# Gender determination and lack of sex-specific West Nile virus mortality in American crows

*Sarah A. Yaremych, Jeffrey M. Levengood, Robert J. Novak, Phil C. Mankin, and Richard E. Warner*

**Abstract** With a set of external measurements from a sample of known-sex American crows (*Corvus brachyrhynchos*) in central Illinois, we determined whether a published discriminant function for sex determination was applicable to crows in a different geographic location than the reference sample. Low classification success with this equation, likely due to geographic size differences in crows between regions, led to the creation of new age-specific discriminant functions, which correctly classified the sex of 100%, 100%, and 89% of hatch-year, subadult, and adult crows, respectively. This technique for gender determination is of timely importance as the American crow is a sentinel for West Nile virus transmission, and ecological studies of this species are important in understanding transmission dynamics. Gender determination by discriminant function of these Midwestern crows indicated that the 2002 West Nile virus epizootic did not cause sex-biased mortality.

**Key words** American crow, *Corvus brachyrhynchos*, discriminant function analysis, sex-specific mortality, West Nile virus

American crows (*Corvus brachyrhynchos*) (hereafter crows) have been the focus of much epidemiological research since the introduction of West Nile virus (WNV) to the United States in the summer of 1999. This species experiences high mortality from WNV (McLean et al. 2001, Komar et al. 2003) and therefore serves as a sentinel for early detection before transmission of the virus to humans (Eidson et al. 2001). Differences in WNV mortality rates between sexes in the crow are currently unknown, however, because this species exhibits no sexual dimorphism in plumage and only a slight difference in size between sexes. Disease-related mortality rate differences between sexes are of evolutionary interest, and information in this area will provide insight into the population effects of WNV on American crows.

Discriminant function analysis (DFA) is a multivariate statistical tool that combines predictor variables to produce a function that maximizes the statistical separation of groups. Through this technique an unknown observation is assigned to a group with a low error rate on the basis of data related to the group, with the assumption that the initial data that formed the groups was classified correctly (Lachenbruch 1975). This technique

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allowed us to rapidly assign sexes to field-captured crows with little specialized equipment and may be more desirable than invasive methods such as endoscopy or more costly methods such as karyotyping.

Clark et al. (1991) used a discriminant function to determine the gender of crows in Saskatchewan. However, this function may not be suitable for sex determination of crows from outside the geographic area of the reference crows used to create the function, as crows display geographical variations in size (Johnston 1961, Verbeek and Caffrey 2002).

Because of the ecological and epidemiological need to determine the sexes of crows in conjunction with a broader WNV study (Yaremych et al. 2003), we conducted a study with the following specific objectives: 1) determine the applicability of a published discriminant function (Clark et al. 1991) for east-central Illinois crows; 2) create age-specific discriminant functions for sexing crows from east-central Illinois and evaluate the performances of these functions; 3) use the age-specific discriminant functions we created to predict the sex of a cohort of crows in east-central Illinois that were live-trapped as part of a WNV study; and, 4) determine whether sex-based differences in WNV-mortality rates of crows exist.

## Materials and methods

We studied crows in Champaign County in east-central Illinois from fall 2001 through 2002. We examined 2 cohorts of crows: (a) dead crows measured and gender-determined for creation of a discriminant function ($n=38$), and (b) live-captured crows ($n=148$) measured and a subsample radio-tracked ($n=56$) to determine mortality rates due to WNV. Capture and handling of wild birds was authorized through Institutional Animal Care and Use Protocol number 1062.

### *Processing of crow carcasses*

We solicited a sample of crows ($n=38$) from a hunter in central Illinois during winter 2001–2002. Before dissection, we collected mensural characteristics according to the methods utilized by Clark et al. (1991) and Johnston (1961), with slight modifications. To minimize observer variability, one observer took all measurements. Heavily worn wings or tails or newly growing feathers were not measured. The unflattened wing chord was measured from the blunt end of the wrist bone to the distal tip of the longest primary. Tail length was measured from the point of insertion of the central retricies to the distal tip of the longest retrix. Tarsus length was measured on the right leg from the joint between the tibiotarsus and the tarsometatarsus to the distal edge of the most distal scale covering the base of the forward-pointing toes. Bill length was measured as the chord of the exposed culmen, from the start of the feathering at the base to the distal tip of the bill. Additional measurements included bill depth (at the anterior point of the nostrils), bill width (across the bill above the proximal point of the nostrils), and head-to-bill length (from the occipital ridge of the skull to the tip of the bill). Digital calipers were used for tarsus length, bill length, width, and depth measurements. A plastic ruler was used to measure the tail length, and a wooden ruler with a perpendicular stop nailed at zero was used to measure wing chord and head-to-bill length. The weight of each crow was taken with a 600-g spring scale, after live crows were immobilized by wrapping a cloth bandage around the wings and body and clipping the scale to the cloth.

We placed crows into 1 of the following 3 age categories based upon upper mandible lining coloration and plumage characteristics (Emlen 1936, Pyle 1997): hatch-year (HY), describing a bird from fledging until the following spring; subadult (Sub-A), describing a bird at least 1 year old and possibly in the 2–3-year-old range; or adult (Ad), describing a bird that likely was >3 years of age. Hatch-year crows typically have a bright pink palate just after fledging that will develop some dark mottling by the first fall. The shape of the tail feathers on HY crows are diagnostic as they are bluntly pointed or rounded when compared to adults, and after the first prebasic molt, these retained feathers on HY crows will appear more worn when compared to newly replaced counterparts on Ad crows after the complete prebasic molt. Additionally, very young crows have a bluish iris and an overall brown cast to their appearance when compared to glossy black adults. Subadult crows have a mixture of pink and black spotting of the palate. Adult crows typically have an entirely black lining of the palate, with glossy black wing coverts. Our age-classification scheme combined palate coloration and plumage characteristics for age determination of the crows into 1 of 3 categories. We determined the sexes of dead crows through gross investigation in which we documented the presence of testes or ovary.

### *Processing of live-captured crows*

We applied the same methods for measuring and aging to live crows. We captured 148 crows, including 18 recaptures for a total of 130 individual crows, in Australian crow traps (Caffrey 2002) on the South Farms property of the University of Illinois in Champaign County, Illinois (latitude 40.14030 north, longitude 88.19610 west) from February–October, 2002. We banded crows with aluminum United States Fish and Wildlife Service bands and fitted a subsample ($n=56$) with tail- or collar-mount radiotransmitters. Intensive radiotracking of the crows allowed recovery of carcasses upon death; ≤5 of these dead crows were sexed upon necropsy.

American crow in east-central Illinois, 2002. Photo by Gabe Hamer, field assistant for the project.

### *Applicability of Clark et al. (1991) equation in east-central Illinois*

Clark et al. (1991) published an equation based on DFA to determine the sex of adult crows in Saskatchewan, yielding 92% classification success based on wing length, tarsus length, and head-to-bill length. They had limited success with attempting to sex younger-than-adult crows and did not report a function for this age class. We applied measurements from the crow carcasses of central Illinois to the published equation. We used a 3-tier aging classification (HY, Sub-A, and Ad), and Clark et al. (1991) used a 2-tier aging classification (HY and Ad). A comparison of aging methods revealed that our Sub-A would have been classified as Ad in the Clark et al. (1991) system; therefore, both our Sub-A and Ad were applied to their published equation for adults. Using the published equation to determine the DFA-predicted sexes of known-sex crow carcasses allowed for determination of applicability of the function to east-central Illinois crows.

### *Sex determination of Illinois crows using discriminant function analysis*

To create age-specific discriminant functions for the Illinois crows, we analyzed linear measurements and body mass using a stepwise discriminant function analysis, performed using the DISCRIMINANT procedure of SPSS 11.0 for Windows (SPSS 2001). The analysis was performed using F-to-enter set at 0.05 and F-to-remove set at 10.0. Stepping was accomplished using the WILKS specification of the METHOD subcommand; thus, the variable that minimized Wilks' lambda was entered. We set prior probabilities equal to sample sizes to account for unequal group sizes. We calculated descriptive statistics using DESCRIPTIVES and employed a 2-way ANOVA with Bonferroni post-hoc testing available through UNIANOVA for comparisons of measurements between sexes and among age classes. Multiple comparisons of measurements taken twice from recaptured crows were accomplished with a paired-samples $t$-test available through T-TEST. We evaluated the performance of these discriminant functions by cross validation, using the leave-one-out procedure of the CLASSIFY subcommand of DISCRIMINANT, yielding an almost unbiased estimate of the expected actual error rate (Lachenbruch 1975).

We created discriminant functions using measurement and age data collected from an initial sample of 47 known-sex crows. This included the 38 crow carcasses collected during winter, as well as 9 known-sex live-captured crows from the summer. Because gonads were reduced in some crows carcasses and in some cases internal damage from shot pellets was present, we classified 4 crows as unknown sex. Additionally, we classified 5 crows as unknown age, allowing data from 29 of these dead birds to be utilized. The addition of the 9 live-captured crows created more robust functions by inclusion of birds from multiple seasons. We determined sexes of these live-captured crows by necropsy upon death (males and females) or observation of brood patch upon capture and incubation on a nest upon release (females only).

### *Measurement repeatability*

Crows captured more than once (recaptures) allowed opportunities for remeasuring them. A statistical comparison of initial measurements with subsequent measurements demonstrated the repeatability of measurements over the season.

### West Nile virus mortality

Monitoring of radiotracked crows allowed for recovery and WNV testing upon death. Assuming that the sex of all captured crows could be accurately predicted through DFA, we determined whether the sex composition of crows that died of WNV was different from the sex composition of the entire cohort of study crows. Kaplan-Meier survival analysis (Kaplan and Meier 1958), using the staggered-entry method (Pollock et al. 1989), served as a temporal comparison of male and female crow survival.

## Results

### Applicability of Clark et al. (1991) equation in east-central Illinois

We assigned the known-sex Sub-A and Ad crow carcasses ($N=21$) from central Illinois a sex using the Clark et al. (1991) equation. This equation correctly classified 71.4% of the crow carcasses, with misclassifications of 4 Sub-A and 2 Ad.

### Sex determination of Illinois crows using discriminant function analysis

Of our sample of 38 crow carcasses plus 9 live-trapped crows for use in creating discriminant functions, 4 carcasses were classified as unknown sex and 5 as unknown age class and thus were removed from analysis, leaving 38 crows for DFA with the following age and sex composition: HY, 6 females, 11 males; Sub-A, 8 females, 4 males; Ad, 5 females, 4 males. Initially, we created 1 function based on measurement data from all 38 known-sex crows of all age classes, with 82% correct classification. Higher classification success occurred when we created age-specific functions (Table 1). Because of the similarity in measurements of Sub-A and Ad, a function was calculated based on measurements of Sub-A and Ad combined; however, this function correctly classified only 76% because weight was the only variable retained in the analysis. Accordingly, we present 3 separate functions for the 3 age classes (Table 1). Cross validation of the crows in each age group yielded 94%, 100%, and 78% correct classification for the HY, Sub-A, and Ad, respectively. For the Sub-A, to determine whether the mass of the reference collection cohort of crows differed from the mass of the live-captured cohort, we used sex-specific Mann-Whitney $U$-tests, with no significant differences detected (females: $U=5.5, P=0.11$; males: $U=22.5, P=0.76$).

### Size comparison between sexes and ages

We assigned sexes of the 148 captured crows based on DFA: we sexed 122 HY crows as 107 female and 15 male, 13 Sub-A crows as 5 female and 8 male, and 13 Ad crows as 11 female and 2 male. Male crows were significantly larger ($P<0.001$) than female crows of the same age class with respect to all morphometric measures except bill length in the Sub-A crows. There were significant differences between age classes for all measurements ($P<0.001$) except tarsus length ($P=0.29$). For all measurements aside from tarsus length, HY crows averaged smaller than Ad crows. In some cases, HY crows were smaller on average than Sub-A crows. However, Sub-A crows did not differ ($P\geq 0.10$) from Ad for any of the characters except mass. Accordingly, we were unable to pool measurements by age, and we report the age- and sex-stratified descriptive statistics of the hunted plus live-captured crows of central Illinois (Table 2).

### Measurement repeatability

With 18 recaptures the average number of days between the initial capture and any subsequent recaptures of a crow was 21 days. In 14 cases we acquired pairs of measurements, upon initial capture and upon recapture. Paired $t$-tests indicated that the only difference detected was in mass ($P=0.01$); other mensural characters did not differ

Table 1. Age-specific discriminant functions for determining the sex of American crows captured in central Illinois, 2001–2002; substituting original measurements to solve the equations yields the discriminant score (D). Values for males are positive and values for females are negative.

| Age | *n* | Discriminant function | DFA classification success |
|---|---|---|---|
| HY | 17 | D = −64.2857920 + 0.54511177 (bill length) + 2.67337499 (bill width) | 100% |
| Sub-A | 12 | D = −16.1101054 + 0.08395439 (mass) − 1.60064349 (bill width) | 100% |
| Ad | 9 | D = −35.0857758 + 0.38368406 (head-to-bill length) | 89% |

Table 2. Age-stratified descriptive statistics for male and female American crows hunted ($n$ = 33) and live-trapped ($n$ = 148) in central Illinois, 2001–2002. Standard errors are in parentheses. In the hunted cohort, 5 crows of unknown age were omitted and 4 crows of unknown sex were assigned a sex by DFA.

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | HY $n$ = 24 | Sub-A $n$ = 14 | Ad $n$ = 5 | HY $n$ = 112 | Sub-A $n$ = 12 | Ad $n$ = 14 |
| Wing chord (mm) | 297.6 (9.52) | 306.1 (7.57) | 315.0 (6.40) | 290.3 (13.47) | 287.0 (12.81) | 306.6 (12.46) |
| Tail length (mm) | 170.1 (6.88) | 176.1 (8.00) | 178.0 (10.12) | 167.3 (7.95) | 166.3 (9.19) | 176.4 (8.19) |
| Tarsus length (mm) | 58.5 (2.84) | 58.5 (2.21) | 58.8 (1.33) | 56.1 (3.09) | 54.9 (2.72) | 57.4 (3.39) |
| Head-bill length (mm) | 91.2 (2.84) | 90.6 (2.98) | 94.0 (1.03) | 86.0 (3.18) | 87.3 (3.02) | 88.9 (2.74) |
| Bill depth (mm) | 17.5 (1.12) | 17.9 (0.62) | 18.1 (0.57) | 16.6 (0.76) | 17.1 (0.76) | 17.4 (0.88) |
| Bill length (mm) | 49.2 (1.42) | 49.3 (2.24) | 50.2 (1.67) | 45.0 (2.32) | 47.0 (1.98) | 48.6 (2.54) |
| Bill width (mm) | 14.3 (0.62) | 13.7 (0.75) | 14.8 (0.46) | 13.1 (0.71) | 13.6 (1.20) | 13.8 (0.83) |
| Mass (g) | 454.7 (48.08) | 491.4 (24.99) | 505.0 (57.45) | 409.4 (37.59) | 429.3 (34.7) | 437.9 (40.60) |

between first and second measurements ($P \geq 0.16$). In no case was the difference in measurements of any mensural character enough to cause a crow to be classified as a different sex by DFA upon subsequent capture.

### *West Nile virus mortality*

Excluding 18 recaptures, we live-captured 130 individual crows, consisting of 20 males and 110 females by DFA. A total of 25 live-captured crows died of WNV from May–October, 2002; 4 of 25 (16%) WNV deaths were male and 21 of 25 (84%) WNV deaths were female. The proportion of males and females that died due to WNV was 0.20 and 0.19, respectively. There was no significant difference in mortality rates between males and females for all age classes combined ($\chi^2 = 0.097$, $P = 0.76$), for HY age class only ($\chi^2 = 0.018$, $P = 0.89$), or for Sub-A and Ad (combined to increase sample size) ($\chi^2 = 0.006$, $P = 0.94$). Sex-specific survival curves for those crows that died of WNV were not significantly different using the Wilcoxon signed-rank test ($P = 0.25$) (Figure 1). Males and females experienced high survival in May and June; WNV deaths began in July.

## Discussion

In such a widely distributed species as the American crow, there exists an intraspecific geographical variation in morphology of individuals (Johnston 1961). Because DFA is based upon morphological measurements, this variation leads to decreased success in classifying the sex of crows from different geographic areas than the reference crows used to create the equations. This may explain the relatively low classification success of the Clark et al. (1991) equation for determining the sex of the central Illinois known-sex crows. Discriminant function analysis has been used previously to classify the sex of American crows (Clark et al. 1991, Caffrey 1992). In the latter study, a subsample of crows was sexed behaviorally or through necropsy, and 5 morphological measurements were taken. A discriminant function was generated and used to sex 17 individuals; however, juvenile crows could not be sexed with this approach due to overlap in body size. This function is not intended for application to other populations of crows, because the values distinguishing males from females were not reported.

In our study the sample sizes used to create the age-specific discriminant functions were small, with the following age and sex composition: HY, 6 females, 11 males; Sub-A, 8 females, 4 males; Ad, 5

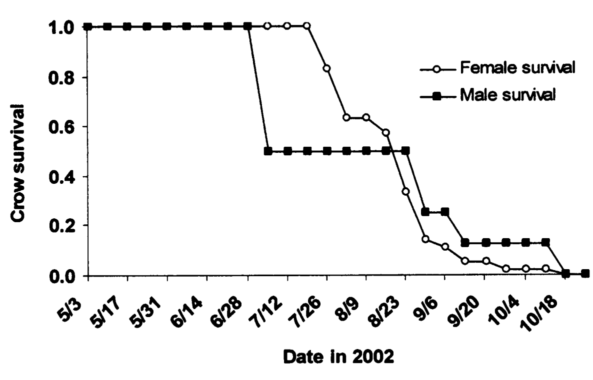


Figure 1. Survival curves (Kaplan-Meier) using the staggered-entry method for male ($n = 4$) and female ($n = 21$) American crows in east-central Illinois in the spring and summer of 2002. All crows were live-captured and released, radiotracked, and later found dead and WNV-positive.

females, 4 males. To provide sufficient power and avoid rejection of the homogeneity of variance-covariance matrices, it is recommended that the number of cases in the smallest group exceed the number of dependent variables (Tabachnik and Fidell 1983). The number of dependent variables (discriminant variables) retained in the discriminant function analysis after stepping was 2 for the HY and Sub-A age classes, and 1 for the Ad age class. Accordingly, the sample sizes in each sex by age group exceeded the number of discriminant variables returned by the stepwise procedure, and so sufficient power should exist to provide some confidence in our ability to discriminate between sexes within age classes. Comparison of original and cross-validation runs for HY and Sub-A crows indicated a high degree of accuracy and consistency in discriminating between male and female crows. The classification success rate for Ad in the cross-validation run was lower than the original run, indicating that further analysis of this age class with additional reference specimens is warranted.

With the exception of mass, measurements did not vary upon recapture, suggesting that these methods of measurement were repeatable and that variation in measurements upon recapture was not sufficient to cause decreased gender-classification success. The molt of crows in Illinois begins in June (Graber et al. 1987) and did not affect our measurements of wing chord or tail length because heavily worn or newly growing feathers were not measured. Mass was retained, along with bill width, in the discriminant function analysis for classifying Sub-A. Mass is expected to vary daily and seasonally due to feedings, growth, and capture collection status. However, the recapture data indicated that mass increased between first and second capture, though this difference was not enough to change the sex classification of the birds.

Male vertebrates tend to exhibit higher rates of disease than females, driven by either ecological or physiological mechanisms, yet the epidemiological consequences of such differences in disease prevalence are unknown (Rizzoli et al. 2002). Higher amounts of testosterone may result in higher disease incidences in male members of a species, as testosterone is known to be immunosuppressive (Grossman 1985). In cooperative breeding systems, such as that of the American crow, adults often mate for life and both sexes provide parental care, while offspring from previous years may delay their own breeding to help rear the new young. In such a system, sex-biased disease-induced mortality may have a destabilizing effect, and a reduction in the production of offspring may result. Before introduction of WNV, there was no evidence for differential survivorship between male and female American crows; one study reported 94.3% and 95.1% survivorship of female and male breeding American crows in Los Angeles, California, respectively (Caffrey 2000). Based on our sample and analysis of dead crows, we determined that the 2002 WNV epizootic in east-central Illinois did not affect one sex of crow disproportionately. Data such as these will be necessary in determining the population-level impact of WNV in American crows. No sex-biased mortality rates of birds are reported from other viral diseases including duck plague, inclusion body disease of cranes, or avian pox (Brand et al. 1987).

We present age-specific discriminant functions with high classification success for use in determining the sex of Midwestern American crows. This rapid, inexpensive, and non-invasive method of gender determination can be performed easily in the field with a calculator. Reference samples can be solicited from hunters and sex determined through necropsy, or can be acquired by trapping, with gender determinations made through karyotyping or endoscopy. This technique for determining gender of American crows should be useful in field-based studies when a low-cost, non-invasive method of sex determination is necessary.

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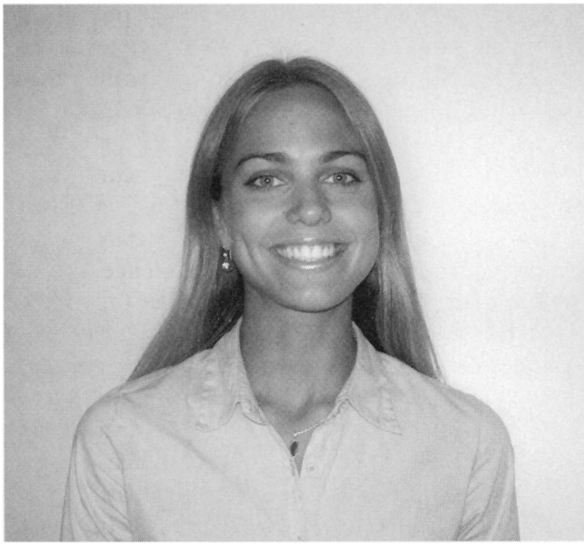

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