

## GENDER IDENTIFICATION OF CASPIAN TERNS USING EXTERNAL MORPHOLOGY AND DISCRIMINANT FUNCTION ANALYSIS

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**ABSTRACT.**—Caspian Tern (*Sterna caspia*) plumage characteristics are sexually monochromatic and gender cannot easily be distinguished in the field without extensive behavioral observations. We assessed sexual size dimorphism and developed a discriminant function to assign gender in Caspian Terns based on external morphology. We collected and measured Caspian Terns in San Francisco Bay, California, and confirmed their gender based on necropsy and genetic analysis. Of the eight morphological measurements we examined, only bill depth at the gonys and head plus bill length differed between males and females with males being larger than females. A discriminant function using both bill depth at the gonys and head plus bill length accurately assigned gender of 83% of terns for which gender was known. We improved the accuracy of our discriminant function to 90% by excluding individuals that had less than a 75% posterior probability of correctly being assigned to gender. Caspian Terns showed little sexual size dimorphism in many morphometrics, but our results indicate they can be reliably assigned to gender in the field using two morphological measurements. Received 19 April 2007. Accepted 15 August 2007.

Many seabird species are sexually monomorphic in plumage characteristics and gender cannot easily be assigned without extensive behavioral sampling (such as gender-specific breeding behaviors or vocalizations) (Coulter 1986, Chardine and Morris 1989, Phillips and Furness 1997, Casaux and Baroni 2000), laparotomy (Quinn 1990, Stern and Jarvis 1991) or subsequent laboratory genetic analyses (Jodice et al. 2000, Genovart et al. 2003, Quintana et al. 2003, Devlin et al. 2004, Setiawan et al. 2004). Discriminant analysis of external morphometrics is a mathematical approach that has been widely used to assign gender of seabirds, including several gulls (*Larus* spp.) (Hanners and Patton 1985, Evans et al. 1993, Mawhinney and Diamond 1999, Torlaschi et al. 2000), terns (*Sterna* spp. and

*Chlidonias* spp.) (Coulter 1986, Stern and Jarvis 1991, Devlin et al. 2004, Fletcher and Hamer 2003, Bluso et al. 2006), petrels (*Fulmarus* spp., *Thalassoica* spp., *Daption* spp., and *Pagodroma* spp.) (Van Franeker and Ter Braak 1993, Weidinger and Van Franeker 1998), jaegers (*Stercorarius* spp.) (Phillips and Furness 1997), shearwaters (*Puffinus* spp.) (Genovart et al. 2003), noddy (*Anous* spp.) (Chardine and Morris 1989), shags (*Phalacrocorax* spp.) (Casaux and Baroni 2000, Quintana et al. 2003), kittiwakes (*Rissa* spp.) (Jodice et al. 2000), and Yellow-eyed Penguins (*Megadyptes antipodes*) (Setiawan et al. 2004). Discriminant analysis produces a linear combination of morphological variables that best describes the distinction between known males and females (Khattree and Naik 2000).

One prior study examined gender differences in Caspian Tern (*Sterna caspia*) morphology (Quinn 1990), but the discriminant function, which accurately assigned gender to 77% of the terns, did not incorporate head plus bill length. The distance from the back of the head to the tip of the bill (hereafter head-to-bill length) is widely used in discriminant function analyses to differentiate gender in many Laridae (Hanners and Patton 1985, Stern and Jarvis 1991, Phillips and Furness 1997, Fletcher and Hamer 2003, Devlin et al. 2004). This single measurement has distinguished gender

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with 88-98% accuracy in some gull species (Coulson et al. 1983, Mawhinney and Diamond 1999, Jodice et al. 2000, Torlaschi et al. 2000) and linear combinations of head-to-bill length with other morphological characteristics has increased the ability to accurately assign gender of several tern species (Stern and Jarvis 1991, Fletcher and Hamer 2003, Devlin et al. 2004). Our objective was to examine sexual size dimorphism of Caspian Terns in San Francisco Bay, California, USA, and develop a discriminant function incorporating head-to-bill length and several other external measurements.

#### METHODS

*Study Area.*—San Francisco Bay, California (37.8° N, 122.3° W) is the largest estuary on the west coast of North America. Caspian Terns breed at as many as 13 different sites in San Francisco Bay (Strong et al. 2004). We sampled Caspian Terns near three breeding colonies, including a site in the North Bay (Napa River near the Carquinez Strait) and two sites in the South Bay on the Don Edwards San Francisco Bay National Wildlife Refuge (Alviso Slough and Coyote Hills Pond 2A).

*Collections and Measurements.*—We collected terns by shotgun under California Department of Fish and Game Scientific Collection permits (SC-801034-05 and SC-007250), U.S. Fish and Wildlife Service permit (MB120154-2), and guidelines of the USGS, Western Ecological Research Center Animal Care and Use Committee. We measured culmen length, bill depth at the gonys, head-to-bill length, tarsus length (tarsometatarsus bone), wing length (carpal joint to the end of the longest straightened primary), length of rectrices R1 and R6 (R1 was the central most rectrix on right side, R6 was the outer most rectrix on right side), and body mass for each tern. We measured tern morphology to the nearest 0.01 mm with digital calipers (Fisher Scientific, Hampton, NH, USA), except wing chord and tail measurements, which were measured to the nearest 1.0 mm with a stopped wing rule. We measured body mass to the nearest 1.0 g with a 1-kg Pesola spring scale (Pesola AG, Baar, Switzerland). One of two researchers measured terns to reduce any observer related variation in measurement er-

ror. We examined the gonads of each tern via necropsy and verified gender by genetic analysis. A drop of blood was collected from each tern for gender analysis of the chromo-helicase-DNA binding protein gene (e.g., Jodice et al. 2000, Quintana et al. 2003) at Zoogen Services Inc.<sup>®</sup>, Davis, California, USA.

*Analyses.*—We used analysis of variance to test differences in morphological measurements between male and female terns. We examined sexual size dimorphism (SSD) in terns by calculating the absolute value of the difference between the mean morphological measurement for females and males, and dividing this quantity by the mean value for males. We calculated the best measurements for classifying gender of Caspian Terns with a forward stepwise discriminant function analysis using PROC STEPDISC in SAS software (Morrison 1990, SAS Institute 2004). We used a criterion based on an *F*-test of Wilks' lambda ( $\Lambda$ ) at each step of the analysis to enter the variable contributing the most or to remove the variable contributing the least discriminatory power to the model until no further variables could be entered or removed at the 0.15 significance level. We believed that a randomly captured tern was equally likely to be female or male in the absence of morphometric data; therefore, we used *a priori* probability of 50% for the likelihood of being female. We used five of the eight morphological measurements recorded in our discriminant function analysis; we excluded body mass and rectrices R1 and R6, because they are more pliable and can vary over time (Kaufman 1983, Voelker 1997). We defined discriminant scores (hereafter *D*) as  $D = -0.5(x - \mu)' \Sigma^{-1}(x - \mu)$ , where  $(x - \mu)' \Sigma^{-1}(x - \mu)$  represented the squared distance from a tern with measurements *x* to a subpopulation with mean  $\mu$  and variance matrix  $\Sigma$  (Khattree and Naik 2000, SAS Institute 2004). We calculated discriminant scores,  $D_{Female}$  and  $D_{Male}$ , using the mean and variance of the respective genders. We classified terns into the gender for which the smallest squared distance, or the largest *D* score was measured. We classified terns as males if  $D_{Male-Female}$ , defined as  $D_{Male} - D_{Female}$ , was  $>0$  and as females if  $D_{Male-Female}$  was  $<0$ . We simplified discriminant scores into linear expressions without changing the effect of scoring between genders by assuming the var-

TABLE 1. Morphological measurements (mean  $\pm$  SD) and sexual size dimorphism (SSD) of Caspian Terns in San Francisco Bay, California, 2006.

Measurement	Female <sup>a</sup>	Male <sup>a</sup>	$F_{1,38}$	$P$	SSD (%)
Bill depth (mm)	15.19 $\pm$ 0.66	16.60 $\pm$ 0.99	27.87	<0.0001	8.49
Head-to-bill (mm)	134.00 $\pm$ 3.24	137.22 $\pm$ 3.23	9.92	0.003	2.35
Tarsus (mm)	45.30 $\pm$ 2.36	46.45 $\pm$ 3.22	1.66	0.21	2.48
Culmen length (mm)	67.27 $\pm$ 2.28	68.29 $\pm$ 2.96	1.49	0.23	1.49
Wing chord (mm)	429.10 $\pm$ 6.95	430.80 $\pm$ 10.60	0.36	0.55	0.39
Tail retrix 1 (mm)	105.90 $\pm$ 4.46	109.65 $\pm$ 7.44	3.74	0.06	3.42
Tail retrix 6 (mm)	150.10 $\pm$ 8.60	151.50 $\pm$ 9.76	0.23	0.63	0.92
Tail R <sub>6</sub> -R <sub>1</sub>	44.20 $\pm$ 8.55	41.85 $\pm$ 6.48	0.96	0.33	5.62
Mass (g)	669.94 $\pm$ 69.85	662.02 $\pm$ 45.13	0.18	0.67	1.20

<sup>a</sup> Gender confirmed by necropsy and genetic analysis.

iance  $\Sigma$  was constant (SAS Institute 2004). We produced classification error rates with a re-substitution analysis in SAS and validated our discriminant functions using a cross-validation procedure (Lachenbruch and Mickey 1968) where each tern was classified using a function derived from the total sample excluding the tern in question (e.g., Chardine and Morris 1989, Phillips and Furness 1997). We also calculated posterior probabilities of a tern being female (Probability =  $1/[1 + \exp(D_{Male-Female})]$ ) and plotted these values against their corresponding discriminant score. This allowed us to calculate cut-off points for discriminant scores that had a 75% probability of being a female or male.

## RESULTS

We collected 40 Caspian Terns from 19 April to 15 June 2006 in San Francisco Bay. Twenty were females and 20 were males by necropsy with gender confirmed using genetic analysis. Gender assigned via necropsy was 100% in concordance with that from genetic analysis. Two of the eight morphological characteristics we measured differed between males and females with bill depth at the gonys and head-to-bill length showing the least amount of overlap between genders (Table 1). The largest proportional differences in structural measurements between males and females were also greatest in bill depth, followed by tail retrix R1 length, tarsus length, and head-to-bill length (Table 1).

Bill depth at the gonys and head-to-bill length were the best structural measurements separating male and female Caspian Terns (Wilks's  $\Lambda = 0.50$ ;  $F_{2,37} = 18.62$ ,  $P < 0.0001$ ).

The discriminant function with these two morphometrics correctly classified 80% of known females and 85% of known males (83% correct classification rate overall). The leave-one-out cross-validation test (Lachenbruch and Mickey 1968) also correctly classified gender of 83% of the terns. The discriminant scores were:  $D_{Female} = \text{bill depth (22.1297)} + \text{head-to-bill length (12.8779)} - 1,030.9089$ , and  $D_{Male} = \text{bill depth (24.1306)} + \text{head-to-bill length (13.1927)} - 1,105.3964$ .

Thus, Function 1 was:  $D_{Male-Female} = \text{bill depth (2.0008)} + \text{head-to-bill length (0.3148)} - 74.4875$  and we classified terns as males when  $D_{Male-Female}$  was  $>0$  and as females when  $D_{Male-Female}$  was  $<0$ .

There was some overlap in morphological measurements between males and females where the probability of correctly classifying gender was reduced (Fig. 1). Terns with discriminant scores from  $-1.10$  to  $1.10$  had  $<75\%$  probability of being correctly assigned to gender (Fig. 2). Twenty-five percent (10 of 40) of the terns we collected were within this overlapping range. Of the 30 terns with discriminant scores outside these cutoff points, the discriminant function correctly classified 90% of the individuals (Fig. 2).

A second discriminant function using only bill depth at the gonys also was successful in classifying gender (Wilks'  $\Lambda = 0.58$ ;  $F_{1,38} = 27.87$ ,  $P < 0.0001$ ). The discriminant scores for this function were:  $D_{Female} = \text{bill depth (21.4758)} - 163.1193$ , and  $D_{Male} = \text{bill depth (23.4606)} - 194.6647$ . Thus, Function 2 was:  $D_{Male-Female} = \text{bill depth (1.9849)} - 31.5454$ .

This simplified discriminant function correctly classified 80% of known females and

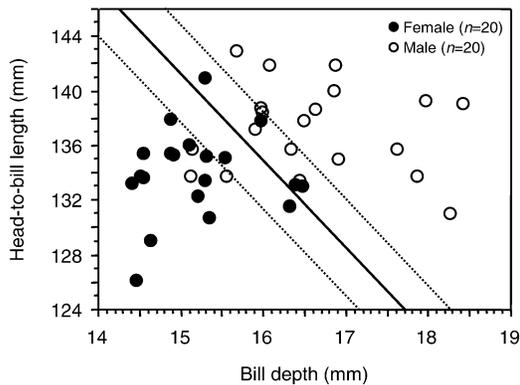


FIG. 1. Discriminant function using bill depth at the gonys and head-to-bill length to classify female (below solid line) and male (above solid line) Caspian Terns in San Francisco Bay, California. Area between the stippled lines indicates morphological overlap where the discriminant function had <75% probability of correctly classifying gender.

75% of known males (78% correct classification rate overall) indicating that bill depth was the most important characteristic for differentiating gender. The cross-validation test also correctly classified 78% of the terns of known gender for Function 2.

#### DISCUSSION

Caspian Terns in San Francisco Bay had only slight sexual size dimorphism in most morphological characteristics we measured (Table 1). However, males were significantly larger than females in bill depth and head-to-bill length. Bill depth at the gonys was the most important morphological measurement in classifying gender of Caspian Terns and this variable alone correctly classified 78% of the terns. A discriminant function incorporating both bill depth and head-to-bill length improved our ability to accurately assign gender of Caspian Terns to 83%. Head-to-bill length and bill depth often are the most important measurements in discriminant functions for seabirds, especially larids (Hanners and Patton 1985, Van Franeker and Ter Braak 1993, Phillips and Furness 1997, Mawhinney and Diamond 1999, Torlaschi et al. 2000, Genovart et al. 2003, Devlin et al. 2004). Our accuracy is slightly better than that reported by Quinn (1990) who developed an earlier discriminant function for Caspian Terns in Texas. He found that 77% of Caspian Terns could be accurately

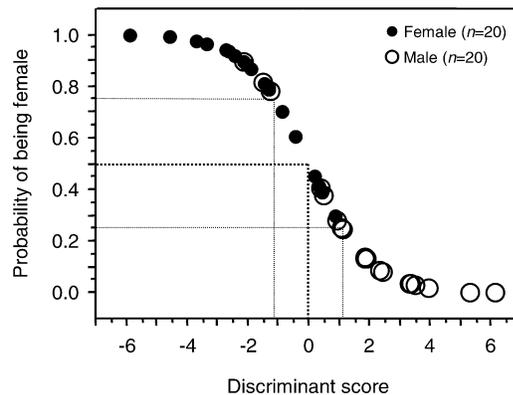


FIG. 2. Probability of being female in relation to the discriminant function scores based on bill depth and head-to-bill length of Caspian Terns in San Francisco Bay, California. All Caspian Terns with discriminant function scores <0 were classified as females and >0 as males in validation procedures. Lines indicate the cutoff points for discriminant scores of 1.10 and -1.10 if the probability of being female was set to 0.25 and 0.75, respectively.

assigned to gender using a function incorporating several morphological measurements including bill depth and culmen length, but not head-to-bill length. Quinn (1990) found that head-to-bill length was significantly larger in male than female Caspian Terns, but this morphometric was not incorporated into his discriminant function.

Our accuracy of 83% for Caspian Terns is comparable to other studies of Laridae. Our discriminant function based on bill depth and head-to-bill length was 5-11% more accurate than functions of multiple morphological characteristics developed for several other tern species (Coulter 1986, Quinn 1990, Stern and Jarvis 1991, Fletcher and Hamer 2003, Devlin et al. 2004), but 3% lower than the function we developed for Forster's Terns (*Sterna forsteri*) (Bluso et al. 2006). For example, using model validation procedures, 73 and 74% of Arctic Terns (*S. paradisaea*) (Fletcher and Hamer 2003, Devlin et al. 2004), 72 and 78% of Common Terns (*S. hirundo*) (Coulter 1986, Fletcher and Hamer 2003), 78% of Black Terns (*Chlidonias niger*) (Stern and Jarvis 1991), and 86% of Forster's Terns (Bluso et al. 2006) were correctly classified as male or female.

Discriminant functions that have been developed for gulls are, in general, more accu-

rate than functions derived for terns. Using multiple morphological measurements, discriminant functions for gulls have accurately classified gender of 90-99% of individuals (Mills 1971, Hanners and Patton 1985, Evans et al. 1993, Mawhinney and Diamond 1999, Torlaschi et al. 2000). The difference in classification rates between Caspian Terns and other Laridae suggests that Caspian Terns exhibit greater sexual size dimorphism than several other terns, but are not as dimorphic as most gull species.

We observed some overlap in morphological measurements between male and female Caspian Terns where the probability of correctly classifying an individual was reduced (Fig. 1). Posterior probabilities can be calculated and individuals with probabilities <75% can be excluded to increase the probability of correctly classifying a tern as male or female. Ten of 40 Caspian Terns we collected were within this range (Fig. 2). Our accuracy increased to 90% after excluding these terns. We caution that excluding terns within this overlapping size range could introduce bias into some studies because researchers would necessarily be classifying only the largest males and smallest females.

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