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Morphometric Sexing of Northwest Atlantic Roseate Terns

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Abstract.—A difficulty in the study of monomorphic species is the inability of observers to visually distinguish females from males. Based on a sample of 745 known-sex birds nesting at Bird Island, MA, USA, a discriminant function analysis (DFA) was used to sex Roseate Terns (*Sterna dougallii*) of the Northwest Atlantic population using morphological measurements. DFA using only the total length of the head (including the bill) correctly identified the sex of approximately 86% of the terns, which increased to 88% if both members of a pair were measured. Including additional measurements increased these percentages slightly, to 87% and 90%, respectively. These levels of accuracy are generally higher than those reported for other species of terns. Because female-female pairs are frequent in this population, one cannot assume that the member of a pair with the larger head is a male, and additional discriminant functions were developed to help separate female-female from male-female pairs. *Received 29 August 2011, accepted 9 April 2012.*

Key words.—discriminant function, female-female pair, morphometric sexing, Roseate Tern, sexual dimorphism, *Sterna dougallii*.

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In most species of terns (Sternidae) the two sexes look identical to human observers. However, subtle sexual dimorphism is present and discriminant function analysis (DFA) can be used to sex terns based on field measurements (e.g. Coulter 1986; Chardine and Morris 1989; Craik 1999; Fletcher and Hamer 2003; Devlin *et al.* 2004; Bluso *et al.* 2006; Nisbet *et al.* 2007; Shealer and Cleary 2007; Reynolds *et al.* 2008). In the Roseate Tern (*Sterna dougallii*) males are, on average, larger than females (Gochfeld *et al.* 1998), and we report here on the first discriminant functions developed for use in sexing this species.

The Roseate Tern is an endangered species in the United States (USFWS 1987) and the subject of ongoing management programs and field research (Nisbet and Spendelow 1999; Spendelow *et al.* 2008). It would be very helpful to be able to identify the sex of the birds using less invasive and costly procedures than molecular sexing. Knowledge of the sex of individual birds is particularly important for this species, because of sex differences in adult mortality (Nichols *et al.* 2004). Many of the Roseate Terns in the Northwest Atlantic population have been sexed using molecular techniques (Sabo *et al.* 1994; Szczys *et al.* 2001) and measured, which provides known-sex individuals on which to base morphometric sexing rules. The Northwest Atlantic Roseate Terns have a female-biased sex ratio with female-female pairs and other multi-female associations (MFAs) such as trios (Nisbet and Hatch 1999), which complicates morphometric sexing when comparing mates. In addition to developing discriminant functions to distinguish females from males, we also tested whether this methodology can be used to separate females mated to males from those paired with other females.

Methods

Study Site

The study was conducted at Bird Island, Buzzards Bay, Massachusetts, USA (area 0.6 ha, maximum elevation 3m above mean high water) in 1987-2000 and 2009 as part of a long-term population dynamics study (Nisbet and Spendelow 1999; Spendelow *et al.* 2008) done as one aspect of the Cooperative Roseate Tern Meta-

population Project (CRTMP) coordinated by the USGS Patuxent Wildlife Research Center. Roseate Terns have been studied at this site since 1970. In recent years, 500-900 pairs have nested on the island and about one third of these are of known age based on banding as nestlings.

Data Collection

Roseate Terns were trapped randomly throughout the colony site, except that in 1992-94 trapping was focused on birds thought to be in multi-female associations (Nisbet and Hatch 1999). Terns were trapped on their nests after ≥15 days of incubation using walk-in traps placed over the nest. Handling time usually did not exceed 5 min. All trapping, handling, blood sampling and (color)banding procedures used as part of the CRTMP were approved by the USGS-PWRC Animal Care and Use Committee and were done under the USFWS Regional Director's Endangered Species Permit (#697823) for this species. Fieldwork from 1987-2000 was done under Nisbet's U.S. Bird Banding Laboratory (BBL) permit (#20589) in addition to federal and state scientific collecting permits. The 2009 work was done under Spendelow's BBL permit (#09801) in cooperation with ongoing Buzzards Bay Tern Project work done by the Massachusetts Division of Fisheries and Wildlife.

The following measurements were taken: head length including the bill, bill length, wing length, tail length and body mass. Head length was measured with calipers to the nearest 0.1mm from the back of the skull to the tip of the bill (Fig. 1a). If they differed in length, the longer of the two mandibles was used to determine the tip of the bill. Bill length (culmen) was measured similarly, but with the lowest point of feathering as the starting point rather than the back of the skull (Fig 1a). Bill length is probably a less precise measurement than head length because of irregular or asymmetric feathering, and we did not measure it in 2009. Wing length was measured in mm with a metal ruler from the carpal joint to the tip of the longest primary on the right wing (unless the right wingtip was broken). The wing was not flattened, thus a natural curve was present in both dimensions. Tail length was measured to the nearest mm from the point of insertion of the central feathers (determined by pushing a metal ruler to the point of resistance) to the tip of the outermost tail feather on each side (Fig. 1b). If one of these feathers was broken or worn, it was not measured and a note was made in the data file. If both outer tail feathers were measured then the mean was used as "tail length", and if only one was measured then that value was used. Birds were weighed to the nearest gram using a Pesola spring scale.

In 1987-2000, 670 birds were sexed using molecular markers (Sabo *et al.* 1994; Szczys *et al.* 2001). In addition, mates of birds known to be male by molecular sexing were assumed to be female (n = 75), resulting in a sample size of 745 birds. The presence of MFAs such as female-female pairs (Nisbet and Hatch 1999) means that mates of females cannot be assumed to be males.

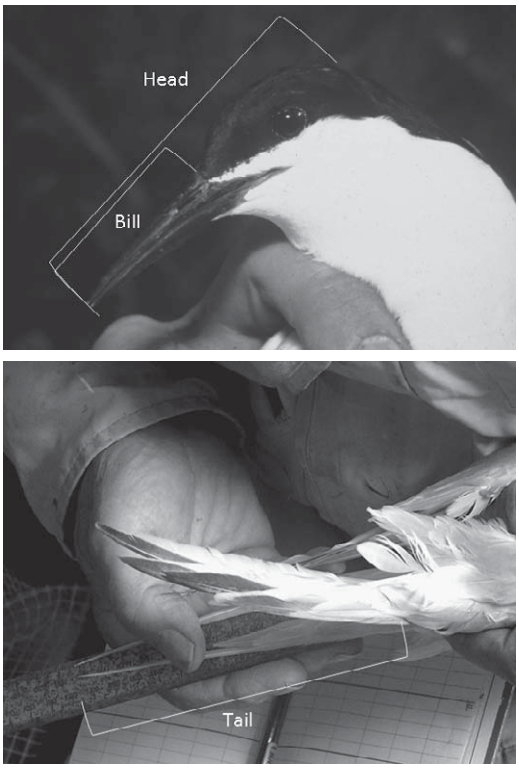

Figure 1. Photographs illustrating measurement landmarks for A) head length and bill length and B) tail length of Roseate Terns. Photo A courtesy of P. J. Lynch; Photo B by B.G.P.

Statistical Methods

Only one encounter per individual was used to eliminate non-independent data from the same individuals measured in more than one year. Selection of encounters was random, except that encounters with complete measurements and/or at the extremes of the age distribution (to provide a complete range of ages) were preferred where available.

Statistical analysis was performed using SPSS. Sex differences in the measured traits were tested with ANOVA, and Pearson correlations were calculated to determine if any traits were highly correlated with each other (Herring *et al.* 2010). Only bill and head length (which includes the bill) were highly correlated (r_{726} = 0.88; for all other pairs of traits $r < 0.4$), therefore we did not include both variables in the same discriminant functions. After demonstrating that head length was a better predictor of sex than bill length (see Results and Discussion), we no longer used bill length in any analyses. Following previous authors (Devlin *et al.* 2004; Bluso *et al.* 2006; Shealer and Cleary 2007; Herring *et al.* 2010), we also did not include body mass, because it varies with factors such as time of day and breeding stage. For the remaining variables, head length, wing length and tail length, we conducted a stepwise discriminant function analysis (DFA) to determine which set of vari-

ables best classified the birds. At each step of the analysis in SPSS, the variable that resulted in the lowest overall Wilks' lambda was entered, with a minimum partial F value to enter the model of 3.84. Whether variables remained in the model was again determined by their partial F values (maximum F to leave = 2.71). A separate DFA was conducted in the same manner with measurements of mates included (mate's head length, mate's wing length, mate's tail length). Because there may be reason to exclude tail length (see Results and Discussion) we also repeated the DFAs with tail length excluded. Additional analyses using a simultaneous rather than stepwise procedure yielded very similar results and are not reported here.

DFAs were cross-validated with the "leave-out-one classification" jackknife procedure. This procedure estimates the percentage of birds correctly classified as male or female by classifying each known-sex tern after that individual's data have been excluded from the function (Dechaume-Moncharmont *et al.* 2011). Despite the presence of a female-biased sex ratio in this population, for the analyses presented here we did not adjust the prior probabilities based on the known number of males and females in our sample. Doing so would have produced slightly better results (unpublished data), but there are several reasons not to adjust the prior probabilities: prior knowledge of the sex ratio is required, the sex ratio may not be unchanging, and the sex ratio in our sample is probably more biased than in nature because it is based in part on sampling that was targeted at MFAs in some years (Nisbet and Hatch 1999).

Cutting values were calculated by averaging the two "functions at group centroids" (mean discriminant scores for each sex), and unstandardized coefficients were used. For ease of interpretation, discriminant functions were simplified to place the cutting value at zero (>0 = male, <0 = female) and to avoid multiplying the first variable by any factor. For example, the function 0.677(head length) – 52.830 with a cutting value of 0.282 simplifies to head length – 78.452 with a cutting value of zero ((-52.830-0.282)/0.677 = -78.452).

RESULTS AND DISCUSSION

Male Roseate Terns had significantly longer heads, bills, tails and wings than did females and were also heavier than females (ANOVA, all $p < 0.0001$, Table 1), although the ranges of the values largely overlapped (Table 1). Studies of other tern species have also found males to be larger than females in mensural characters (Coulter 1986; Chardine and Morris 1989; Craik 1999; Fletcher and Hamer 2003; Devlin *et al.* 2004; Bluso *et al.* 2006; Nisbet *et al.* 2007; Shealer and Cleary 2007; Reynolds *et al.* 2008), although female Common Terns (*S. hirundo*) are often reported to be heavier than males (Nisbet *et al.* 2007).

Similar to other studies of terns and gulls (e.g. Coulson *et al.* 1983; Chardine and Morris 1989; Craik 1999; Fletcher and Hamer 2003; Devlin *et al.* 2004; Bluso *et al.* 2006; Nisbet *et al.* 2007; Shealer and Cleary 2007; Herring *et al.* 2010), we found head length to be the most reliable trait for sexing birds in the hand. Although mean head lengths of males and females differed by only 3.2 mm (approximately a 4% difference), there was less overlap between the sexes in this than in other measurements (Table 1). Using cross-validated DFA, 86% of males and 87% of females were correctly classified based on head length alone (head length > 78.45 = male, head length < 78.45 = female; Table 2). Head length, which includes the bill, performed better than bill alone (which classified 77% of males and 79% of females correctly, Table

Table 1. Morphological measurements (mean ± SD) of female and male Roseate Terns are shown, with lengths in mm and body mass in g. Head length includes the bill. Ranges are given below the means, and sample sizes are in parentheses. F values and df are given for the results of ANOVAs comparing the two sexes (all $p < 0.0001$).

Measurement	Females	Males	F value
Head Length	76.9 ± 1.5 71.9-82.5 (466)	80.1 ± 1.5 75.0-83.4 (269)	$F_{1,733} = 756.7$
Bill Length	38.0 ± 1.1 34.8-42.3 (467)	39.8 ± 1.2 36.1-42.7 (269)	$F_{1,734} = 388.9$
Tail Length	174.8 ± 11.8 134-211 (473)	184.2 ± 12.3 146.5-215 (272)	$F_{1,743} = 105.4$
Wing Length	227.5 ± 4.7 212-242 (455)	229.3 ± 4.3 220-243 (266)	$F_{1,719} = 25.8$
Body Mass	113.1 ± 6.5 92-134 (456)	115.0 ± 6.9 90-133 (264)	$F_{1,718} = 13.4$

Table 2. Discriminant functions for sexing Roseate Terns. Terns with a function <0 are classified as female, >0 as male. Functions are listed in the order they are presented in the text. Additional functions for identifying females paired to other females are not shown here but are discussed in the text.

Function[1]	% Correct classification[2]	N
H – 78.45	86	735
B – 38.90	78	736
H + 0.022T – 82.40	87	712
H + 0.030T – 0.452MH – 48.78	90	552
H – 0.413MH – 46.34	88	564

[1]H = head length including the bill, B = bill length, T = tail length, MH = mate's head length. [2]Classification accuracy is based on the leave-out-one classification procedure. Accuracy for each sex is given in the text.

2). A possibly useful rule of thumb for field sexing with head length is that most Roseate Terns in this population with head length measurements greater than or equal to 79.0 mm were male and less than or equal to 78.0 mm were female. With these cutoffs 8% of females were misclassified as males and 9% of males were misclassified as females, with 12% of males and 13% of females unclassified. By leaving birds with heads longer than 78 mm but shorter than 79 mm unclassified, this sexing rule is more conservative than DFA and produces fewer misclassified birds.

In a stepwise DFA with head length, wing length and tail length, wing length dropped out of the analysis. A discriminant function using the remaining variables, head length and tail length (Table 2), correctly classified 87% of both males and females. Because the improvement in classification accuracy is very small and tail length varies with age (Palestis *et al.*, in press), it may be best to also exclude tail length (Devlin *et al.* 2004; Bluso *et al.* 2006; Herring *et al.* 2010) and simply use head length alone.

If both members of a pair had been measured, including mate characteristics in the discriminant functions increased accuracy, as in other terns (Fletcher and Hamer 2003; Devlin *et al.* 2004; Nisbet *et al.* 2007; Shealer and Cleary 2007). The stepwise analysis produced a discriminant function with the following variables: head length, tail length, and mate's head length (Table 2). This function correctly classified 88% of males and 91% of females. Again the function performed only slightly better than when tail length was excluded (Table 2). With just head length and mate's head length, 86% of males and 90% of females were correctly sexed. Although ideally one would want to correctly identify sex close to 100% of the time, the levels of accuracy reported here are generally higher than those reported in the literature for morphometric sexing of other tern species.

In addition to these discriminant functions, a large number of females can be successfully identified by simply assuming that the member of a pair with the shorter head is a female; this was true 96% of the time in our sample. However, because of the presence of female-female pairs, the individual with the larger head could either be a male or another female. Females in female-female pairs or other MFAs have relatively short tails but do not differ from females mated to males in head length or wing length (Palestis *et al.*, in press). Males have longer tails than females in general (Palestis *et al.*, in press; Table 1), and this difference would be even larger when comparing a male to a female in an MFA. Therefore we predicted that tail length would be useful here despite variation with age.

The following two-step procedure may help identify MFAs: 1) assume that the member of a pair with the smaller head is female, 2) to classify the female's partner as a male or another female, apply the following discriminant function to those with a larger head than their mates: head length + 0.040(tail length) – 86.24. Wing length again dropped out of the analysis. This procedure correctly identified 79% of female members of male-female pairs and 82% of female members of MFAs. This level of accuracy is surprisingly only a slight improvement over 78% and 81%, respectively, when

tail length is excluded (discriminant function: head length – 78.97). Overall, 96% of females but only 79% of males were correctly sexed with tail length included, compared to 95% and 79%, respectively, when tail length is excluded. The large discrepancy between sexes arose because males can be misclassified as females in both steps 1 and 2, whereas no females can be misclassified as males in step 1. Without having measurements on both partners, it is not possible to accurately identify females in MFAs using morphometrics. A three-category discriminant function with head length and tail length included did correctly identify 86% of males, but only 46% of females paired to males and 58% of females in MFAs. With tail length excluded the level of accuracy was similar for males and females in MFAs (87% and 59%, respectively) but was notably lower for females mated to males (38%), because many were misclassified as members of MFAs.

The discriminant functions and rules of thumb we developed are based on terns at one colony. Because of substantial dispersal among colony sites (Spendelow *et al.* 2010 and references therein), they should also be valid for Roseate Terns at other breeding sites in the Northwest Atlantic population, but different functions would need to be developed for use in other populations (Shealer and Cleary 2007; Herring *et al.* 2010). Although the specific functions and sexing rules probably differ in other populations, it is likely that two general conclusions from this and previous papers on morphometric sexing would remain valid: 1) head length is the single most useful trait for sexing terns in the hand, and 2) having measurements on both members of a pair increases the probability that sex is correctly assigned.

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