006. Además, el tamaño de las bandadas observadas en tres sitios tradicionales de descanso comunal monitoreados en Sacramento, California,

¹¹E-mail: spcrosbie@ucdavis.edu

disminuyó dramáticamente después del establecimiento del VON, y dos de estos sitios quedaron vacíos para el verano de 2005 mientras que el tercero disminuyó precipitadamente hasta 2006. De 38 muestras serológicas de 21 individuos obtenidas en 2006 en Davis, California, sólo un individuo demostró producir anticuerpos específicos para el VON. Se requiere realizar un monitoreo a nivel de todo el ámbito de distribución de la especie para detectar y seguir sus tendencias poblacionales. El tamaño poblacional, la diversidad genética y su estructura poblacional, la mortalidad causada por factores específicos, y la viabilidad poblacional deben ser evaluados.

THE YELLOW-BILLED MAGPIE (*Pica nuttalli*; hereafter “magpie”) is a corvid endemic to California, its range limited primarily to the Sacramento and San Joaquin valleys and sections of the Central Coast Ranges (Reynolds 1995; Fig. 1). Habitat conversion, rodent poisoning, and shooting may have contributed to a reduction in numbers and range contraction in the past century (Reynolds 1995). Although the current population size is unknown, Reynolds (1995) estimated the minimum number of breeding pairs at 25,000–50,000, and the National Audubon Society (NAS) and Partners in Flight (PIF) both estimate total population size at ~180,000 (Rich et al. 2004, NAS 2006). However, these estimates are based on extrapolation of data from limited surveys and may over- or underestimate population size.

Although the magpie does not currently have special-status listing under either the state or the federal Endangered Species Act, it is listed as a “watch list” species by NAS and PIF and as a “woodland focal species” by California PIF (California PIF 2005, NAS 2006), primarily because of its restricted range. Here, we focus on the new, emerging threat of West Nile virus (WNV), which was introduced into North America in 1999

and has caused high corvid mortality in several areas (Barker 2003, Komar 2003, Komar et al. 2003, Yaremych et al. 2004, Caffrey et al. 2005), including California (Airola et al. 2007, Koenig et al. 2007). The first record of WNV in the magpie was documented in summer 2004 by the California Department of Health Services (CDHS) Dead Bird Surveillance Program (DBSP), which solicits the public to report dead birds for WNV testing by calling a hotline or using a website reporting system (see Acknowledgments).

Because of the magpie’s restricted range and its apparently high susceptibility to WNV, our goal was to analyze available data to assess the effects that WNV may have had on the magpie to date.

METHODS

California’s Dead Bird Surveillance Program.—In 2000, the CDHS initiated the DBSP throughout California. The CDHS compiles reports of dead birds reported by the public in a database that includes species, carcass location, reporting date, and carcass condition. Only carcasses in good condition and estimated to be <24 h old were picked up by local agency personnel for WNV testing. Carcasses were delivered to the California Animal Health and Food Safety Laboratory System, where a sample of kidney tissue was removed and sent to the Center for Vectorborne Diseases at the University of California, Davis, for testing for the presence of viral RNA specific to WNV using a real-time reverse-transcription polymerase chain reaction (RT-PCR) assay (Reisen et al. 2004) with published primers (Lanciotti et al. 2000). Virus isolation was also attempted from pooled organs of RNA-positive birds using a plaque assay on Vero cell culture (Reisen et al. 2004). We entered DBSP data from 2004–2005 into JMP, version 6.0 (SAS Institute, Cary, North Carolina), and used a binomial logistic regression to compare the proportion of WNV-positive carcasses of magpies with data from other California corvids.

Breeding Bird Surveys.—As an index of magpie populations in California and the potential effects of WNV following the spread of the disease throughout the state in 2004, we examined changes in numbers of magpies counted between successive years during the U.S. Geological Survey’s (USGS) North American Breeding Bird Survey (BBS) from 1995 to 2005 (USGS 2007). The BBS is conducted yearly in June along arbitrarily established roadside routes throughout North America. Surveys start 0.5 h before local sunrise and consist of recording all birds detected during 3-min point counts performed at 50 established sites 0.8 km apart on each route.

Routes were included only if they (1) detected magpies in at least one year from 1995 to 2005, (2) were conducted by the same individual in each of the two successive years being compared (because of interpersonal differences in counting abilities; see Sauer

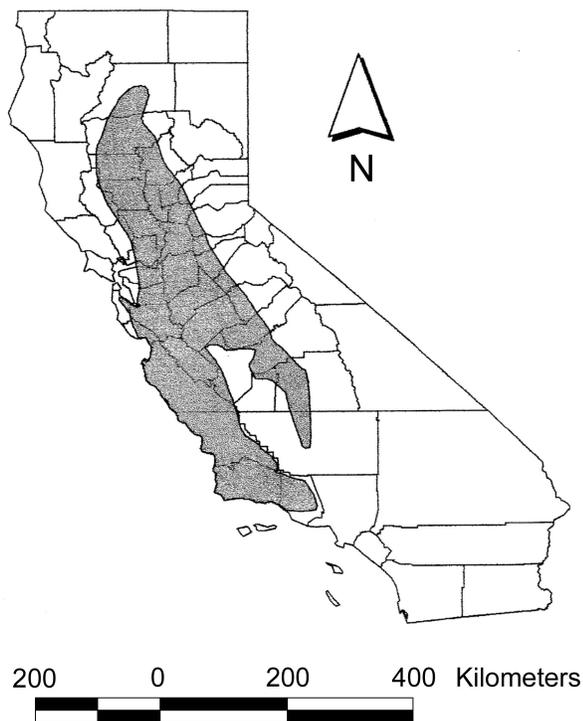


FIG. 1. Map of California with outline of counties and historical distribution of the Yellow-billed Magpie (shaded).

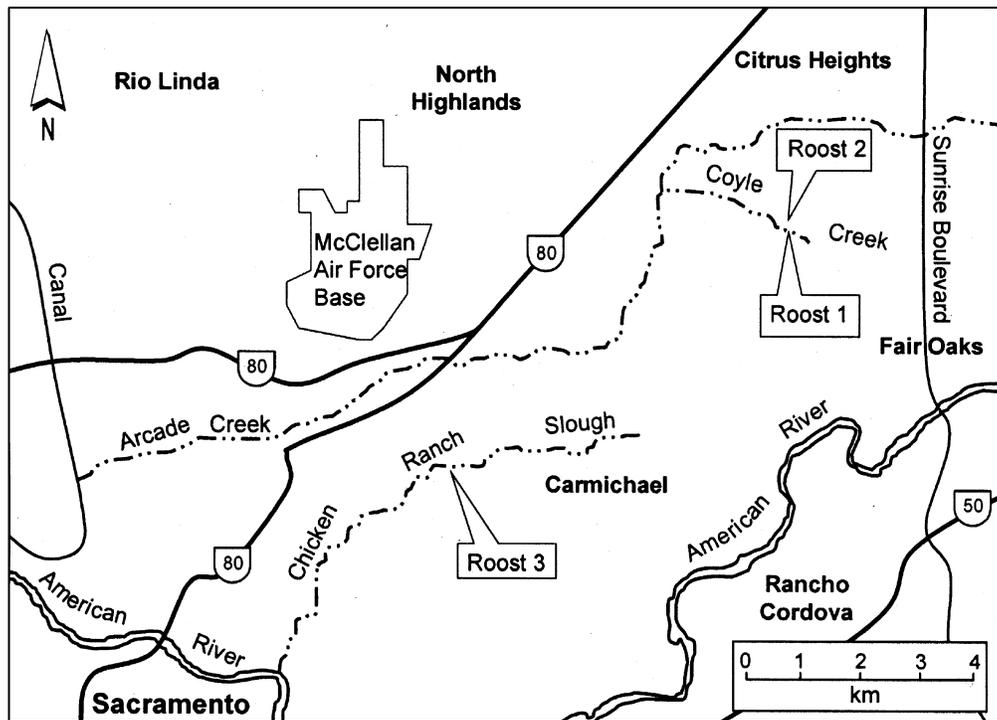


FIG. 2. Location of three Yellow-billed Magpie communal roosts in Sacramento, California, at which roost flock size was estimated weekly from July 2003 to June 2004 and from September 2005 to March 2006.

et al. 1994), and (3) were conducted every year since 1995. Nine routes located throughout the magpie's range met these criteria (route codes 21, 80, 97, 98, 108, 153, 155, 159, and 189). Year-to-year change in magpie detections was analyzed by calculating the difference in mean number of magpies detected per route between successive years.

Christmas Bird Counts.—With the same objectives as the BBS data analysis, we examined changes in mean number of magpies detected per survey party hour in successive years during NAS's Christmas Bird Count (CBC; NAS 2008) in central California from 1995 to 2006. Each CBC is conducted within a 24.1-km-diameter circle that is surveyed annually by volunteers on a single day scheduled during the last two weeks of December or the first week of January (Dunn et al. 2005). Sites were included if they had at least one magpie detection from 1995 to 2006 and were conducted every year since 1995. Twelve sites located throughout the magpie's range met these criteria (site codes CAAA, CACW, CAGV, CALS, CAMD, CAMH, CAMR, CAPI, CAPV, CARE, CASJ, and CAST). Year-to-year change was analyzed by calculating the difference in mean number of magpies detected per party hour between successive years.

To test whether low numbers of magpies detected in 2005 could have been attributable to poor weather conditions (many compilers noted heavy rain), we conducted the same analysis of CBC data for three "control" species, the Mourning Dove (*Zenaidura macroura*), Black Phoebe (*Sayornis nigricans*), and American Robin (*Turdus migratorius*). We chose these species because they are common and widespread within the analyzed count sites, are easy to detect and identify, are found in many of the same habitats as the

magpie, and are generally considered less vulnerable to WNV than corvids (Koenig et al. 2007; note, however, that this study did not include the Mourning Dove).

Communal-roost monitoring.—Magpies are highly social birds that roost in flocks for much of the year (Reynolds 1995, Crosbie et al. 2006). From July 2003 to June 2004, we conducted pre-WNV flock-size estimations ($n = 167$) at three roost sites, at least two of which have been used by magpies over the past 10–20 years (W. Shepard and M. Morris pers. comm.). Flock size was estimated by recording roost arrivals and departures approximately weekly at three urban communal roosts of the magpie in Sacramento, California. Roost 1 was located on Coyle Creek in Fair Oaks, and roost 2 was located ~113 m north of roost 1 (Fig. 2). Roost 3 was ~8.3 km southwest of roost 2, near Chicken Ranch Slough. All roosts were located on or between developed residential lots and were dominated by cultivated shrubs and trees.

For morning observations, we arrived at the roost at least 45 min before sunrise and counted magpies departing the roost (number min^{-1}) until no birds were observed departing the roost for at least 20 min and no remaining birds were heard within the roost. For evening observations, we arrived at the roost at least 1.5 h before sunset and counted magpies arriving at the roost (number min^{-1}) until no further birds arrived for at least 20 min and it was too dark to see birds in flight. The minute-by-minute counts were totaled to estimate roost flock size. Observations of roosts 1 and 2 were made from roofs of nearby houses approximately 40–50 m away, respectively, and observations of roost 3 were made from a sidewalk ~40 m from the roost. In general, the birds were easily visible and countable. Yet, during roost arrivals, there was often

TABLE 1. Locations and captures of Yellow-billed Magpies for collection of serum samples, March–December 2006, in Davis, California.

Location of traps (Latitude, longitude)	Number of new captures	Number of recaptures	Number of hatch-year birds	Number of after-hatch- year birds
El Marcero Country Club (38.5433, -121.6939)	0	3	0	3
University of California, Davis, Agronomy Field Lab (38.5392, -121.7819)	1 ^a	0	NA	NA
Davis Cemetery (38.5533, -121.7269)	20	14	5	29

^aThis individual was not aged.

two-way traffic, with birds coming in and out of the roost (this phenomenon was rarely observed during roost exodus). When roost flock size was high, several successive evening arrivals and morning departures were observed, and comparison of evening and morning counts showed very little difference in total number of birds estimated, which suggests that two-way traffic did not cause over- or underestimation of flock size.

The first case of a WNV-positive bird in Sacramento County was confirmed on 22 July 2004 (Sacramento County Department of Health and Human Services unpubl. data). Post-WNV estimations of roost flock size ($n = 51$) were resumed from late summer 2005 through mid-March 2006, following the second summer of the WNV epizootic in Sacramento.

West Nile virus seroprevalence.—From March through December 2006, one crow trap baited with cat food and sunflower seeds was set at each of three locations in Davis, California (Table 1). Twenty-one magpies were captured, banded, bled, and released. Of these, 17 were recaptured and bled on subsequent occasions ranging from one to eight weeks later. Forty-eight Western Scrub-Jays (*Aphelocoma californica*) were also sampled, 10 of which were recaptured one to seven times over a period of 1–13 weeks. Blood samples (0.1 mL) were taken by jugular venipuncture, diluted 1:10 with phosphate-buffered saline, and clarified by centrifugation. Serum samples were screened for WNV, western equine encephalomyelitis, and St. Louis encephalitis (SLEV) antibodies by an enzyme-linked immunosorbent assay (EIA) using *Flavivirus* and western equine encephalomyelitis virus antigens (Chiles and Reisen 1998). Presumptive EIA positives were confirmed using a plaque-reduction neutralization test (PRNT) on live virus, with positives producing >80% reduction of more than 50–60 plaques on Vero cell culture of the NY99 strain of WNV or the KERN217 strain of SLEV at a $\geq 1:20$ dilution. To identify the infecting virus, an endpoint titer $\geq 4\times$ the competing virus was used to separate antibodies raised by WNV or SLEV.

RESULTS

In 2003, before the arrival of WNV in the magpie’s range, 223 magpie carcasses were reported to the DBSP by the public. Of those, all 66 carcasses tested for WNV were negative. In 2004, the

first year WNV entered the range of the magpie (the first known magpie case occurred in July), 2,667 magpie carcasses were reported to the DBSP and 304 (81%) of 374 carcasses tested WNV-positive. Only 4.1% of these 2,667 carcasses were reported before June, whereas 68.2% were reported from July through September, when WNV transmission rates were generally highest. In 2005, 8,751 magpie carcasses were reported to the DBSP and 363 (82%) of 444 tested WNV-positive. In 2006, 793 magpie carcasses were reported to the DBSP and 122 (65%) of 189 tested WNV-positive. The proportion of carcasses testing positive for WNV among all dead corvids tested by the California Animal Health and Food Safety Laboratory System from 2004 to 2005 is shown in Table 2. A binomial logistic regression showed that Yellow-billed Magpie carcasses were significantly more likely to be WNV-positive than any other corvid tested except for Black-billed Magpies (*Pica hudsonia*) (Table 3).

Results of the 1995–2005 BBS data analysis are shown in Figure 3. The number of magpies detected per route has increased by an average of 0.7% each year since 1995. In 2005, however, 21.6%

TABLE 2. Proportion of carcasses that tested positive for West Nile virus (WNV) among all dead corvids tested by the California Animal Health and Food Safety Laboratory System from 2004 to 2005. Samples were screened for WNV by an enzyme-linked immunosorbent assay and confirmed using a plaque-reduction neutralization test with positives producing >80% reduction of more than 50–60 plaques on Vero cell culture of the NY99 strain of WNV.

Species	Total tested for WNV (n)	WNV positive (n)	Proportion of carcasses testing WNV positive (%)
Pinyon Jay	2	2	100
Black-billed Magpie	8	7	87
Yellow-billed Magpie	818	667	82
Western Scrub-Jay	2,062	1,440	70
American Crow	5,294	2,988	56
Steller’s Jay ^a	184	90	49
Common Raven	336	46	14

^a*Cyanocitta stelleri*; all other scientific names are given in the text.

TABLE 3. Results of a binomial logistic regression comparing the proportion of Yellow-billed Magpie carcasses that tested positive for West Nile virus from 2004 to 2005 with the proportions of five other California corvids that tested positive ($n = 818$ Yellow-billed Magpie carcasses). Data are from the California Department of Health Services Dead Bird Surveillance Program.

Species	WNV positive (n)	P	Odds ratio (95% confidence interval)
Black-billed Magpie	8	0.6499	0.631 (0.055–2.667)
Western Scrub-Jay	2,062	>0.0001	1.908 (1.398–3.129)
American Crow	5,294	>0.0001	3.409 (2.516–5.575)
Steller's Jay	184	>0.0001	4.776 (3.129–7.834)
Common Raven	336	>0.0001	27.848 (18.743–47.658)

fewer magpies were detected per route compared with the 2004 average.

Results of the 1995–2006 CBC data analysis are shown in Figure 4. The number of magpies detected per party hour has decreased by an average of 10.3% since 1995. In 2004, 6.4% more magpies were detected per party hour compared with the 2003 average. From 2004 to 2005, and 2005 to 2006, 29.7% and 16.6% fewer magpies were detected per party hour, respectively. There was no evidence of a decline in the numbers of any of the control species from 2004 to 2005. American Robin, Mourning Dove, and Black Phoebe detections per party hour increased by 288.1%, 30.3%, and 9.0% from 2004 to 2005, respectively. Thus, we assume that any potential change in detectability attributable to poor weather conditions in 2005 was negligible.

Roost flock size fluctuated markedly in the year preceding the arrival of WNV in Sacramento County (Fig. 5A–C). In

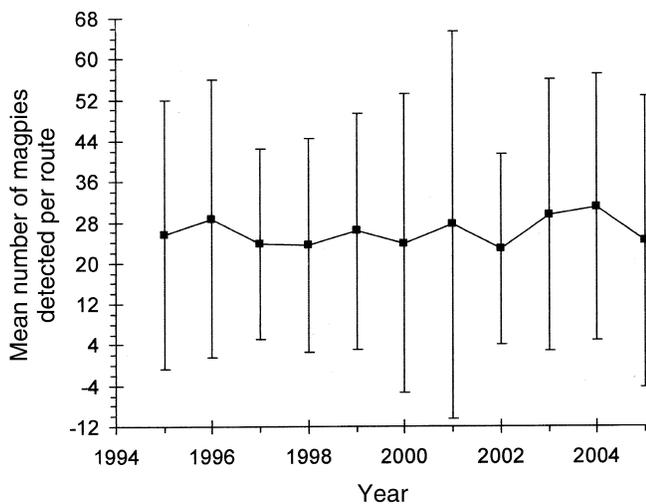


FIG. 3. Mean number of Yellow-billed Magpies detected at nine Breeding Bird Survey routes from 1995 to 2005. Standard-deviation bars are indicated.

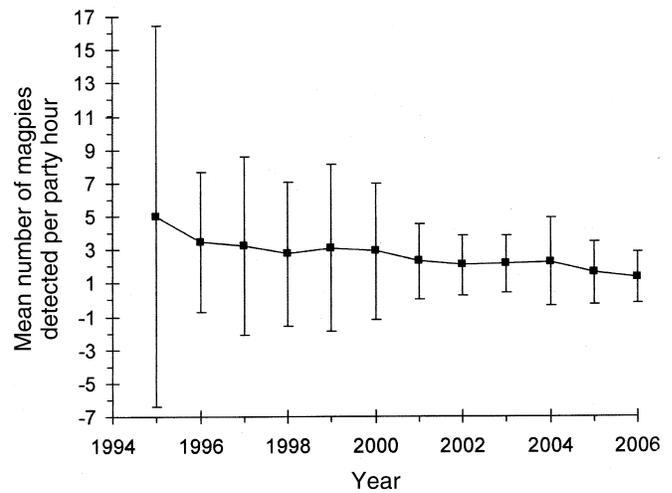


FIG. 4. Mean number of Yellow-billed Magpies detected per party hour at 12 Christmas Bird Counts in California's Central Valley from 1995 to 1996. Standard-deviation bars are indicated.

2003 and 2004, the number of magpies occupying roosts 1, 2, and 3 ranged from 0 to 913 birds, 0 to 133 birds, and 0 to 993 birds, respectively. Roost 1 reached three major peaks during the study, one during mid-November 2003 (913 birds), one during early March 2004 (879 birds), and one during mid-June 2004 (442 birds). Fluctuation in occupancy at roost 2 was similar, but fewer birds used this roost. At roost 3, 993 birds were present during mid-November 2003, 818 during early March 2004, and 762 in mid-June 2004. Roost 3 had an additional peak not represented at the other roosts during mid-January 2004 (651 birds). Peaks in roost occupancy during early March and mid-June corresponded with the prebreeding and postbreeding seasons, respectively. Mid-November peaks followed the wandering phase that occurs during the summer drought in the Central Valley (Reynolds 1995). Roosts 1 and 2 were consistently and completely vacant from late summer 2005 through March 2006 (Fig. 5A, B), the period when use of these roosts was highest in 2003 and 2004. Magpies were still using roost 3, but numbers were considerably lower (Fig. 5C). The peak at roost 3 in November 2005 had 21.8% fewer birds than the peak in 2003, and flock size at roost 3 declined precipitously into 2006. In 2004 and 2005, ≥ 24 WNV-positive magpies were collected in the vicinity of these roosts (CDHS unpubl. data), and, in summer 2005, local residents near roosts 1 and 2 reported finding "dozens" of dead magpies at these roosts (M. Morris pers. comm.).

Of the 38 plasma samples obtained from 21 magpies collected in Davis, only one individual (4.8%) tested positive for WNV-specific antibodies. This bird was negative when first caught in April and then strongly WNV-positive when recaptured in September, with an EIA positive–negative well ratio of 2.21 and a PRNT titer against WNV of $>1:80$ and against St. Louis encephalitis of $<1:20$. Twenty-five percent (12 of 48) of Western Scrub-Jays were WNV-positive.

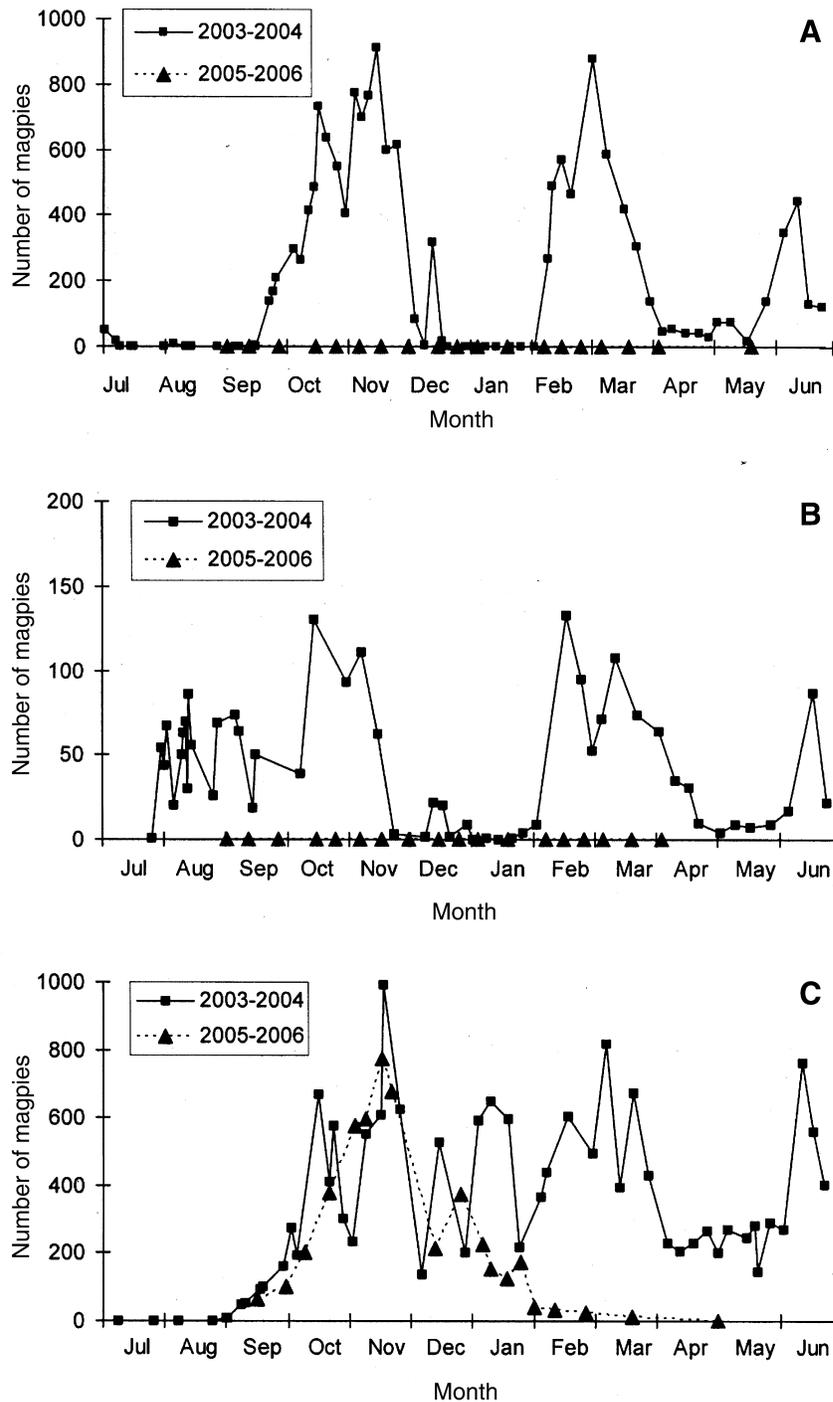


FIG. 5. Flock-size estimations from July 2003 to June 2004 and from September 2005 to March 2006 at communal roosts of the Yellow-billed Magpie in Sacramento, California: (A) roost 1, (B) roost 2, and (C) roost 3.

DISCUSSION

Using data from several independent sources, our results suggest that WNV may have had a significant effect on magpie populations. The DBSP data show a striking increase in the number of

magpie carcasses reported to CDHS from 2003 to 2006, which may be attributable, in part, to an increase in public awareness and vigilance associated with WNV surveillance programs. When comparing the proportion of WNV-positive carcasses among all corvids tested, the highest were found in the Pinyon

Jay (*Gymnorhinus cyanocephalus*) and Black-billed Magpie; yet both had low sample sizes (2 and 8, respectively). Aside from these two species, the Yellow-billed Magpie had the highest proportion of WNV-positive carcasses, and the odds of finding a WNV-positive carcass ranged from 1.9 to 27.8 times higher for magpies than for any other California corvid analyzed (Table 3).

Breeding Bird Survey and CBC data suggest that a population decline has occurred since WNV establishment. Assuming that the population size started at 180,000 (Rich et al. 2004), BBS data show a reduction of 21.6% (38,849 birds) from 2004 to 2005. Christmas Bird Count data from 2004 to 2006 show a larger reduction of 41.7% (74,999 birds) from the pre-WNV estimate. In an independent analysis using a different set of CBC sites, Airola et al. (2007) found a post-WNV decline of 48%, similar to our findings. Although we cannot directly attribute these declines to WNV alone, these data suggest that, when compared with pre-WNV years, a dramatic decline took place after WNV establishment. However, both the BBS and the CBC data (particularly the BBS data) show large standard deviations for most years (see Figs. 3 and 4), which suggests that minor changes from year to year may not be biologically meaningful. Nevertheless, that both sources of trend data analyzed exhibit declines suggests a definite decrease in abundance after WNV establishment.

Examples of published carcass-detection rates may provide further insight on the impact of WNV. Ward et al. (2006) recently assessed the reporting rates of American Crow (*Corvus brachyrhynchos*) carcasses in Georgia, where an active WNV dead-bird surveillance system similar to California's was in place. The researchers put marked American Crow carcasses in the environment, and reporting rates were assessed after the public was solicited to report dead birds. Reporting rates of 17% and 3% were reported for urban and rural areas, respectively. For the present study, we calculated a mean expected carcass reporting rate of 13.8% using BBS, CBC, and DBSP data. This was calculated by dividing the known number of carcasses reported to the DBSP by the expected population decreases calculated from the BBS and CBC data for each year from 2004 to 2006. Assuming a carcass reporting rate of 13.8% throughout the magpie's range, the 12,211 magpie carcasses reported by the public from 2004 to 2006 would equate to a total loss of 88,485 magpies (49.2% decline). Unfortunately, we do not know what carcass-detection and reporting rates are for magpies in California and, more generally, the level of uncertainty in such sources of data is unknown.

Communal-roost surveys before and after WNV establishment in Sacramento showed a dramatic local decline following the establishment of WNV. Communal roosting is a common behavior for many corvids (Goodwin 1986), and some are known to occupy traditional roost-sites for many years. For example, some winter roosts of the American Crow span several human generations (Madson 1976), and Common Ravens (*C. corax*) have exhibited roost fidelity over many years in a variety of locations (Cushing 1941, Temple 1974, Stiehl 1981, Cotterman and Heinrich 1993). Judging from historical observations of magpie roosts (Linsdale 1946, Verbeek 1973) and what is known of the roosts in the present study, it seems that magpies are quite faithful to roost-sites. Residents living at or near the roosts we observed confirmed that magpies have used roost 1 consistently for at least 20 years and roost 2 for 10 years. Furthermore, magpies are known to have occupied

the neighborhood encompassing these roosts for at least the past 50 years. Unfortunately, no history of roost occupancy was available for roost 3. Except for the establishment of WNV in the Sacramento area, no other factors were obviously responsible for the decline; no changes in roost structure were evident, nor were there known attempts to evict the birds. In 2005, high incidence of viral infection in mosquitoes, birds, and humans in central California led to the characterization of the area as a WNV epicenter (State of California WNV maps and data; see Acknowledgments). The disappearance of magpies from the roosts observed in the present study in 2005 and 2006 suggests that a dramatic local population decline occurred, rather than a pattern of shifting use. However, it is possible that use patterns changed in response to observed deaths or to other factors not measured in our study.

If a large proportion of the population had survived WNV infection, we would expect to find a larger number of individuals with WNV antibody production. That only 1 of 21 individuals (4.8%) had WNV-specific antibodies suggests that the species may have a low rate of survival of WNV infection. Granted, this was a small sample of birds from a limited geographic area that may not be representative range-wide. Studies in Illinois (Beveroth et al. 2006), New York (Komar et al. 2001), and Georgia (Gibbs et al. 2006) found mean seroprevalence rates of 6.6%, 23.0%, and 6.2%, respectively, in resident (nonmigratory) birds. That seroprevalence in magpies was found to be lower by an average of 7.1% suggests that they experience higher mortality from WNV (see Beveroth et al. 2006).

Gibbs et al. (2006) and Beveroth et al. (2006) reported seroprevalence rates of 3.4% (9 of 261) for the American Crow and 2.2% (1 of 46) for the Blue Jay (*Cyanocitta cristata*), respectively. These rates are similar to what we observed for magpies. A seroprevalence rate of 25.0% was found in the concurrently sampled Western Scrub-Jay, which suggests that it may be less prone to WNV-related mortality than the magpie (assuming that rates of serum antibody decay, exposure, survival, immunocompetence, and re-immunization are equivalent between the two species). None of these studies, including ours, tested for cell-mediated responses that may play a role in fighting WNV infection (Samuel and Diamond 2006). In addition, carcass submissions themselves may be biased by anything affecting carcass-detection rates (color or size of species, species-specific behaviors, etc.).

In the magpie's limited range, there are, presumably, no refuges where WNV-vectoring mosquitoes are absent. West Nile virus-positive pools of *Culex tarsalis*, *C. pipiens*, and *C. quinquefasciatus* have been reported throughout the species' range (State of California "positive mosquito pools by species" table; see Acknowledgments), and these mosquitoes are considered primary vectors in the enzootic maintenance and transmission of WNV (Goddard et al. 2002, Reisen et al. 2005). Additionally, the behavioral characteristics of magpies may put them at greater risk of WNV infection than some other corvids, for several reasons: (1) magpies form large roost flocks from dusk to dawn, often near water (Crosbie et al. 2006); (2) they are highly social, and there is evidence that oral or direct exposure can serve as WNV-transmission mechanisms (Peterson 2004); and (3) WNV may be transmitted by ingestion of infected carrion (Peterson 2004), a food that magpies will consume and generally consume more often than jays (see Reynolds 1995, Curry et al. 2002). Although the North American strain of WNV appears to be more virulent for birds

than other strains (Peterson et al. 2004), genetic resistance may develop and lead to lower mortality rates for magpies in the future. Nonetheless, in the meantime, a range contraction and genetic bottleneck may result from high WNV-associated mortality rates, predisposing the population to inbreeding depression and a decline toward extinction vortex (Gilpin and Soulé 1986).

On 15 December 2005, the above data (available to that date) were presented at a Yellow-billed Magpie Working Group meeting at the University of California, Davis. Participants included local and state agency and institutional representatives, and experts on the magpie, WNV, and population monitoring. Discussion at the meeting led to a consensus letter that was presented to state, federal, and nongovernmental agencies, with the following conclusions. (1) West Nile virus may endanger the persistence of the magpie. (2) Listing the species may be warranted. (3) Initiating range-wide monitoring is critical, and studies that should be considered include estimation of current population size and changes in abundance and distribution over time, comparison of pre- and post-WNV population genetic diversity, monitoring of nesting success and use of traditional communal roosts, and estimation of cause-specific mortality. (4) Computer-based modeling should be used to predict population viability and test potential management options. (5) Protection and management of habitat should occur, including reducing the risk of mosquito-borne diseases. And, (6) assessment of the need, benefits, risks, and practicality of a captive breeding program may need consideration.

While much of the natural history of this species remains unknown, the magpie may play an important role in California. Richardson and Bolen (1999) found that the presence of magpies was positively correlated with the nesting success of Bullock's Orioles (*Icterus bullockii*); whether such a benefit is to be had by other species is unknown but plausible. Furthermore, because of their avid acorn-caching behavior (Linsdale 1946) and the evidence that acorn caching favors oak (*Quercus* spp.) seedling establishment (Johnson et al. 2002), magpies may contribute to the abundance and dispersal of native oak species. Losses or extirpation of magpies may be detrimental to oak recruitment and functioning of oak woodland and savanna ecosystems in California. These ecosystems are already affected by land conversion (Bolsinger 1988), overgrazing (Welker and Menke 1990), competition with introduced annuals (Welker and Menke 1990), and fire suppression (Mensing 1992). Because of the magpie's limited distribution and population decline, monitoring and conservation of this species should be considered a high priority by wildlife managers.

ACKNOWLEDGMENTS

We thank L. Woods of the California Animal Health and Food Safety Laboratory System and I. Holser of the California Department of Health Services (CDHS) for their efforts and coordination; members of the Yellow-billed Magpie Working Group for collaboration; Magpie Monitor (www.magpiemonitor.org) volunteers for their assistance; M. Schlenker, W. Shepard, and M. Morris for allowing access to roosts; the U.S. Geological Survey, Canadian Wildlife Service, and Breeding Bird Survey (BBS) participants for their efforts in collecting, organizing, and allowing access to BBS data; the National Audubon Society for their efforts in collecting, organizing, and allowing access to Christmas Bird

Count data; from the Center for Vectorborne Diseases, S. Wheeler and V. Armijos, who collected blood samples, and S. Garcia and Y. Fang, who tested these samples for antibodies; J. Well of the University of California (UC) Davis Wildlife and Ecological Genetics Laboratory for helping compile data; N. Yanga for field assistance and critical review; A. Engilis and M. Truann of UC Davis Museum of Wildlife and Fish Biology, W. Boyce of UC Davis, S. Torres and D. Steele of the California Department of Fish and Game, M. Reynolds of The Nature Conservancy, and D. Airola of Airola Environmental Consulting, for valuable insight; and the anonymous reviewers for their improvement of the manuscript. West Nile virus (WNV) antibody testing was funded in part by grant ROI-A155607 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, along with funds awarded to W.K.R. for mosquito research. S.P.C. conducted the communal-roost monitoring as part of a thesis presented to the Department of Biological Sciences at California State University, Sacramento, in partial fulfillment of the requirements for the M.S. degree. He is currently a doctoral student at UC Davis, working with H. Ernest to research Yellow-billed Magpie ecology and population health. The CDHS Dead Bird Surveillance Program's website reporting system is at westnile.ca.gov/dead_bird_report.php. For state of California WNV maps and data, go to westnile.ca.gov/maps_data.htm. For the "positive mosquito pools by species" table, see westnile.ca.gov/website/maps_data/mosquito_species.pdf.

LITERATURE CITED

- AIROLA, D. A., S. HAMPTON, AND T. MANOLIS. 2007. Effects of West Nile virus on sensitive species in the lower Sacramento Valley, California: An evaluation using Christmas Bird Counts. *Central Valley Bird Club Bulletin* 10:1–22.
- BARKER, I. 2003. West Nile virus and animals: Reservoirs, sentinels and victims. Pages 21–22 *in* Mosquitoes and West Nile Virus: Present Situation and Perspective (T. D. Galloway, Ed.). Proceedings of the 59th Annual Meeting of the Entomological Society of Manitoba, Winnipeg, Manitoba.
- BEVEROTH, T. A., M. P. WARD, R. L. LAMPMAN, A. M. RINGIA, AND R. J. NOVAK. 2006. Changes in seroprevalence of West Nile virus across Illinois in free-ranging birds from 2001 through 2004. *American Journal of Tropical Medicine and Hygiene* 74:174–179.
- BOLSINGER, C. L. 1988. The hardwoods of California's timberlands, woodlands, and savannas. U.S. Department of Agriculture, Forest Service, Resource Bulletin PNW-RB-148.
- CAFFREY, C., S. C. R. SMITH, AND T. J. WESTON. 2005. West Nile virus devastates an American Crow population. *Condor* 107:128–132.
- CALIFORNIA PARTNERS IN FLIGHT. 2005. Executive steering committee meeting minutes, November 2, 2005. [Online.] Available at www.prbo.org/calpif/pdfs/exec05minutes.pdf#search=%22audubon%20species%20of%20concern%20yellow-billed%20magpie%22.
- CHILES, R. E., AND W. K. REISEN. 1998. A new enzyme immunoassay to detect antibodies to arboviruses in the blood of wild birds. *Journal of Vector Ecology* 23:123–135.
- COTTERMAN, V., AND B. HEINRICH. 1993. A large temporary roost of Common Ravens. *Auk* 110:395.
- CROSBIE, S. P., D. A. BELL, AND G. M. BOLEN. 2006. Vegetative and thermal aspects of roost-site selection in urban Yellow-billed Magpies. *Wilson Journal of Ornithology* 118:532–536.

- CURRY, R. L., A. T. PETERSON, AND T. A. LANGEN. 2002. Western Scrub-Jay (*Aphelocoma californica*). In *The Birds of North America*, no. 712 (A. Poole and F. Gill, Eds.). Birds of North America, Philadelphia.
- CUSHING, J. E., JR. 1941. Winter behavior of ravens at Tamales Bay, California. *Condor* 43:103–107.
- DUNN, E. H., C. M. FRANCIS, P. J. BLANCHER, S. R. DRENNAN, M. A. HOWE, D. LEPAGE, C. S. ROBBINS, K. V. ROSENBERG, J. R. SAUER, AND K. G. SMITH. 2005. Enhancing the scientific value of the Christmas Bird Count. *Auk* 122:338–346.
- GIBBS, S. E. J., A. B. ALLISON, M. J. YABSLEY, D. G. MEAD, B. R. WILCOX, AND D. E. STALLKNECHT. 2006. West Nile virus antibodies in avian species of Georgia, USA: 2000–2004. *Vector-Borne and Zoonotic Diseases* 6:57–72.
- GILPIN, M. E., AND M. E. SOULÉ. 1986. Minimum viable populations: Processes of species extinction. Pages 19–34 in *Conservation Biology: The Science of Scarcity and Diversity* (M. E. Soulé, Ed.). Sinauer Associates, Sunderland, Massachusetts.
- GODDARD, L. B., A. E. ROTH, W. K. REISEN, AND T. W. SCOTT. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerging Infectious Diseases* 8:1385–1391.
- GOODWIN, D. 1986. *Crows of the World*, 2nd ed. St. Edmundsbury Press, Suffolk, United Kingdom.
- JOHNSON, P. S., S. R. SHIFLEY, AND R. ROGERS. 2002. *The Ecology and Silviculture of Oaks*. CABI, New York.
- KOENIG, W. D., L. MARCUS, T. W. SCOTT, AND J. L. DICKINSON. 2007. West Nile virus and California breeding bird declines. *Eco-health* 4:18–24.
- KOMAR, N. 2003. West Nile virus: Epidemiology and ecology in North America. *Advances in Virus Research* 61:185–234.
- KOMAR, N., J. BURNS, C. DEAN, N. A. PANELLA, S. DUSZA, AND B. CHERRY. 2001. Serologic evidence for West Nile virus infection in birds in Staten Island, New York, after an outbreak in 2000. *Vector-Borne and Zoonotic Diseases* 1:191–196.
- KOMAR, N., S. LANGEVIN, S. HINTEN, N. NEMETH, E. EDWARDS, D. HETTLER, B. DAVIS, R. BOWEN, AND M. BUNNING. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerging Infectious Diseases* 9:311–322.
- LANCIOTTI, R. S., A. J. KERST, R. S. NASCI, M. S. GODSEY, C. J. MITCHELL, H. M. SAVAGE, N. KOMAR, N. A. PANELLA, B. C. ALLEN, K. E. VOLPE, AND OTHERS. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase–PCR assay. *Journal of Clinical Microbiology* 38:4066–4071.
- LINSDALE, J. M. 1946. Yellow-billed Magpie. Pages 155–183 in *Life Histories of North American Jays, Crows and Titmice* (A. C. Bent, Ed.). U.S. National Museum Bulletin 191.
- MADSON, J. 1976. The dance on Monkey Mountain and other crow doings. *Audubon* 78:52–61.
- MENSING, S. A. 1992. The impact of European settlement on Blue Oak (*Quercus douglasii*) regeneration and recruitment in the Tehachapi Mountains, California. *Madroño* 39:36–46.
- NATIONAL AUDUBON SOCIETY. 2006. Audubon's WatchList 2002–2006 in taxonomic order by geographic region. [Online.] Available at <http://audubon2.org/webapp/watchlist/AudubonWatchList2002.pdf>.
- NATIONAL AUDUBON SOCIETY. 2008. [Christmas Bird Count] Historical Results. [Online.] Available at www.audubon.org/bird/cbc/hr/index.html.
- PETERSON, A. T., N. KOMAR, O. KOMAR, A. NAVARRO-SIGÜENZA, M. B. ROBBINS, AND E. MARTÍNEZ-MEYER. 2004. West Nile virus in the New World: Potential impacts on bird species. *Bird Conservation International* 14:215–232.
- REISEN, W. K., Y. FANG, AND V. M. MARTINEZ. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. *Journal of Medical Entomology* 42:367–375.
- REISEN, W. [K.], H. LOTHROP, R. CHILES, M. MADON, C. COSSEN, L. WOODS, S. HUSTED, V. KRAMER, AND J. EDMAN. 2004. West Nile virus in California. *Emerging Infectious Diseases* 10:1369–1378.
- REYNOLDS, M. D. 1995. Yellow-billed Magpie (*Pica nuttalli*). In *The Birds of North America*, no. 180 (A. Poole and F. Gill, Eds.). Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, D.C.
- RICH, T. D., C. J. BEARDMORE, H. BERLANGA, P. J. BLANCHER, M. S. W. BRADSTREET, G. S. BUTCHER, D. W. DEMAREST, E. H. DUNN, W. C. HUNTER, E. E. IÑIGO-ELIAS, AND OTHERS. 2004. *Partners in Flight North American Landbird Conservation Plan*. Cornell Lab of Ornithology, Ithaca, New York.
- RICHARDSON, D. S., AND G. M. BOLEN. 1999. A nesting association between semi-colonial Bullock's Orioles and Yellow-billed Magpies: Evidence for the predator protection hypothesis. *Behavioral Ecology and Sociobiology* 46:373–380.
- SAMUEL, M. A., AND M. S. DIAMOND. 2006. Pathogenesis of West Nile virus infection: A balance between virulence, innate and adaptive immunity, and viral evasion. *Journal of Virology* 80:9349–9360.
- SAUER, J. R., B. G. PETERJOHN, AND W. A. LINK. 1994. Observer differences in the North American Breeding Bird Survey. *Auk* 111:50–62.
- STIEHL, R. B. 1981. Observations of a large roost of Common Ravens. *Condor* 83:78.
- TEMPLE, S. A. 1974. Winter food habits of ravens on the Arctic Slope of Alaska. *Arctic* 27:41–46.
- U.S. GEOLOGICAL SURVEY. 2007. North American Breeding Bird Survey internet data set. [Online.] Available at <http://www.pwrc.usgs.gov/bbs/index.html>.
- VERBEEK, N. A. M. 1973. *The exploitation system of the Yellow-billed Magpie*. University of California Publications in Zoology 99:1–58.
- WARD, M. R., D. E. STALLKNECHT, J. WILLIS, M. J. CONROY, AND W. R. DAVIDSON. 2006. Wild bird mortality and West Nile virus surveillance: Biases associated with detection, reporting, and carcass persistence. *Journal of Wildlife Diseases* 42:92–106.
- WELKER, J. M., AND J. W. MENKE. 1990. The influence of simulated browsing on tissue water relations, growth and survival of *Quercus douglasii* (Hook and Arn.) seedlings under slow and rapid rates of soil drought. *Functional Ecology* 4:807–817.
- YAREMYCH, S. A., R. E. WARNER, P. C. MANKIN, J. D. BRAWN, A. RAIM, AND R. NOVAK. 2004. West Nile virus and high death rate in American Crows. *Emerging Infectious Diseases* 10:709–711.