Sex Determination for the Great Egret and White Ibis

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Abstract.—Most species of wading birds are monomorphic and present few or no external characteristics to allow for sex determination in the field. We used standard morphometric measurements and discriminant function analysis to determine the sex of Great Egrets (*Ardea alba*) and White Ibises (*Eudocimus albus*). The models were validated based on sex determination from DNA. Two functions were created for Great Egrets; mass reliably discriminated 88% of our samples, while wing chord separated 81% of our samples. We included mass in the discriminant function analysis for Great Egrets because mass did not vary between years or within our pre-breeding sampling period. Mass was not included in our analysis of White Ibis because it differed by year and within our pre-breeding sampling period. White Ibis samples were separated by a discriminant function using the length of curved bill and tarsus. This function correctly classified 78% of our samples. We provide simplified linear equations to calculate the sex of Great Egrets and White Ibises as well as cut off points where the probability of correctly sexing individuals drops below 75%. Our model can be used to reduce the costs of sex determination by allowing researchers to use expensive DNA analysis techniques only for those individuals that cannot be reliably classified using the simple statistical model. *Received 16 July 2007, accepted 2 December 2007.*

Key words.—*Ardea alba*, discriminant function analysis**,** *Eudocimus albus*, Everglades, Florida, Great Egret, sex determination, White Ibis.

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Research on avian species commonly relies on the ability to accurately determine the sex of a species to understand sex specific life history traits (e.g., body condition; Herring and Collazo 2006, sex ratios; Maness *et al.* 2007, spacing patterns; Rodway 2006). While the sex of many bird species (e.g., waterfowl) can be easily determined from sexual dimorphic plumage or morphology, it cannot be determined for many monomorphic species. Wading birds (Ciconiiformes) are a group of species whose sex cannot be easily determined in the field; so most studies have relied on invasive techniques (e.g., Heath *et al.* 2003), or expensive DNA analysis (e.g., Hylton *et al.* 2006) to identify the sex of individuals. Two species of wading birds, the Great Egret (*Ardea alba*) and White Ibis (*Eudocimus albus*), have been the focus of considerable research, however there are no accurate field techniques for determining their sexes (Kushlan and Bildstein 1992; McCrimmon *et al.* 2001). Published mass and morphometric data for these two species overlap greatly and do not provide a clear separation of sexes.

Analyzing morphometric measurements with discriminant function analysis (DFA) has proved to be an effective technique to accurately sex other species of waterbirds (e.g., Forster's Terns *Sterna forsterni*; Bluso *et al.* 2006, Dovekies *Alle alle*; Jakubas and Wojczulanis 2007, Imperial Shags *Phalacrocorax atriceps*; Svagelj and Quintana 2007). We assessed the utility of a discriminant function model that predicts gender based on six morphometric measurements for Great Egrets and White Ibises. We validated our model with samples of birds whose sex was known based on a DNA analysis.

STUDY AREA AND METHODS

Adult pre-breeding Great Egrets and White Ibises were captured between 10 Jan and 23 Mar during 2006-2007 as part of a larger project focusing on habitat selection and physiological condition of these two species in the Florida Everglades. Great Egrets and White Ibises were captured in the Arthur R. Marshall Loxahatchee National Wildlife Refuge and Water Conservation Areas 2 and 3 using a net gun and a modified flip trap (Herring *et al.* 2008). Each bird was banded with a U.S. Fish and Wildlife Service band, radio-tagged, and measured. Tarsus length (middle of midtarsal joint to the end of tarsometatarsus), wing chord, wing flattened, exposed culmen length, bill depth, and mass were recorded for both species, and additionally curved bill length for White Ibis. All measurements were to the nearest one mm using calipers or a wing ruler, except mass, which was measured to the nearest five g using a spring scale. All measurements were taken by trained individuals and supervised by GH. All birds were captured during the morning, mostly while they were enroute to foraging

sites, thus it is assumed that masses were not biased by ingested food.

Up to 2 ml of blood from the brachial vein was sampled from all birds using a 27.5-gauge syringe. Blood samples were stored on ice in the field and frozen at -20°C in the lab until DNA analysis. DNA sexing was conducted by Zoogen Services, Inc. (Davis, California), with a reported sex identification accuracy of 99.9% (Zoogen 2007). Zoogen uses a polymerase chain reaction (PCR) to visualize segments of DNA from the sex chromosomes of each bird, where females are heterogametic (WZ) and males homogametic (ZZ; Zoogen 2007). The gene chromo-helicase-DNA binding protein (CHD) is found on both the W and Z chromosomes as a pair of replicated gene loci, CHD-W and CHD-Z (Zoogen 2007). While the coding DNA of these two loci is conserved, the lengths of the non-coding DNA vary (Zoogen 2007). Using a pair of CHD primers, the PCR enzyme effectively replicates these segments of DNA thousands of times until they can be imaged (Zoogen 2007). The sizes of the two PCR products from the W and Z CHD loci differ, so females and males can be readily identified and then compared with DNA from birds of known sex to determine the results (Zoogen 2007).

Statistical Analyses

Differences between sexes for all measurements were tested using a t-test after determinination that data met equal variance assumptions (Levene's homogeneity of variance test; JMP, SAS Institute 2001) and univariate normality (Shapiro-Wilks test statistic; JMP, SAS Institute 2001). Because mass has been found to be an unreliable discriminator between sexes for species whose weights fluctuate seasonally (see Devlin *et al.* 2004; Bluso *et al.* 2006; Svagelj and Quintana 2007) mass data by species was analyzed separately for each sex to determine if it varied by year and Julian date using an analysis of covariance (ANCOVA). An index of body size was included as a covariate in the model to control for differences in structure size. The body size index consisted of the standardized scores of the first principal component (PC1) obtained from a principal component analysis on six (Great Egret) and seven (White Ibis) morphometric measurements taken from each bird (Afton and Ankney 1991; Esler *et al.* 2001). PC1 explained 43% and 49% of the overall variation among morphometric measurements for White Ibises and Great Egrets respectively. All ANCOVA tests met the requirement of parallelism. Because White Ibis mass was found to differ by either year or Julian date (see Results) this measurement was dropped from subsequent DFA.

Pearson's correlation analysis (PROC CORR: SAS Institute 2003) was then used to examine all variables for multicollinearity. If two or more variables were found to be highly correlated ($r \geq 0.7$; McGarigal *et al.* 2003) univariate ANOVAs were calculated for each variable and compared their respective F-values (PROC GLM: SAS Institute 2003). Variables with the highest F-value were retained for the discriminant function analysis (DFA; Noon 1981). Stepwise DFA (PROC STEPDISC, proc discrim; SAS Institute 2003) was then used to select the best single or combination of measurements that separated sexes (Klecka 1982; McGarigal *et al.* 2003). The residuals of the canonical scores were examined for normality to meet the assumption of multivariate normality (McGarigal *et al.* 2003). A jackknife procedure (leave-one-out; PROC DISCRIM: SAS Institute 2003) was used to validate the results (Phillips and Furness 1997; McGarigal *et al.* 2003). The posterior probability of each bird's classification was calculated and plotted against their respective discriminant scores. All groups met minimum appropriate sample sizes based on $N \geq 3P$, where P is the number of discriminating variables (Williams and Titus 1988; McGarigal *et al.* 2003).

RESULTS

Two hundred and nine adults (79 egrets and 130 ibises) were captured between 26 Jan 2006 and 23 Mar 2007. Twenty-nine and 47 male and female Great Egrets were identified, respectively, and 62 and 68 male and female White Ibis respectively using DNA techniques. Five out of six Great Egret morphometric measurements differed significantly between sexes (Table 1). All White Ibis morphometric measurements differed significantly between sexes (Table 1). Mass of female and male Great Egrets did not differ between years (P = n.s.) or by Julian date (P = n.s.; Table 2). Mass of female White Ibis was higher during 2006 than 2007 ($P < 0.05$), but did not differ by Julian Date ($P > 0.05$; Table 2). Male White Ibis mass did not differ by year (P = n.s.), but mass decreased with Julian Date ($P < 0.05$; Table 2).

The discriminant function model for the Great Egret contained only a term for mass (Wilks $\lambda = 0.58$: $F_{1,74} = 54.13$, $P < 0.0001$), and correctly classified 88% of the 76 known egret sexes (84% male and 92% female; Fig. 1). The jackknife procedure showed no drop in correct classification (87% male and 92% female) and indicated that the model was stable. The discriminant functions for Great Egret mass were: $D_{Female} = \text{mass}\ (0.13) - 58.48$ and $D_{Male} = \text{mass}\ (0.15) - 83.01$.

For comparative purposes, a DFA for the Great Egret was also conducted without mass because in other studies where mass varies seasonally, it has proved unreliable (see Devlin *et al.* 2004; Bluso *et al.* 2006; Svagelj and Quintana 2007). The best model without mass consisted only of wing chord (Wilks $\lambda = 0.68$: $F_{1,74} = 36.17$, $P < 0.0001$) and correctly classified 81% of the 76 known Great Egret sexes (84% male and 78% female; Fig. 2). The jackknife validation procedure showed only a slight drop in correct classification of

Table 1. Male and female body measurements (mean ± SE) of all Great Egrets and White Ibises captured during 2006-2007. Differences between sexes were determined using t-tests. All measurements are presented in mm, except mass, which is in g.

Species	Measurement	Female (±SE)	N	Male (±SE)	N	t-value	P
Great Egret							
	Wing chord	372.0 (1.78)	47	391.4 (2.89)	29	-5.68	<0.0001
	Wing flat	376.0 (1.72)	47	394.4 (3.34)	29	-4.87	<0.0001
	Culmen	107.6 (0.80)	47	112.1 (1.13)	29	-3.28	<0.01
	Bill depth	19.6 (0.24)	47	20.7 (0.21)	29	-2.48	<0.05
	Tarsus	145.2 (1.41)	47	153.2 (5.05)	29	-1.53	n.s.
	Mass	883.3 (11.0)	47	1,048.3 (22.40)	29	-6.61	<0.0001
White Ibis							
	Wing chord	277.0 (1.42)	68	291.3 (1.53)	62	-6.82	<0.0001
	Wing flat	281.2 (1.43)	68	295.4 (1.5)	62	-6.85	<0.0001
	Culmen	136.2 (1.78)	68	155.3 (2.0)	62	-7.15	<0.0001
	Bill depth	21.1 (0.27)	68	22.3 (0.25)	62	-3.00	<0.01
	Curved bill	133.1 (1.7)	68	152.2 (1.95)	62	-7.34	<0.0001
	Tarsus	88.4 (0.86)	68	97.4 (1.0)	62	-6.89	<0.0001

the sexes (82% male and 79% female), suggesting the model was stable. The discriminant functions for wing chord were: D_{Female} = wing chord (1.93) – 359.34 and D_{Male} = wing chord (2.03) – 397.69.

These discriminant functions were simplified into a linear expression where if $D_{Male-Female}$ was >0, Great Egrets were classified as males, and if $D_{Male-Female}$ was <0 then Great Egrets were classified as females. These linear functions were defined as: $D_{Male-Female}$ = mass (0.02) – 24.83 and $D_{Male-Female}$ = wing chord (0.1) – 38.35.

Male and female Great Egret morphometric measurements overlapped resulting in a low probability of correct sex classification. Great Egrets with a mass or wing chord function from ≤-0.2 to ≥0.82 (mass), and ≤-0.64 to ≥0.90 (wing chord) had a lower than 75% chance of being sexed correctly (Figs. 1 and 2). Seventeen and 19 of the sampled Great Egrets were within this range respectively. After removing those individuals, the mass and wing chord functions correctly classified the sex of 92% and 87% of the samples respectively.

The White Ibis DFA determined that the combination of curved bill and tarsus best discriminated sexes (Wilks $\lambda = 0.66$: $F_{1,124} = 30.74$, $P < 0.0001$). The combination of White Ibis curved bill and tarsus only correctly classified 78% of the known sexes

Table 2. Analyses of covariance model results for pre-breeding Great Egret and White Ibis mass by year and Julian date. The covariate was the first principal component 1, obtained from six and seven morphometric variables measured from Great Egrets and White Ibises respectively.

	Female			Male		
Effect	df	*F*	*P*	df	*F*	*P*
Great Egret						
Year	1,43	0.64	0.42	1,25	1.53	0.22
Julian date	1,43	0.93	0.33	1,25	0.002	0.96
PC1[a]	1,43	16.84	<0.05	1,25	16.61	<0.05
White Ibis						
Year	1,63	8.87	<0.05	1,59	0.14	0.70
Julian date	1,63	1.21	0.27	1,59	5.39	<0.05
PC1	1,63	9.94	<0.05	1,59	58.59	<0.05

[a]Principal component 1.

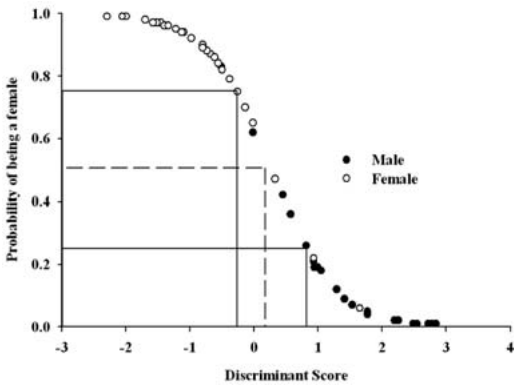

Figure 1. Probability of being a female Great Egret after applying the mass discriminant function. Broken line indicates the 0.50 probability of being a female. Solid lined indicates the 0.75 probability. Great Egrets with a discriminant function ≤-0.27 would be classified as females and ≥0.82 as males.

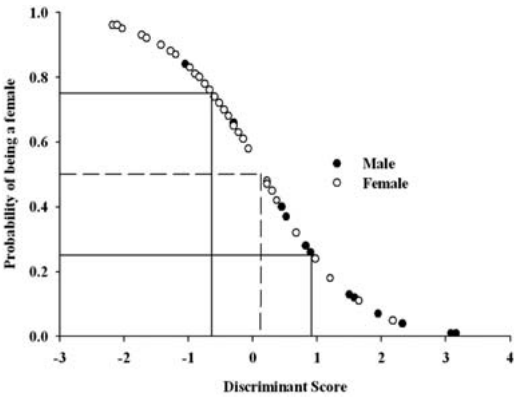

Figure 2. Probability of being a female Great Egret after applying the wing chord discriminant function. Broken line indicates the 0.50 probability of being a female. Solid lined indicates the 0.75 probability, Great Egrets with a discriminant function ≤-0.64 would be classified as females and ≥0.90 as males.

(79% male and 75% female; Fig. 3). The jackknife procedure estimate showed only a slight drop in the overall percent correct classification (77%; 80% male and 75% female), indicating that the model was stable. White Ibis discriminant functions were: D_{Female} = bill curved (-0.01) + tarsus (1.66) – 72.20 and D_{Male} = bill curved (0.04) + tarsus (1.73) – 87.77. The simplified linear form of this discriminant function was: $D_{Male\text{-}Female}$ = bill curved (0.03) + tarsus (0.07) – 15.57.

Male and female White Ibis morphometric measurements also overlapped resulting in a low probability of correct sex classification. White Ibises with a bill curved/tarsus function from -0.75 to 0.75 had a lower than 75% chance of being sexed correctly (Fig. 3). Twenty-four of the sampled White Ibises were within this range. After removing those individuals, the bill curve/tarsus function correctly classified the sex of 88% of the remaining 106 samples.

DISCUSSION

Great Egret discriminant functions indicated that there were large differences between sexes for both mass and wing chord, with minimal overlap. While these results demonstrated that the highest discrimination of sexes occurred when using mass (88% correct classifications), the less variable wing chord measurement also provided a reliable sexing criterion (81% correct classifications). While other researchers have excluded mass due to concerns over increased variance associated with seasonal mass changes (see Devlin *et al.* 2004; Bluso *et al.* 2007; Svagelj and Quintana 2007), we included this measurement because it did not vary during our sampling time frame and little is known about seasonal or intraspecific mass change in Great Egrets (Dunning 1993; McCrimmon *et al.* 2001). Although our results represent a large sample of Great Egrets captured outside the breeding season, additional sampling of egrets across all seasons could prove beneficial in understanding species specific seasonal mass changes and could validate the use of mass to predict sex of Great Egrets.

White Ibis discriminant functions proved less efficient at separating sexes with only 78% of our measured individuals being classified correctly using the combination of curved bill length and tarsus length. While these results were not as effective as the Great Egret discriminant functions (e.g., mass), they do provide a quick and reliable method to determine sex for 88% of all White Ibis captured after removing overlapping individuals. Using this study as an example with an overlap of 18% of sexes, only 24 of our samples would have to be sexed using DNA tech-

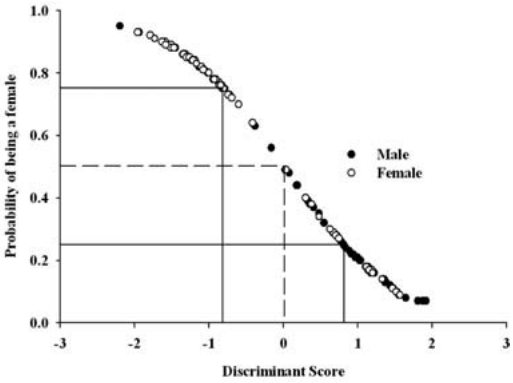

Figure 3. Probability of being a female White Ibis after applying the curved bill/tarsus discriminant function. Broken line indicates the 0.50 probability of being a female. Solid lined indicates the 0.75 probability. White Ibis with a discriminant function ≤-0.75 would be classified as females and ≥0.75 as males.

niques. This represents an 82% savings in cost for the analysis of sex when compared to having DNA sexing done for all samples. Similarly for Great Egrets, using either our mass or wing chord discriminant functions would results in a 75%-78% reduction in cost respectively from using DNA sexing exclusively.

We caution that direct application of the equations derived in this study may not be suitable for all Great Egrets and White Ibises. Given the large geographic area that both species exist in (see Kushlan and Bildstein 1992; McCrimmon *et al.* 2001) it is expected that geographic variation in size may occur and should be considered before application of these equations to birds captured elsewhere (van Franeker and ter Braak 1993).

Error in measurements between researchers could also be important in determining the applicability of these discriminant functions in other studies. During this study six individuals took measurements on both species. We considered any differences between observers to be within measurement error and reflected normal field conditions. This inherent variation likely improves the applicability of these discriminant functions to other studies (Devlin *et al.* 2004; Bluso *et al.* 2006) where minor errors may occur due to researcher or equipment inaccuracy.

The results of this study provide a valuable tool for current and future research on Great Egrets and White Ibises. Using Great Egret and White Ibis morphometric measurements and DFA produced reliable, accurate functions for sexing both species outside of the range where their measurements overlap. Future research might examine seasonal and intraspecific fluctuations in Great Egret mass to validate its use across other seasonal periods for DFA sexing. Examining possible differences in all measurements between adults and subadults may also be important in determining the applicability of these models to all captured Great Egrets and White Ibises.

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