Morphological Description of American Crow, *Corvus brachyrhynchos,* Populations in Southern Québec

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The American Crow has always been a much scrutinized bird in North America but, since the emergence of West Nile Virus (WNV) in North America in 1999, public health authorities' attention to it has been raised another notch. In Québec, like everywhere else in North America, part of the WNV surveillance programme was based on detection of WNV mortality in crow populations. During the summer of the 2005 surveillance season, we followed an age and gender determination protocol, as well as a morphological measurement protocol, on dead crows sent in for WNV status determination, to improve our knowledge of the crow population in Québec. Statistical analysis of the measurements revealed that age and gender were important factors in the morphological characterisation of the American Crow. Bill depth and head-to-bill length appeared as the most important morphological variables for gender prediction through a discriminant function analysis. We also realized that, in adult age groups, our WNV positive carcasses had lower mean weights than carcasses that tested negative for WNV, in adult age groups.

Depuis toujours, la corneille d'Amérique est un oiseau très étudié, mais, depuis l'apparition du virus du Nil occidental (VNO) en Amérique du Nord en 1999, l'attention des autorités en santé publique sur cet oiseau a encore augmenté. Au Québec, comme ailleurs en Amérique du Nord, une part importante du programme de surveillance pour la détection du VNO a été basée sur la détection des mortalités liées au VNO dans les populations de corneilles. Pour améliorer notre connaissance de cette espèce au Québec, nous avons mis à profit la récolte des carcasses au cours de l'été 2005 dans le cadre du programme de surveillance en instaurant un protocole de détermination de l'âge, du genre ainsi qu'une prise des mesures morphologiques sur ces mêmes carcasses. L'analyse statistique des résultats a montré qu'à la fois l'âge et le genre étaient des facteurs importants dans la caractérisation morphologique de la corneille d'Amérique. À l'aide de l'analyse discriminante, il est apparu que la profondeur du bec ainsi que la distance tête-bec étaient les mesures les plus importantes pour prédire le genre de l'oiseau. Nos analyses nous ont également permis d'observer que, dans les groupes d'oiseaux adultes, les carcasses positives pour le VNO étaient en moyenne moins lourdes que les carcasses négatives.

Key Words: American Crow, *Corvus brachyrhynchos*, intraspecific variations, morphological analysis, West Nile virus, age and gender effect, Quebec.

In the last century, the American Crow (*Corvus brachyrhynchos*) was the subject of research in North America (Emlen 1938; Emlen 1940; Good 1952; Johnson 1994). Interest was shown in the ecology of the species because of its important interactions with humans and crops. Other studies demonstrated the richness of crow biology by focusing on the complex social structure of the population, and on the frequent movements of crows during the year, depending on the age of an individual and the season (Verbeek and Caffrey 2002).

Since the emergence of West Nile Virus (WNV) in North America in 1999, the life history and ecology of American Crows have been scrutinized more intensely (Eidson et al. 2001a). Because the species has been found particularly susceptible to this virus (Komar et al. 2003), crows are an important epidemiological sentinel for WNV in public health surveillance systems of the United States of America and of Canada (Eidson et al. 2001b; Beroll et al. 2007). Despite this increased attention from public health authorities, very little is known about crow populations in Québec, Canada, where WNV infections have been observed since 2002 in crows (Brown and Dallaire 2002*; Health Canada 2006*). Carcasses collected in 2005 during the province's WNV epidemiological surveillance program provided an opportunity to improve our knowledge of the crow population in Québec. A descriptive study was carried out on the carcasses of dead crows that were submitted. The objective of the study was to characterize the crow population in Québec, while taking into account the WNV status of the carcasses, by means of gender determination and external measurements on submitted carcasses.

Materials and Methods

Data collection

The carcasses were collected in southern Québec, Canada, between 6 June 2005 and 15 September 2005 during the WNV surveillance program carried out conjointly by provincial and federal agencies (Québec Ministère de la sante et des services sociaux, 2005*). People were advised to report dead or sick crows via a central telephone line, so the distribution of the carcasses sampled was dependent on human activity in a given area and motivation of the people in that area to participate in surveillance activities. Reported carcasses were collected by wildlife conservation officers and, if judged in good enough conditions, were shipped to the Centre québécois pour la santé des animaux sauvages – Canadian Cooperative Wildlife Health Center in Saint-Hyacinthe, where samples were taken for detection of WNV. A total of 332 crows were received during the sampling period. Ten crows were rejected from the study due to advanced decomposition. A direct diagnostic test (VecTest®, Medical Analysis Systems, Inc.) was performed on each carcass to determine its status for WNV. This WNV antigen detection test was chosen because of its specificity between 79% and 100% and sensitivity between 70.4% and 92.8% (Lindsay et al. 2003; Stone et al. 2004; Stone et al. 2005; Padgett et al. 2006).

Age determination

Carcasses were grouped in three age classes: hatching year birds (HY), second-year birds, i.e., birds that hatched in 2004 (SY), and after-second-year birds, i.e., birds that hatched before 2004 (ASY). Age class was determined in 322 carcasses according to the following criteria: eye colour, oral mucosa colour, feather colour, and feather shape (Emlen 1936; Good 1952; Rea 1967; Pyle 1997; Madge and Burn 1999). Colour of the iris is blue in the HY and black in the SY and the ASY. Oral mucosa is pink in the HY, is marbled pink and black in the SY and completely black in the ASY. The colour of oral mucosa was examined on both the mandible and the maxilla (floor and roof of mouth). Feather coloration is faded brown in the HY and becomes glossy black in the SY and ASY. Feather shape was also examined. In the HY, the rectrices initially have an irregular outline as well as a narrow and pointed shape. In the ASY, the rectrices become squared off or truncated with a smooth outline. Finally, the feathers of the alula (wrist of the bird) are downy and matte in the HY and glossy-black in the ASY. The morphologic characteristics corresponding to SY are intermediate between the characteristics of HY and ASY. If, for an individual carcass, some criteria pointed toward different age classes, we retained the age class indicated more frequently.

Morphometric measurements

It was not possible to determine the gender and complete the morphological measurements for all of the 322 carcasses during the limited time available at the BL3 facilities (biosecurity level). A secondary sample of 138 was selected from the 322 carcasses, using a stratified non-proportional random sample. The selected carcasses were frozen for conservation. Stratification was done according to age and WNV status. Six age-by-WNV-infection-status groups were created (HY positive, HY negative, SY positive, SY negative, ASY positive, and ASY negative). To guarantee reasonable statistical precision in further analysis, all the carcasses were kept from the HY positive group (one carcass), SY positive group (30), and ASY positive group (30). Systematic random samples were selected within the HY, SY and ASY negative groups (giving sample size of 23 carcasses for HY, and 27 for both SY and ASY groups). At this stage, we had no knowledge of the gender of carcasses within the six different age-status groups. Morphological measurements were taken on the 138 selected carcasses by one observer (Antoinette Ludwig) in order to minimize observer variability. A dial calliper and a metallic ruler were used for external measurements, and carcasses were weighted using electronic scales (Sartorius L610; precision: 10^{-3} grams). If poor condition of the carcass had a negative effect on one or more of the measurements, those measurements were excluded from the analysis.

The methods of measuring the tail, bill, tarsus and wing were as described by Pyle (Pyle 1997). Flattened wing length was taken from the blunt end of the wrist joint to the tip of the longest primary feather. Tail length was measured between the tip of the longest rectrix and the point of insertion of the two central rectrices (this insertion point corresponds to the distal end of the uropygial gland). Tarsus length was measured between the intertarsal joint and the distal end of the last scale before the toes emerge. Bill length was measured as the exposed culmen, between the tip of the feathering at the base of the bill and the bill's tip. It was important to take the feathers at the base of the bill into consideration because these feathers can be rather long in the crow. Bill depth (height of the bill) and bill width (across the bill) were taken at the anterior point of the nostril. Head-to-bill length was taken from the occipital ridge of the skull to the tip of the bill. The gender of each crow was determined via necropsy after all external measurements had been recorded.

Statistical methods

The frequency distribution of each morphometric variable was obtained for each gender-and-age class group and compared to the Gaussian distribution. General linear regression was performed on morphological variables using gender by age classes as the independent variable. The residual distribution for each morphological variable was studied after removing age and gender effect in order to detect non-normality. The analysis was performed using PROC GLM in SAS (9.1 – SAS Institute Inc., Cary, North Carolina, USA).

FIGURE 1: Spatial localisation of the crow carcasses collected during the 2005 West Nile Virus surveillance program in South of Québec.

The confounding effect of West Nile Virus (WNV) status on gender-and-age class group comparison was evaluated using a regression model that took into account differences in sample sizes of the gender-age-and-status groups. The dependent variables were the eight morphological variables. The values for each morphological variable were compared between both statuses in each age-and-gender groups.The analyses were performed using PROC MIXED in SAS (9.1 – SAS Institute Inc., Cary, North Carolina, USA).

We calculated our mean values and standard deviations of all the morphological variables for the 6 age and gender groups, taking into account the non-proportional stratified sampling strategy (Cochran 1977). Linear regression, taking into account differences in sample sizes of the gender-and-age class groups, was used to evaluate the effect of age, gender and age* gender interaction (independent variables) on the eight morphological variables (dependent variables). The analyses were performed using PROC MIXED in SAS (9.1 – SAS Institute Inc., Cary, North Carolina, USA).

Discriminant analysis for gender prediction from morphological variables was developed in two steps: variable selection and discriminant function construction. The stepwise selection procedure of the key variables for gender determination was performed using PROC STEPDISC in SAS (SAS 9.1 – SAS Institute Inc., Cary, North Carolina, USA). The analysis was performed using 0.15 as the significant level for adding variables in the forward selection mode, and the significant level for retaining variables in the backward elimination mode was set at 0.15. To create the discriminant functions we used PROC DISCRIM in SAS (SAS 9.1 – SAS Institute Inc., Cary, North Carolina, USA). Discriminant functions were built to predict the sex, both by specific age class and by all age classes confounded.

Results

Spatial distribution of the randomly selected carcasses covered a large part of southern Québec (from the USA border to Québec city), providing a reliable overview of the morphological characteristics of the crow population from that territory (Figure 1).

The frequency distribution for the following variables did not have a completely normal distribution:

bill width and wing length for the SY and the ASY, and bill depth and tarsus length for the ASY. Using general linear regression for those variables, we found the residuals had a slightly bimodal distribution frequency, even after removing age and gender effect. This bimodal distribution frequency was most evident for wing length for the SY and the ASY groups, and tarsus length for the ASY group.

Evaluation of WNV status as a confounding variable

The tests were performed only for the SY and the ASY age classes because the number of carcasses of both positive and negative WNV status in the HY group was very low ($N_{\text{HY WNV+ female}} = 0$; $N_{\text{HY WNV- female}} = 5$; $N_{\text{HY WNV+ male}} = 1$ and $N_{\text{HY WNV- male}} = 18$). WNV status appeared as a significant variable for weight in the SY group (P for male = 0.0019 and P for female = 0.0003) and in the male ASY group (P= 0.0162).

Age and gender effect on the morphological variables

The values of all morphological variables for all the six age and gender groups are presented in Figure 2. The age effect was statistically significant for all of the morphological variables. The effect of age on weight has to be considered with caution because WNV status is a potential confounding variable for weight determination by age class. The gender effect was only statistically significant for the following variables: bill depth, head-to-bill length, and weight. Regarding the mean values presented in Figure 2, gender effect corresponds to larger morphological measurements for males in comparison with females except for wing length in the HY group, and age effect corresponds to larger morphological measurements for older carcasses. The effect of interaction between age and gender was never significant (lowest $P = 0.0941$).

Gender prediction

During the stepwise selection procedure of the key variables for gender prediction, no variables were retained for the HY age group. Two variables were selected for the SY age group (Bill depth (P=0.0017), and Head-to-bill length (P=0.129)), and three variables were selected for the ASY age group (Head-to-bill length (P<=0.001), Tarsus length (p=0.1248), and Weight (P=0.1226)). For the total analysis performed without age-class distinction, three variables were selected: Head-to-bill length (P=0.003), Wing length (P=0.0056), and Weight (P=0.0725). The discriminant equations created are presented in Table 1, along with the success of classification within each age-class group, which ranges from 64.37% to 88%.

Gender proportion in the dead crow population

In the randomly sampled age-status groups, we observed that males were much more frequent than females, especially in HY age group (20.8% females in the HY group, 36.8% females in the SY group and 43.8% in the ASY group).

Discussion

Evaluation of WNV status as a confounding variable

When comparing the mean values for weight in male and female SY and ASY crows with both positive and negative WNV status, we observed that mean values were lower for carcasses positive for WNV than for those negative for WNV. As all crows in our study were picked up dead, it was difficult to know if the lower weight of the WNV positive carcasses appeared before or after their infection with WNV.

Let us consider that the weight loss preceded West Nile virus infection. In this case, weight loss could correspond to a chronic disease or a period of starvation or even coinfection of WNV and another disease that would have weakened its immune system and predisposed it to a viral infection. However, no reference currently exists in the literature in favour of such a hypothesis.

We could therefore speculate that weight loss follows WNV infection in American Crows. It has been observed that some bird species of the Passeriformes order present a lower body condition after WNV infection (Steele et al. 2000; Gibbs et al. 2005). As of yet, this observation was rare for the American Crow (Dallaire, A.D., Centre québécois pour la santé des animaux sauvages, 2007, personal communication). This is due to the acute nature of the disease in the American Crow, not allowing enough time for a change in body condition, except dehydration, that could be responsible for the weight loss (Komar et al. 2003).

However, some observations about the WNV status of wild crows, based on serological studies, suggest that this phenomenon is evolving. Serological studies conducted since 1999 have demonstrated that the proportion of crows in the population that were seropositive for WNV was increasing in North America (Gibbs et al. 2006, Ringia et al. 2004)). The two studies support the hypothesis of development of an increased capacity for resistance against West Nile Virus infection in the crow population, allowing for the possibility that resistant individuals could stay alive for a longer time after infection than has been previously observed. This phenomenon could help explain the lower weight observed in part of our adult carcasses: the more resistant adults to WNV had a longer WNV clinical period before dying allowing for the change in body condition.

Age and gender effect on the morphological variables

The important results reported in Table 1 concern the role of the bill in gender differentiation in the American Crow and the role of both the bill and the tail in age differentiation in the American Crow (the older the crows were, the longer were their tails and their bills). It is already known that young crows are smaller than adult crows (Gauthier and Aubry 1995; Verbeek and Caffrey 2002). Males had a longer and a deeper bill than females, consistent with Clark's study on crows in Saskatchewan (Clark et al. 1991) and with

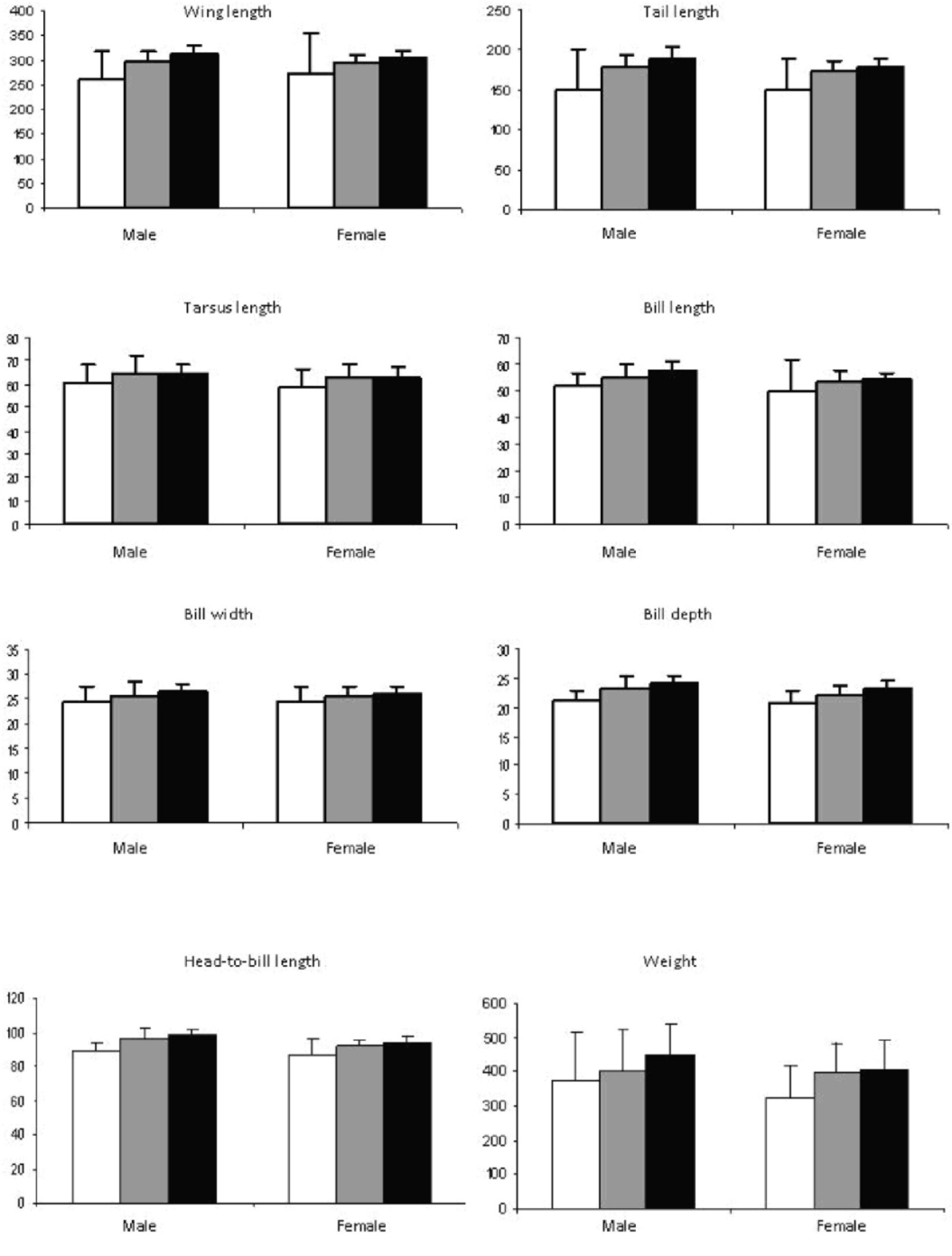

FIGURE 2 : Mean morphological values of crows from Québec within each gender and age group, in 2005.

Note: Arithmetic mean length values are given in mm and weight are given in grammes. HY, SY and ASY age groups are represented by the white, grey and black bars respectively. Sample sizes for each group are the following: 19 HY Male (1 WNV+), 5 HY Female (0 WNV+), 36 SY Male (20 WNV+), 21 SY Female (10WNV+), 32 ASY Male (16 WNV+) and 25 ASY Female (14 WNV+).

TABLE 1: Total and age-specific discriminant functions for gender determination of American Crow carcasses in Québec.

Age	Sample size	Discriminant function	Correct classification of the carcasses
SY	57	Male=-468.9136+142.61782*Bill depth+64.58749*Head-to-bill length	69.44%
		Female=-439.19922+135.51637*Bill depth+63.10948*Head-to-bill length	76.19%
ASY	57	Male=-871.69621+14.69643*Tarsus length+171.68221 *Head-to-bill length-0.08483*Weight	81.25%
		Female=-800.73918+17.71192*Tarsus length+162.80922 *Head-to-bill length-0.09624*Weight	88%
All	138	Male=-250.17699-1.39873*Wing length+60.47778 *Head-to-bill length-0.07532*Weight	64.37%
		Female=-238.90005-1.06270*Winglength+58.46411 *Head-to-bill length-0.08124*Weight	80.39%

Note: Substituting original measurements into both equations (for males and females) results in a score. The highest score obtained from the two discriminant functions identifies to the gender category of the carcass. HY crows were not included into the analysis because of the small size group 19 HY Male (1 WNV+), 5 HY Female (0 WNV+).

Yaremych's study on crows in Illinois (Yaremych et al. 2004). Another characteristic that has been proposed as being gender dimorphic in crows is the observation of cloacal protuberance or brood patches in females (Pyle 1997). However, these structures can be observed only during the breeding season, which is outside the period when the crows from our study were collected. In addition, these characteristics have also been reported in males due to the presence of brooding activity in both gender (Good 1952; Clark et al. 1991). The fact that interaction between age and gender was never significant demonstrated that growth was not different between sexes, and that gender dimorphism was not different between age classes. In conclusion, age and gender affect external measurements in the American Crow but growth follows a similar pattern in the male and female groups, and gender differentiation involves the same external structures regardless of the age class of the individual.

Gender prediction

One objective of discriminant function analysis was to find the external morphological variables that most useful in predicting the gender of a living bird. In previous studies, accounts of sexual variations in birds were limited to describing females as slightly smaller than males: the morphometric variables which differed between sexes and the extent of those differences were not precisely explained (Good 1952; Gauthier and Aubry 1995; Pyle 1997; Verbeek and Caffrey 2002). Our data provided a good opportunity to build discriminant functions for the SY and ASY age classes. It was impossible to build a discriminant function for HY age class, because of the small group size. The variables selected by discriminant analysis for the SY and the ASY age groups were the most significant when evaluating the gender effect in the crow population except for the tarsus length in the ASY. Bill length was also an important variable in gender dimorphism according to our gender effect analysis but was not conserved in the discriminant function because of its strong association with head-to-bill length. Classification successes in gender determination of carcasses were fairly good for both age classes (more than 70% of correct classification), and were best for ASY birds (more than 81% of correct classification). This could be explained by stronger sexual dimorphism in adult crows compared with younger crows. The discriminant function built while including all carcasses (without distinguishing age class) indicated that wing length, head-to-bill length and weight were the most useful variables for gender determination. According to gender and age effect analysis, wing length, weight and head-to-bill length are variables that discriminate both for age and gender in crows. From our analysis, age emerged as a determinant variable for increasing the gender predicting precision for American Crow.

Gender proportion in dead crow population

Male carcasses were much more frequent than female carcasses, especially in the HY age group. No differences in survivorship and life span between male and female have been reported in the literature (Verbeek and Caffrey 2002), nor do the differences have anything to do with carcasses' WNV status, as no association has been found between WNV status and gender (A. Ludwig, unpublished data). As, in our study, the carcasses were collected by humans, we surmised that the larger number of male carcasses collected could be linked to observed but not clearly established behavioural differences between males and females: males are more reckless than females (Verbeek and Caffrey 2002), and therefore have a greater probability of dying in a human-occupied area and of being picked up as part of the surveillance program.

Limits

Apart from the HY female group, which consisted of only five carcasses, and the HY WNV positive group, which had only had one carcass, the number of carcasses by age class, status and gender group was large enough to allow for good statistical precision for

the mean value estimation of the morphological variables. For the two HY groups, statistical conclusion must be made with caution, because such a quasi-complete separation of the data (i.e. sparse data) can create errors in statistical tests due to small group size (Dohoo et al. 2003; Mather et al. 2007).

The objective of this study was to carry out morphometric measurements on crows from the entire province of Québec. However, this was not achieved as crows were collected during a governmental surveillance program (people phoning to signal the presence of dead birds to be picked up) rather than actively and randomly sampling across the target territory.

The crows sampled were carcasses rather than live birds. The frozen carcasses that we manipulated were not always in good condition (feathers were sometimes damaged; in some cases the internal organs were putrid). All this may have generated an underestimation for all of our measurements (information bias). As an example, the bill of a carcass in bad condition tends to come off, which makes measurements on the bill less precise. But as no gender or age specific decomposition process is known for crows, the underestimation of the measurements that could result from this phenomenon was considered to be uniform for all the carcasses, and therefore, comparisons among them continued to be acceptable.

This study emphasizes that age and gender are important factors in describing morphology of crows in Québec, as in other regions of North America (Yaremych et al. 2004, Clark et al. 1991). The West Nile Virus status was a confounding factor for weight comparison among the SY and ASY age groups, because the WNV positive birds seemed to be thinner than WNV negative birds in this adult group.

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