

Sexing Forster's Terns using Morphometric Measurements

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Abstract.—Forster's Terns (*Sterna forsteri*), like most seabirds, are monomorphic and are difficult to sex without extensive behavioral observations or genetic sexing. We conducted the first morphological study and discriminant function analysis on Forster's Terns to develop a method to accurately identify their sex in the field. A sample of 84 terns from the San Francisco Bay estuary were captured or collected, measured, and the sex of 40 female and 44 male terns was confirmed by genetic analyses or via necropsy. Male Forster's Terns were larger than females for 7 of 9 morphological measurements, with head-bill length showing the least amount of overlap between the sexes, followed by culmen length and culmen depth at the gony. Sexual size dimorphism was greatest with retrix R₆ length, followed by culmen width, and culmen depth. A discriminant function including only head-bill length accurately sexed 82% of Forster's Terns, whereas a second discriminant function incorporating both head-bill length and culmen depth at the gony increased sexing accuracy to 87%. When we used a 75% posterior probability or greater of accurately sexing Forster's Terns, we excluded only 18% of the sample that overlapped and accurately sexed 94% of the remaining individuals. Our results indicate that Forster's Terns can be accurately sexed in the field using only 2 morphological measurements. *Received 25 March 2006, accepted 11 September 2006.*

Key words.—Forster's Tern, *Sterna forsteri*, sexual size dimorphism, discriminant function analysis, sex differences, San Francisco Bay.

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The ability to sex captured birds is often necessary in studies of marked populations, but sexing seabirds is difficult because most species are monochromatic and exhibit only slight size dimorphism. Although sex can be determined with genetic methods, these results are relatively costly to apply over large samples and are not immediately available to field researchers. Alternatively, many Laridae have been effectively sexed using discriminant function analyses of morphological characteristics (Evans *et al.* 1993; Phillips and Furness 1997; Mawhinney and Diamond 1999; Jodice *et al.* 2000; Torlaschi *et al.* 2000; Fletcher and Hammer 2003; Devlin *et al.* 2004). In some gull species, the distance from the back of the head to the tip of the bill (hereafter head-bill length) distinguishes an individual's sex 88–98% of the time (Coulson *et al.* 1983; Mawhinney and Diamond 1999; Jodice *et al.* 2000, Torlaschi *et al.* 2000), but, in others, multiple morphologi-

cal characteristics are necessary to accurately sex a similar proportion of individuals (Hanners and Patton 1985; Evans *et al.* 1993).

Although discriminant function analyses have not been as successful at correctly identifying sexes for terns as they are for other larids, they have proven useful in sex determination of some species. Coulter (1986) accurately sexed 73–80% of Common Terns (*Sterna hirundo*) with discriminant functions of bill measurements and mass, while Quinn (1991) accurately sexed 77% of Caspian Terns (*S. caspia*) using a discriminant function derived from bill and tarsus measurements. Seventy-eight percent of Black Terns (*Chlidonias niger*) were accurately sexed by a discriminant function of head-bill and culmen length (Stern and Jarvis 1991). Fletcher and Hammer (2004) used head-bill and tail fork measurements to accurately sex 77–78% of Common Terns and 72–73% of Arctic Terns (*S. paradisaea*) in Britain. Using head-

bill and culmen depth measurements, Devlin *et al.* (2004) reported similar accuracy (73-74%) sexing Arctic Terns in North America. Despite the difference in sexing accuracy between terns and other larids, discriminant functions based on bill measurements, specifically head-bill length, have been the most widely used technique for sexing many Laridae.

Forster's Terns (*S. forsteri*), like many Laridae, are monomorphic and are difficult to sex in the field (McNicholl *et al.* 2001) without extensive behavioral observations or genetic sexing (e.g., Jodice *et al.* 2000). Although several discriminant functions have been developed to sex larids, no study has developed models for sexing Forster's Terns. In this paper, we present the first data describing sexual size dimorphism in Forster's Terns and develop a discriminant function to reliably sex Forster's Terns in the San Francisco Bay using morphological characteristics.

METHODS

Study Area and Species

Forster's Terns are medium-sized terns whose annual range is limited to North America (McNicholl *et al.* 2001). About 30% of the Pacific Coast breeding population nests within the estuary of the San Francisco Bay, California (hereafter SFBay; McNicholl *et al.* 2001; Strong *et al.* 2004). The SFBay (37.8°N, 122.3°W) is the largest estuary on the west coast of North America. Artificial salt evaporation ponds cover 40% of its 115,000 ha (Goals Project 1999) and provide nesting and foraging habitat for Forster's Terns (Harvey *et al.* 1992; U.S. Geological Survey, unpubl. data). Presently, there are 10 Forster's Tern colonies within SFBay (Strong *et al.* 2004; C. Strong, pers. comm.). In 2005, we captured or collected terns at nine sites within the Napa-Sonoma Marshes State Wildlife Area (Ponds 1, 2, 3), the Eden Landing Ecological Reserve (Pond 7), and the Alviso complex of the Don Edward's San Francisco Bay National Wildlife Refuge (New Chicago Marsh and Ponds A1, AB2, A8, A16).

Data Collection

Pre-breeding Forster's Terns were captured with remotely detonated net-launchers (Coda Enterprises, Mesa, AZ) and collected by shotgun. Breeding terns were captured on the nest with treadle-activated bow-nets. Terns were collected as part of a larger study examining contaminant levels in SFBay birds (Schwarzbach *et al.* 2005). Morphological characters measured were culmen length, culmen depth, and culmen width at the gony, head-bill length, tarsus length (tarsometatarsus

bone), wing length (carpal joint to the end of the longest straightened primary), length of tail rectrices R_1 and R_6 (R_1 being the central most rectrix, R_6 the outer most rectrix), and mass. Live terns were captured, measured, and released whereas collected terns were measured after death. All measurements were made to the nearest 0.01 mm with digital calipers (Mitutoyo Corporation, Kanagawa, Japan) or vernier calipers (ST Industries, St. James, Minnesota), except wing chord and tail measurements, which were measured to the nearest 1.0 mm with a stopped wing rule. Mass was measured to the nearest 1.0 g with a 300-g Pesola spring scale (Pesola AG, Baar, Switzerland). While variation may exist between the four different observers (Barrett *et al.* 1989) who measured terns, that variation was incorporated into the discriminant functions to increase the general application of the function to other researchers in the field (Devlin *et al.* 2004). Therefore, any variation between researchers was considered to be measurement error and individual differences were not corrected for in the analyses. To verify the sex of terns, a drop of blood was collected from all captured birds for genetic analysis (Zoogen Services, Inc.®, Davis, CA). Collected specimens were sexed via necropsy. Early in the breeding season when follicles were still developing, some terns were difficult to sex by necropsy. Therefore, blood was also collected from these terns for genetic sexing.

All research was conducted under the guidelines of the Western Ecological Research Center Animal Care and Use Committee. Forster's Terns were collected under California Department of Fish and Game Collection permit SC-007250 and Fish and Wildlife Service permit MB102896-0.

Statistical Analysis

Analysis of variance (ANOVA) was used to test differences in morphological measurements between males and females terns at the 0.05 significance level. Sexual size dimorphism was assessed by calculating the absolute value of the difference between the mean morphological measurement for females and males and dividing this quantity by the mean male value. The best measurements for sexing Forster's Terns were determined using a forward stepwise discriminant function analysis (Morrison 1990) with a prior probability of being female of 50% using SAS software (SAS Institute 1999). Discriminant scores (hereafter D) were defined as $D = -0.5(x - \mu)' \Sigma^{-1}(x - \mu)$, where $(x - \mu)' \Sigma^{-1}(x - \mu)$ represented the squared distance of a tern with measurements x from a subpopulation with mean μ and variance matrix Σ (Khattree and Naik, 2000; SAS Institute, 2004). The scores, D_{Female} and D_{Male} , were calculated using the mean and variance of the respective sexes. Terns were then classified into the sex for which the smallest squared distance, or the largest D score, was measured. Equivalently, if $D_{\text{Male-Female}}$, defined as $D_{\text{Male}} - D_{\text{Female}}$, was greater than 0, then terns were classified as males and if $D_{\text{Male-Female}} < 0$ then terns were classified as females. Assuming the variance S was constant, then discriminant scores could be simplified into linear expressions without changing the effect of scoring between sexes (SAS Institute 2004). Three of the 9 morphological measurements in our discriminant function, body mass and rectrices R_1 and R_6 , were excluded because they can vary over time (Kaufman 1983; Cramp 1985; Voelker 1997). At each step of the analysis, a criterion based on Wilks' lambda (λ) was used to enter the variable contributing

the most discriminatory power to the model until no further variables satisfied the criterion. Classification errors were produced using SAS's resubstitution analysis and discriminant functions were validated using a cross-validation procedure (Lachenbruch and Mickey 1968), in which 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). Posterior probabilities of being female were calculated for each tern and plotted against their corresponding discriminant score. This function determined cut-off points for discriminant scores with a 75% probability of being a female or male.

RESULTS

Eighty-four Forster's Terns were captured or collected from 23 March to 28 June 2005 in the SFBay. Of these, 40 females and 44 males were identified with genetic sexing ($N = 64$) or by necropsy ($N = 20$). The sex of 14 collected terns that were difficult to sex by necropsy was confirmed using genetic sexing.

Seven of the 9 morphological characteristics measured differed significantly between sexes, with head-bill length showing the least amount of overlap, followed by culmen length and culmen depth at the gonys (Table 1). Sexual size dimorphism was greatest with retrix R₆ length, followed by culmen width, and culmen depth.

A discriminant function analysis determined that two functions best separated the sexes of Forster's Terns. A discriminant function using head-bill length only correctly classified 82% of the 84 known sex terns (Wilks's $\lambda = 0.5035$: $F_{1,82} = 80.85$, $P < 0.0001$), indicating that head-bill length was the most important characteristic for differentiating sex. A leave-one-out cross-validation test

(Lachenbruch and Mickey 1968) correctly classified 82% of the terns' sexes. The discriminant scores were:

$$D_{\text{Female}} = \text{head-bill} (24.4575) - 975.0818$$

$$D_{\text{Male}} = \text{head-bill} (25.5562) - 1064.6590$$

The discriminant scores were simplified into linear expressions where if $D_{\text{Male-Female}}$ was greater than 0, then terns were classified as males and if $D_{\text{Male - Female}} < 0$ then terns were classified as females. The equation for the first discriminant function (Function 1) was:

$$D_{\text{Male - Female}} = \text{head-bill} (1.0987) - 89.5772.$$

A second discriminant function including head-bill length and culmen depth at the gonys improved our ability to separate the sexes (Wilks' $\lambda = 0.4338$: $F_{2,81} = 52.87$, $P < 0.0001$). The discriminant scores were:

$$D_{\text{Female}} = \text{head-bill} (23.8072) + \text{culmen depth} (43.5172) - 1118.4435$$

$$D_{\text{Male}} = \text{head-bill} (24.8635) + \text{culmen depth} (46.2995) - 1226.8711$$

or (Function 2):

$$D_{\text{Male - Female}} = \text{head-bill} (1.0563) + \text{culmen depth} (2.7823) - 108.4276$$

This discriminant function correctly classified 85% of known females and 89% of known males (87% correct classification rate overall). The cross-validation test correctly classified 83% of females and 89% of males (86% overall).

Table 1. Morphological measurements (mean \pm SD), ANOVA F-tests, and sexual size dimorphism (SSD) of Forster's Terns in San Francisco Bay. Terns were sexed using either genetic analysis or by necropsy.

Measurement	Female	SD	Male	SD	F _{1,82}	P	SSD
N	40.00		44.00				
Head-bill (mm)	79.74	1.64	83.30	1.96	80.85	<0.0001	4.27%
Culmen length (mm)	37.07	1.11	39.03	1.51	45.28	<0.0001	5.02%
Culmen depth (mm)	7.78	0.41	8.26	0.40	30.21	<0.0001	5.81%
Culmen width (mm)	3.94	0.41	4.21	0.40	8.70	0.004	6.41%
Tarsus (mm)	24.31	0.73	24.69	0.81	5.06	0.03	1.54%
Wing (mm)	267.35	6.04	269.59	5.02	3.44	0.07	0.83%
Tail R1 (mm)	66.65	4.71	68.93	4.48	5.17	0.03	3.31%
Tail R6 (mm)	168.97	16.92	181.93	17.94	11.53	0.001	7.12%
Mass (g)	136.15	12.79	135.31	10.21	0.11	0.74	0.62%

The posterior probability of a tern being female was described by the equation:

$$\text{Probability} = 1/[1+\exp(D_{\text{Male}} - D_{\text{Female}})]$$

There was some overlap in morphological measurements between tern sexes (Fig. 1) where the probability of correctly classifying the sex was low. Terns with discriminant scores (Function 2) from -1.10 to 1.10 had less than a 75% probability of being sexed correctly (Fig. 2). However, only 15 of the 84 terns (18%) were captured fell within this range. Of the 69 terns with discriminant scores outside these cutoff points, Function 2 correctly sexed 94% of the individuals (Fig. 2).

DISCUSSION

Male Forster's Terns in SFBay were, on average, significantly larger than females for most morphological measurements. Retrix R₆ showed the most sexual size dimorphism (Table 1) but was excluded from the discriminant function analysis. Our goal was to create a discriminant function capable of sexing Forster's Terns throughout the year and among observers. Therefore we did not include tail R₁ or R₆ lengths in the discriminant

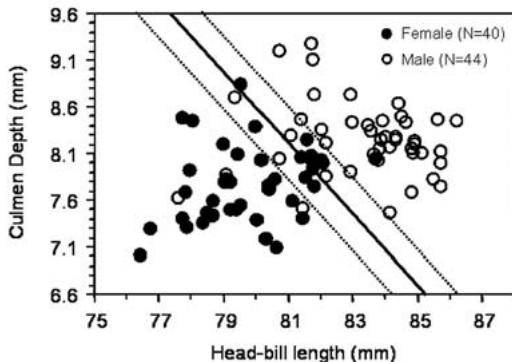


Figure 1. Discriminant function using head-bill length and culmen depth at the gonyx to sex female (below solid line) and male (above solid line) Forster's Terns in San Francisco Bay. Discriminant function line (solid) is defined as $D_{\text{Male}} - D_{\text{Female}} = \text{head-bill} (1.0563) + \text{culmen depth} (2.7823) - 108.4276$. Area between the stippled lines indicates morphological overlap in sexes where the discriminant function had <75% probability of correctly classifying the sex; stippled lines were calculated by setting $D_{\text{Male}} - D_{\text{Female}} = -1.10$ or 1.10 in the discriminant function equation (see Fig. 2).

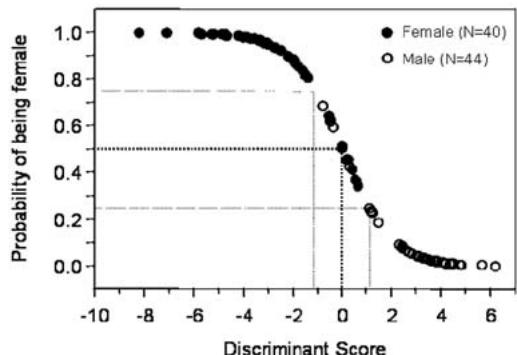


Figure 2. Probability of being female in relation to the discriminant function scores based on head-bill length and culmen depth of Forster's Terns. Discriminant function scores were calculated using the equation: $D_{\text{Male}} - D_{\text{Female}} = \text{head-bill} (1.0563) + \text{culmen depth} (2.7823) - 108.4276$. All Forster's Terns with discriminant function scores <0 were classified as females and >0 as males; actual sexes of terns determined using genetic sexing or necropsy are shown. Lines indicate the cutoff points for discriminant scores of -1.10 and 1.10 if the probability of being female were set to 0.25 and 0.75 respectively ($\text{Probability} = 1/[1+\exp(D_{\text{Male}} - D_{\text{Female}})]$); scores < -1.10 would be classified as females and >1.10 as males. This leaves 15 of 84 terns between -1.10 and 1.10 as not classified and 94% of the individuals outside of the cutoff points correctly classified.

function analysis because their lengths may vary intra-annually due to seasonal molts (Kaufman 1983; Cramp 1985); may wear or break throughout the year; and may vary inter-annually as a result of age, as demonstrated in Arctic Terns (Voelker 1997). Additionally, Fletcher and Hammer (2004) found the tail fork measurement (the difference between tail R₆ and tail R₁) was the least repeatable measurement between observers. Furthermore, our sample included individuals captured early in the breeding season that had not completed their pre-alternate tail molt, consequently, the measurements reported for R₆ include some terns without fully grown tail streamers.

Head-bill length was the most important morphological measurement in sexing Forster's Terns; a discriminant function using only head-bill length (Function 1) accurately sexed 82% of Forster's Terns. Head-bill length often is the most important measurement in discriminant functions for larids (Coulson *et al.* 1983; Hanners and Patton 1985; Coulter 1986; Stern

and Jarvis 1991; Mawhinney and Diamond 1999; Jodice *et al.* 2000; Torlaschi *et al.* 2000; Fletcher and Hammer 2003; Devlin *et al.* 2004) and is considered a stable and repeatable measurement (Barrett *et al.* 1989; Devlin *et al.* 2004). In contrast to tail measurements, head-bill measurements tend to be consistent between observers (Barrett *et al.* 1989) and show little interannual variation in Arctic Terns (Devlin *et al.* 2004). We found that a discriminant function incorporating multiple morphometric characteristics best discriminated between the sexes; a function based on head-bill length and culmen depth (Function 2) accurately sexed 87% of Forster's Terns. Despite the increase in sexing accuracy, culmen depth could be a potential source of error in some studies. Unlike head-bill length, culmen measurements at the gony can be difficult to repeat between observers (Barrett *et al.* 1989; Evans *et al.* 1993). Additionally, culmen depth measurements might vary with age as shown in Herring Gulls (*Larus argentatus*; Coulson *et al.* 1983). While this pattern has not been confirmed in other Laridae, more research is needed to test the usefulness of culmen depth measurements in morphometric studies of larids.

Our discriminant functions successfully sexed 82-87% of SFBay Forster's Terns. This rate is comparable to other sexing studies on Laridae. Our discriminant function based on head-bill length and culmen depth sexed Forster's Terns with 6-13% greater accuracy than functions of multiple morphometric characters developed for other tern species (Coulter 1986; Stern and Jarvis 1991; Quinn 1990; Fletcher and Hammer 2003; Devlin *et al.* 2004). Our discriminant function with head-bill length alone sexed Forster's Terns with 9-10% greater accuracy than other studies on tern species (Fletcher and Hammer 2003; Devlin *et al.* 2004). However, our accuracy rates for sexing Forster's Terns were less than the 88-99% sexing accuracy reported for gulls when multiple morphological measurements are incorporated into discriminant functions (Fox *et al.* 1981; Coulson *et al.*

1983; Hanners and Patton 1985; Evans *et al.* 1993; Hatch *et al.* 1993; Mawhinney and Diamond 1999; Jodice *et al.* 2000; Torlaschi *et al.* 2000). The difference in classification rates between Forster's Terns and other Laridae suggests that Forster's Terns exhibit greater sexual size dimorphism than other terns but are not as dimorphic as some gull species.

To increase sexing accuracy of Forster's Terns, researchers can determine posterior probability values of correctly sexing a tern and set discriminant score cut-off values to exclude cases with more equivocal discriminant scores (see Hatch *et al.* 1993; Phillips and Furness 1997; Devlin *et al.* 2004). For example, in Function 2, increasing the posterior probability to 75% gives new cut off points, which increases sexing accuracy to 94% when applied to our sample of SFBay Forster's Terns (Fig. 2). Eighteen percent of terns remained unclassified when applying the cut-off points of 75% posterior probability and would require sexing via DNA, dissection (laparotomy or necropsy), or behavioral observation (courtship prey deliveries or copulatory events) if applied under field conditions. Although it is beyond the scope of this study, within-pair morphological comparisons may further improve the sexing accuracy of Forster's Terns as they have for other species (Coulter 1986; Stern and Jarvis 1991; Fletcher and Hammer 2003; Devlin *et al.* 2004).

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