

Sexing California Gulls Using Morphometrics and Discriminant Function Analysis

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Abstract.—A discriminant function analysis (DFA) model was developed with DNA sex verification so that external morphology could be used to sex 203 adult California Gulls (*Larus californicus*) in San Francisco Bay (SFB). The best model was 97% accurate and included head-to-bill length, culmen depth at the gonyes, and wing length. Using an iterative process, the model was simplified to a single measurement (head-to-bill length) that still assigned sex correctly 94% of the time. A previous California Gull sex determination model developed for a population in Wyoming was then assessed by fitting SFB California Gull measurement data to the Wyoming model; this new model failed to converge on the same measurements as those originally used by the Wyoming model. Results from the SFB discriminant function model were compared to the Wyoming model results (by using SFB data with the Wyoming model); the SFB model was 7% more accurate for SFB California gulls. The simplified DFA model (head-to-bill length only) provided highly accurate results (94%) and minimized the measurements and time required to accurately sex California Gulls. Received 29 January 2009, accepted 29 November 2009.

Key words.—California, discriminant function, *Larus californicus*, San Francisco Bay, sex determination.

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Sex determination of birds is a critical component of most field studies examining potential differences between sexes in their behavior, habitat use, parental duties and physiological condition. For many species of gulls, terns, shorebirds (Charadriiformes) and wading birds (Ciconiiformes), sex determination in the field requires intensive behavioral observations or invasive laparotomy procedures. However, models that identify relatively small differences in measurements between males and females can be beneficial for determining the sex of species that would otherwise be considered monomorphic in terms of plumage characteristics or gross morphological differences.

Recent papers have capitalized on the approach of using morphometric measurements in conjunction with DNA sex validation for sex determination of relatively monomorphic species (e.g. Dovekies *Alle alle*: Jakubas and Wojczulanis 2007; Imperial Shags *Phalacrocorax atriceps*: Svagelj and Quin-

tana 2007; Sooty Terns *Onychoprion fuscatus*: Reynolds *et al.* 2008). An important issue with these morphometric models is that they may not necessarily be appropriate throughout the range of a species because of potential geographic variation in bird size. There are few studies that have examined the applicability of a previously-developed sex determination model to other regions.

California Gulls (*Larus californicus*) are considered a monomorphic species with similar plumage and only slight differences in body size between sexes. They are widely distributed, with breeding sites ranging from the southwestern extreme of California, east to North Dakota, and north to the Northwest Territories, Canada (Jehl 1987; Winkler 1996). Rodriguez *et al.* (1997) developed a previous sex determination model for a Wyoming population of California Gulls (hereafter, Wyoming model), but this model has not been validated in other breeding areas. However, California Gulls are known to vary

in their morphology throughout their range (Jehl 1987). Although the Wyoming model was accurate for the Wyoming population, the sex of <9% of the gulls used in that study were validated via laparotomy, while the remainder were sexed based on behavior. A critical element of recently-developed sex determination models has been conducting validation with DNA testing. Thus, in light of apparent geographical differences in measurements across the range of California gulls, results from the Wyoming model were compared with data from SFB that were validated with DNA testing.

The objectives of this study were to: 1) develop a discriminant function analysis (DFA) using DNA sex validation to construct a sex determination model for California Gulls in San Francisco Bay (SFB); 2) determine whether the Wyoming model could be replicated by testing the same measurements using SFB data and a new DFA; and 3) test the Wyoming model functions by using SFB California Gull morphometric data, and determine the difference in sexing accuracy between models.

METHODS

Study Area and Field Methods

The San Francisco Bay area supports seven breeding colonies of California Gulls, with recent estimates of >46,000 breeding gulls in the region (Strong *et al.* 2004; Ackerman *et al.* 2006; J. Bluso-Demers, San Francisco Bay Bird Observatory, unpublished data). Gulls were captured between 6 March and 30 May during 2007 and 2008 using net launchers (Coda Enterprises, Mesa, AZ) and rocket nets (Parris 1977; Heath and Frederick 2003) during the pre-breeding season, and net launchers and bow nets (Bub 1991) during the breeding season. Gulls were captured at the two largest breeding colonies in San Francisco Bay: Pond A6 and N2A/3A on the Don Edwards San Francisco Bay National Wildlife Refuge (37°26'N, 121°58'W). Measurements were taken for each gull, including exposed culmen length (tip of upper mandible to the base of feathers on the forehead), culmen depth at the gonys, culmen width at the gonys, head-to-bill length (tip of upper mandible to the occipital crest), short tarsus length (middle of midtarsal joint to the end of tars-metatarsus), length of rectrices R1 and R6, flattened wing length, and body mass. All measurements were recorded to the nearest 0.01 mm using digital calipers, except flattened wing length and tail feather lengths which were measured with a stopped wing ruler to the nearest 1 mm. Body mass was measured to the nearest 1 g using a Pesola spring scale (Pesola AG, Baar, Switzerland). All measurements were

taken by closely-supervised, trained biologists following an established protocol. One drop of blood was collected from each gull from the brachial vein. Blood samples were stored on ice in the field and frozen at -20°C in the lab for analysis. DNA sex determination was conducted by Zoogen Services, Inc. (Davis, California), with a reported sex identification accuracy of 99.9% (Zoogen 2008). Each gull was banded with USGS aluminium leg bands and released at the capture site.

Statistical Analyses

Analysis of variance (ANOVA) was used to test for differences between sexes for all external measurements after it was determined that data met equal variance assumptions (Levene's homogeneity of variance test; JMP 2001) and residuals were normal. The percent sexual size dimorphism (SSD) was determined by subtracting the mean morphological values of females from the mean values of males and then dividing the absolute difference by the mean male value.

In the next stage of the analysis, DFA was used to identify the measurements that best separated the sexes of California Gulls. Pearson's correlation analysis (PROC CORR; SAS Institute 2003) was used to determine if variables were highly correlated (multicollinearity; $R^2 \geq 0.70$; McGarigal *et al.* 2003). No variables were highly correlated so they were all retained for the DFA (Noon 1981). Of the nine measurements recorded, body mass and rectrices (R1 and R6) were excluded from the DFA models because mass can change seasonally (see Devlin *et al.* 2004; Bluso *et al.* 2006; Svagej and Quintana 2007) and feathers can wear over time (Voelker 1997).

Stepwise DFA (PROC STEPDISC, PROC DISCRIM; SAS Institute 2003) was then used to identify the measurements that best separated the sexes (Klecka 1982; McGarigal *et al.* 2003), using the unequal covariance matrices option (Khattree and Naik 2000). The leave-one-out procedure (jackknife; PROC DISCRIM; SAS Institute 2003) was used to validate results (Phillips and Furness 1997; McGarigal *et al.* 2003). Posterior probabilities were calculated for each gull's classification and plotted against their individual discriminant scores. The 75% cutoff points were calculated for discriminant scores to represent 75% probabilities of being either male or female. To determine if a simplified model could be developed that accurately sexed California Gulls based on just one measurement, DFA models were run iteratively with individual measurements until it was established which measurement most accurately predicted sex of California Gulls.

After establishing the DFA model to sex California Gull's in SFB, a DFA model was then run that included only the measurements that the Wyoming model used for sex determination. This model evaluated whether the DFA model selection process would select the same measurements as the Wyoming model. The measurements used in the Wyoming study were culmen depth at the gonys, culmen length, head-to-bill length, tarsus length and mass. However, it was determined that the DFA would not converge on the same set of measurements that the Wyoming model used (see Results). Next, to determine whether the Wyoming sex determination model could be replicated, only the measurements specified by the final Wyoming model (head-to-bill length, culmen depth at gonys and tarsus length) were used, but this time using DFA and SFB gull data.

Lastly, the SFB morphometric data were fit to the published sex determination model functions of the Wyoming model to determine whether that model was accurate compared to the new DFA. The Wyoming model classification function was:

$$\hat{y} = -117.241 + 2.286 (\text{culmen depth at gonys}) + 0.604 (\text{head-to-bill length}) + 0.319 (\text{tarsus})$$

where \hat{y} is the discriminant score. California Gulls in Wyoming had a $\hat{y} < 0$ (negative) for females and $\hat{y} \geq 0$ for males (Rodríguez *et al.* 1997). A χ^2 test was used to analyze the frequency of correct versus incorrect sex classifications for SFB gulls using the new DFA developed and the Wyoming model functions with our SFB data.

RESULTS

A total of 203 adult California Gulls (90 males and 113 females) were captured and sexed using DNA. All nine measurements differed significantly between sexes, with males being consistently larger than females (Table 1). Sexual size dimorphism between female and male California Gulls was largest for body mass, followed by culmen length, culmen depth at the gonys, culmen width, and head-to-bill length (Table 1). However, the greatest statistical difference between males and females among the measurements was for head-to-bill length, followed by culmen depth at gonys, wing length, culmen length, and body mass. The relationship between head-to-bill length and culmen depth at gonys is presented to demonstrate those differences between males and females (Fig. 1).

SFB California Gull DFA Sexing Model

The DFA determined that the combination of head-to-bill length, culmen depth at the gonys, and flattened wing length best discriminated sexes of California Gulls (Wilks $\lambda = 0.24$; $F_{6,197} = 203.14$, $P < 0.0001$). This DFA correctly classified 97% of the known sexes (96% female and 98% male; Fig. 2A). The leave-one-out procedure also estimated a 96% correct classification (97% female and 96% male), demonstrating that the model was stable. Gull discriminant functions were:

$$D_{\text{male}} = \text{head-to-bill length (4.8417)} + \text{culmen depth at gonys (16.2935)} + \text{flattened wing length (4.7736)} - 1325.0$$

Table 1. Sexual size dimorphism (SSD) and body measurements (mean \pm SD) of California Gull males (N = 90) and females (N = 113) in San Francisco Bay, CA, during 2007 and 2008 (in order of greatest statistical difference). Wyoming measurements presented to demonstrate geographic variation in morphology. All measurements in mm, except mass (g).

Measurement	California			Wyoming ¹			
	Females (\pm SD)	Male (\pm SD)	$F_{1,202}$	P	% SSD	Females (\pm SD)	Male (\pm SD)
Head-to-bill length	102.8 (3.4)	112.4 (4.1)	461.5	<0.0001	8.5	100.7 (3.3)	110.1 (2.6)
Culmen depth at gonys	15.0 (0.6)	16.7 (0.8)	287.3	<0.0001	10.2	14.6 (0.7)	16.7 (0.9)
Wing length	387.0 (9.0)	407.4 (0.8)	279.0	<0.0001	5.0		
Culmen length	44.5 (2.4)	49.6 (2.5)	210.2	<0.0001	10.3	43.2 (2.0)	48.2 (2.2)
Mass	599.1 (4.8)	700.8 (51.3)	193.5	<0.0001	14.5	538.0 (53.6)	644.0 (63.1)
Tarsus length	55.5 (2.3)	60.0 (3.3)	114.3	<0.0001	7.5	53.3 (2.8)	57.6 (2.8)
Tail R1 length	134.7 (8.4)	144.8 (1.2)	51.1	<0.0001	7.0		
Tail R6 length	141.2 (10.0)	150.4 (10.7)	42.4	<0.0001	6.1		
Culmen width	6.6 (0.6)	7.3 (0.6)	42.2	<0.0001	9.6		

¹Wyoming measurement data from Rodríguez *et al.* (1996).

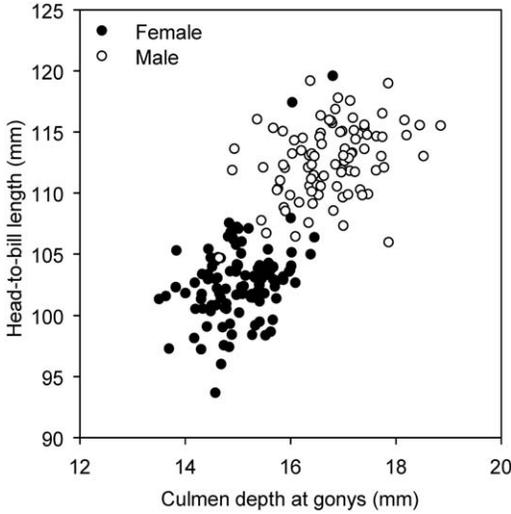


Figure 1. Sexual size dimorphism based on head-to-bill length and culmen depth at gonyx to sex female (filled circles) and male (hollow circles) California Gulls.

$$D_{\text{Female}} = \text{head-to-bill length (4.2761)} + \text{culmen depth at gonyx (14.2344)} + \text{flattened wing length (4.6350)} - 1172.0$$

The simplified linear form of this discriminant function was:

$$D_{\text{Male-Female}} = \text{head-to-bill length (0.5656)} + \text{culmen depth (2.0591)} + \text{flattened wing length (0.1386)} - 153.0$$

where $D_{\text{Male-Female}} > 0.2 = \text{males}$ and $D_{\text{Male-Female}} < 0.2 = \text{females}$.

Of all the individual measurements, head-to-bill length most accurately predicted sex (94% overall; 92% female, 96% male; Fig. 2B). The leave-one-out procedure estimated a 95% correct classification (96% female and 93% male), demonstrating that the model was stable. The discriminant functions for the simplified model were:

$$D_{\text{male}} = \text{head-to-bill length (10.7982)} - 608.2516$$

$$D_{\text{Female}} = \text{head-to-bill length (9.8585)} - 506.9981$$

and the simplified linear form of this discriminant function was:

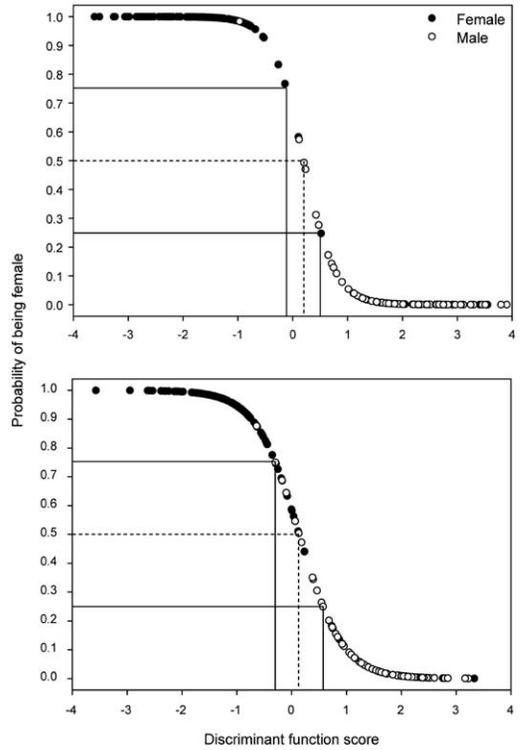


Figure 2. A) Probability of being a female California Gull after applying the best discriminant function model that included culmen depth at gonyx, head-to-bill and wing length. The broken line at 0.20 separates the 0.50 probability of being a female or male. The solid line designates the 0.75 probability of being correctly classified; California Gulls with a discriminant function ≤ -0.10 would be classified as females and ≤ 0.50 as males. B) Probability of being a female California Gull after applying the discriminant function model iteratively and determining that head-to-bill length best discriminated sex. The broken line at 0.13 separates the 0.50 probability of being a female or male. The solid line designates the 0.75 probability of being correctly classified; California Gulls with a discriminant function ≤ -0.31 would be classified as females and ≥ 0.57 as males.

$$D_{\text{Male-Female}} = \text{head-to-culmen length (0.9397)} - 101.2535$$

where $D_{\text{Male-Female}} > 0.13 = \text{males}$ and $D_{\text{Male-Female}} < 0.13 = \text{females}$.

Wyoming Sexing Model

The measurements that the Wyoming model originally specified were used initially to sex California Gulls (culmen length, culmen depth at gonyx, head-to-bill length, tar-

sus length, mass), and the DFA did not converge on the same final measurements as the Wyoming model. Starting with the same measurements used in Wyoming, the new SFB DFA model determined that the combination of head-to-bill length, culmen depth at gonys, culmen length, and body mass best discriminated sexes of gulls (Wilks $\lambda = 0.27$: $F_{4,199} = 132.54$, $P < 0.0001$), whereas the final Wyoming model chose culmen depth at gonys, head-to-bill length, and tarsus. Unlike the Wyoming model, the new SFB DFA model did not identify tarsus as an important sex determinant of California Gulls, but included additional variables not in their model: culmen length and body mass.

An additional DFA model was then tested, starting with the final measurements used in the Wyoming model (culmen depth at gonys, head-to-bill length, and tarsus length); however, this procedure also did not result in a model that converged on the measurements that the Wyoming model used. Instead, this model converged using only head-to-bill length and culmen depth at gonys (Wilks $\lambda = 0.26$: $F_{2,200} = 277.60$, $P < 0.0001$). Thus, the Wyoming model could not be replicated.

Lastly, the sexing accuracy of the Wyoming model was determined for SFB data. The SFB data were fitted to the Wyoming model functions; the sex classification rate was reduced to 90% (89% female and 91% male). The proportion of correct sex classifications was lower using the Wyoming model compared to the SFB DFA model ($\chi^2_{203} = 134.67$, $P < 0.0001$).

DISCUSSION

Male California Gulls were significantly larger than females in SFB, similar to California Gull populations measured in Wyoming (Rodríguez *et al.* 1997). Whereas sexual size dimorphism was greatest in body mass and culmen length, head-to-bill length and culmen depth at the gonys statistically differed the most between sexes in SFB. Accordingly, the DFA model selected head-to-bill length, culmen depth at gonys, culmen length, and flattened wing length. These differences be-

tween sexes yielded a discriminant function model with a very high correct sex classification rate (97%). This discriminant function model significantly improved (by 7%) the classification rate of SFB California Gull sexes compared to the Wyoming model when SFB data were fit to the Wyoming model functions. Additionally, using data from SFB, it was not possible to replicate the Wyoming model to sex California Gulls despite using similar body measurements.

The Wyoming sex determination model could not be replicated, suggesting that some degree of geographic difference in measurements exist. The final DFA model for California Gulls in SFB used different measurements than the Wyoming model. Comparing the size of gulls indicated that SFB California Gulls were larger in most measurements (except male culmen depth at gonys) in both sexes, compared to Wyoming California Gulls by about 4%. Jehl (1987) previously demonstrated these geographic differences, suggesting that there were, in fact, a larger northern (*L. c. albertaensis*) and smaller southern (*L. c. californicus*) subspecies. Although SFB California Gulls were generally larger than gulls breeding at the largest southern breeding colony at Mono Lake in California, they were smaller than the proposed northern subspecies that breeds primarily in Canada (Jehl 1987). Evans *et al.* (1993) found that a model developed to sex Laughing Gulls (*L. atricilla*) in Florida misidentified the sex of 40% of Laughing Gulls from a site in New York State. Further, Ruiz *et al.* (2008) observed that a sex determination model developed for a specific Audouin's Gulls (*L. audouinii*) colony failed to correctly identify 44% of the gulls in the same colony 13 years later. Most sex determination models are derived from data collected over short periods of time (e.g. two to three years; Liordis and Goutner 2008; Wallace *et al.* 2008). The Wyoming model was developed using data that spanned over one decade (Rodríguez *et al.* 1997), and there is potential for temporal variation to have influenced the sexing model (Coulson *et al.* 1982; Ruiz *et al.* 2008).

Several other DFA models have been developed for other species of gulls, with a similarly high rate of correct sex classification (e.g. $\geq 99\%$ for Yellow-legged Gull [*L. cachinnans*], Bosch 1996; $>99\%$ for Great Black-backed Gull [*L. marinus*], Mawhinney and Diamond 1999; and 97% for Kelp Gull [*L. dominicanus*] Torlaschi *et al.* 2000). Common among all of these studies were discriminant functions that included head and culmen measurements. In particular, head-to-bill length and culmen depth at the gonyes are often the most important measurements for discriminating sexes in Larids (Bosch 1996; Mawhinney and Diamond 1999; Torlaschi *et al.* 2000; Bluso *et al.* 2006; Ackerman *et al.* 2008). Jodice *et al.* (2000) and Rodriguez *et al.* (1997) are the only studies that found tarsus to be an important measurement for discriminating sex in gulls.

Compared to other recently developed sex determination DFA models for other species of waterbirds, the model developed for SFB California Gulls provided a very high degree of accuracy. For example, Bluso *et al.*'s (2006) Forster's Tern (*Sterna forsteri*) and Ackerman *et al.*'s (2008) Caspian Tern (*Hydroprogne caspia*) sex determination models predicted sex 87% and 83% correctly, and Herring *et al.*'s (2008) models for Great Egrets (*Ardea alba*) and White Ibis (*Eudocimus albus*) were only correct 88% and 78% of the time, respectively. Collectively, these studies demonstrate the utility of DFA sex determination models, especially for gull species, where a high degree of accuracy can be expected relative to other species.

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