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Visual Observation to Identify Sexes in Adult Black Skimmers

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RUNNING HEAD: VISUALLY SEXING BLACK SKIMMERS

Abstract.—Identifying sexes in birds from visual observations is a very useful and inexpensive method. While sexual dichromatism and ornaments are readily used by observers, sexual size dimorphism can also be used to identify sexes in some bird species. This study assessed the applicability of visual observation of size differences in order to identify sexes in adult Black Skimmers (*Rynchops niger*). Black Skimmers do not have sexual dichromatism however males are larger in size and weight than females. The study focused on two sub-species: Amazonian (*R. n. cinerascens*) and South American (*R. n. intercedens*) Black Skimmers. Sex identified by visually observating size differences was consistent with the sex identified at specimen preparation from examining gonads (R_{GLMM} = 0.996 ± 0.004). The identification of sexes from photographs using visual observation of size had a very high within- (R_{GLMM} = 0.995 ± 0.001) and between- (R_{GLMM} = 0.984 ± 0.002) observer repeatability. Non-invasive methods for identifying sex by visual observation may allow enhanced use of data from photographic datasets, citizen science projects, and surveys using direct observation or images.

Key words.—non-invasive sex assessment, *Rynchops niger*, sexual size dimorphism.

The ability to identify sexes of animals is essential in many biological studies. Sexual dichromatism and ornaments in birds are easily perceived by observers and so can provide an appropriate tool for sex determination. However, many species show little or no sexual dimorphism in color or ornamentation. In these cases, sex can be determined with confidence by molecular analysis from blood or other tissues, and sometimes by biometrics or sex-specific behaviors such as egg laying and certain vocalizations (Griffiths *et al.* 1998; Redman *et al.* 2002; Serrano-Meneses and Székely 2006). However, not all species display sex-specific vocalizations, and egg laying happens only a very specific times at the breeding sites, for example. Moreover, molecular methods and biometrics require capture and handling of individuals to obtain measurements or tissue samples, which is not always possible (Genovart *et al.* 2003; Dechaume-Moncharmont *et al.* 2011). Hence information on sex might only be available for a sub-set of the data and methods that can readily sex all observed birds would be advantageous for many field studies..

Black Skimmers (*Rynchops niger*) were thought to be monomorphic with no significant visual characteristics to identify sexes (Zusi 1996; Scherer *et al.* 2013). However, many studies have reported significant differences in body size measurements between male and female Black Skimmers. Black Skimmer males are heavier than females already at chick age of 23 days (Schew and Collins 1990). Head length, bill length, bill depth at base, wing length, and body mass are all between 9 to 35% larger in adult males than in adult females, with very little or no overlap between the sexes in some of these metrics (Burger and Gochfeld 1990; Quinn 1990; Mariano-Jelicich *et al.* 2007; Scherer *et al.* 2013). Our objective was to assess whether the size differences between sexes in two sub-species from South America can be reliably detected by visual observation.

METHODS

Specimens from museums

We checked the reliability of visually identifying sex without having to measure the bird by using a three-step process of one observer first identifying the sexes of specimens in a museum without knowledge of the sex recorded on the label, later taking measurements, and finally looking at the labels. The specimens used in this study were South American (*Rynchops niger intercedens*) or Amazonian (*R. n. cinerascens*) Black Skimmer skins held at the British Natural History Museum. The 23 South American specimens were collected in Brazil, Argentina, Paraguay, British Guiana, Venezuela, Suriname, and Peru. The 23 Amazonian specimens were collected in Chile, Peru, British Guiana, Venezuela, and Paraguay.

First, one observer (BPV) visually assigned the sex to each specimen based entirely on the perceived sizeof that specimen and not comparing it to others in order to avoid bias from size comparisons between individuals. The observations were made 50 cm away from the specimen arranged on its side and showing full profile in good light. Each specimen was assessed once only.

Secondly, the same observer (BPV) took measurements of all specimens. These measures were compared to the ones available in the literature to develop the discriminant function. We compiled the measurements of South American and Amazonian Black Skimmers for body mass, culmen length, lower bill, head + bill length, bill depth at base, tarsus length, and wing chordaccording to sex and sub-species (Table 1). The same measurements were also taken from the museum specimens except tarsus length and body mass.

Finally, the observer checked the label for information on sex. Sex recorded on labels was determined by the collector based on examining the gonads.

Photographs

After verifying the feasibility of identifying sexes by the perceived body size of specimens, we also tested the within- and between-observer repeatability for identifying size differences in individuals from photographs taken in the field. One hundred photographs containing a total of 165 individuals were selected from the Wikiaves web dataset. The actual sex of birds in photographs could not be assessed and we assumed that a consistent size difference both within- and between-observers is an indirect measure of sexes; considering males are larger than females (Burger and Gochfeld 1990; Quinn 1990; Mariano-Jelicich *et al.* 2007; Scherer *et al.* 2013). Consistent differences in size of individuals from images were assessed independently by the same observer who identified the sex of museum specimens twice six months apart plus by another two observers. Birds detected in the images were South American and Amazonian sub-species. We did not run separate tests for each sub-species because size differences between adult males and adult females were significant for both sub-species with very similar measurements within males and within females (Table 1).

Analysis

We formally used the biometric measurements (lower bill, culmen, head + bill length, bill depth at base, tarsus length, and wing chord) to determine a discriminant function. We tested collinearity between the biometric measurements using a Spearman test in the corrplot package (Wei and Simko 2016) in R (R Core Development Team 2016) and considering a variable collinear when $r > 0.4$ (Booth *et al.* 1994). The only variables that did not correlate in both sub-species were head + bill length and depth at base (Amazonian: $r = 0.39$, $n = 23$; South American: $r = 0.37$, $n = 23$). For the two variables (bill depth at base and head + bill length), we tested the multivariate normality with a Henze-Zirkler's test (Amazonian: HZ = 0.65, $P = 0.11$; South American: HZ = 0.52, $P = 0.24$) and the homoscedasticity with a box's M test (Amazonian: $\chi^2_3 = 5.49$, $P = 0.13$; South American: $\chi^2_3 = 3.37$, $P = 0.33$) using MVN (Korkmaz *et al.* 2014) and biotools (Silva *et al.* 2017) packages, respectively. We conducted the linear discriminant analysis using the package MASS (Venables and Ripley 2002) with a jackknife cross-validation as suggested in Dechaume-Moncharmont *et al.* (2011). The performance of the linear discriminant function was assessed with a Wilks' Lambda test using the rrcov package (Todorov and Filzmoser 2009) which varies from 0 to 1 with lower values indicating higher discriminant power. We also ran a t-test between males and between females of both sub-species to test if differences in measurements were significant.

To determine the concordance between the three assessment methods of sex in specimens from the museum, we used the Bray-Curtis dissimilarity index which varies from 0 to 1 with the maximum value meaning full similarity (Bray and Curtis 1957). We also tested the repeatability of binomial data (sex) between labels, biometric measurements, and visual determination with an additive generalized linear mixed-effects model (GLMM) with binomial error structure, logit link function, 1,000 bootstraps, and 1,000 permutations using the rptR package (Stoffel *et al.* 2017).

The repeatability of assigning sexes based on perceived size in photographs within- and between-observers was also tested using the additive generalized linear mixed-effects model (GLMM) with binomial error structure, logit link function, 1,000 bootstraps, and 1,000 permutations.

RESULTS

The pooled mean and standard deviation for all body size measurements are shown in Table 1 and did not differ between Amazonian and South American Black Skimmer males ($t_{1,6} = -0.05$, $P = 0.92$) nor between Amazonian and South American Black Skimmer females ($t_{1,6} = 0.01$, $P = 0.97$). Head + bill length and bill depth at base were 15.1% and 24% greater in males than in females in the Amazonian and 17.6% and 18.2% in the South American sub-species (Table 1).

The linear discriminant function analysis of head + bill length and bill depth at base was accurate to identify sexes in all Amazonian and South American Black Skimmer museum specimens (Fig. 1); the jackknife cross-validation predicted sexes with 98% and 96% accuracy. The discriminant function of 0.02 * (head + bill length) + 0.34 * (depth at base) – 12.05 predicted the sex of 95% of the Amazonian males and 100% of the Amazonian females with a very low Wilks' Lambda of 0.02 ($\chi^2_2 = 30.38$, $P < 0.001$). The discriminant function of 0.05 * (head + bill length) + 0.44 * (depth at base) – 18.71 predicted the sex of 92% of the South American males and 100% of the

South American females also with a very low Wilks' Lambda of 0.01 ($\chi^2_2 = 35.81$, $P < 0.001$).

The Bray-Curtis dissimilarity index presented a full correspondence of 1 between visual determination, biometric measurements, and labels for both studied sub-species (Table 2). The repeatability between the different sexing methods was very high ($R_{GLMM} = 0.996 \pm 0.004$, 95% confidence interval = 0.991 – 0.999, $P < 0.001$).

Both the within- ($R_{GLMM} = 0.995 \pm 0.001$, 95% confidence interval = 0.993 – 0.998, $P < 0.001$) and the between-observer repeatability ($R_{GLMM} = 0.984 \pm 0.002$, 95% confidence interval = 0.981 – 0.994, $P < 0.001$) of perceiving size differences from photographs were very high.

DISCUSSION

Black Skimmers males are skeletally larger (6.7 – 31.7% depending on trait) and 33.3 – 37.5% heavier than females. The visual observation of sex of museum specimens agreed with the known sex of the specimen. From photographs, both the within- and between-observer repeatability of visual identification of sexes based on perceived body size was very high and statistically significant. Although we cannot be completely sure which sex each individual in photographs had, differences in size between individual Black Skimmers were perceived consistently. Because of the clear and non-overlapping size differences in Black Skimmers these size differences very likely represent different sexes.

The discriminant analyses based on two size measurements (head + bill length, bill depth at base) had very low Wilk's lambda in both sub-species. Other studies had created discriminant functions for the North American Black Skimmer sub-species (Quinn 1990) and non-breeding populations of mixed sub-species in Argentina (Mariano-Jelicich *et al.* 2007) and southern Brazil (Scherer *et al.* 2013). However, accuracy and variables used varied between studies and none addressed the Amazonian and South American Black Skimmers separately. Moreover, Burger (1981) also visually assigned sexes to North American Black Skimmer (*R. n. niger*) although she did not present a formal test of reliability for such method.

Sexual size dimorphism varies considerably among species. Some groups (e.g. gulls) have bigger males than females and others (e.g. skuas) the opposite (Fairbairn and Shine 1993; Serrano-Meneses and Székely 2006). Seabirds, such as King Penguin (*Aptenodytes patagonicus*), Herring Gulls (*Larus argentatus*), Great Frigatebird (*Fregata minor*), and Great Skua (*Stercorarius skua*) where the sexes differ by 2% to 24% in size had been reported to be assigned to sex by sexual size differences with careful observation and experience (Burger and Gochfeld 1981; Fairbairn and Shine 1993; Serrano-Meneses and Székely 2006). Sexual size differences in Black Skimmers are towards the upper end of sexual size differences in other species that are successfully sexed by size but don't present an extreme case.

Other studies have used relative size between nearby birds to assign sex. Hamer and Furness (1991) reported in Great Skuas that there was good agreement between sexing by visual observation of the two members of breeding pairs and results from a discriminant analysis from their biometrics, with about 90% of visual assignments in accordance with the discriminant function. Burger and Gochfeld (1981) were comfortable assigning sexes to Herring Gulls visually by comparing the members of a pair or adjacent birds for unpaired birds.

Visual observation to identify sexes in lone individual Black Skimmers is reliable, but their flock behavior when resting may improve the observer's ability to identify sexes. Comparisons between males and females is facilitated, thus sex identification might be easier as it allows using other individuals as a scale although we did not formally test it in this study. Flocking behavior, however, may have been selected to confuse predators and to diffuse individuality (Landeau and Terborgh 1986) and may therefore also confuse human observers without much experience. It is possible that observer's experience may also affect the reliability of visually assigning sexes. In this case, observing mainly the head + bill length and bill depth at base and taking photographs may help break the sensation of uniformity in the flock. This non-invasive method using visual observation for identifying sex may provide more detailed use of data from photographic datasets, citizen science projects, and surveys using images or direct observation.

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Table 1. Biometric measurements (mean ± standard deviation) for adult male and female South American (*Rynchops niger intercedens*) and Amazonian (*R. n. cinerascens*) Black Skimmer sub-species (summarised in Vieira 2016). All linear measurements in mm; body mass in grams.

Sub-species	Sex	Character (Mean ± SD)						
		Mass	**Culmen length**	**Lower bill**	**Head + bill length**	**Depth at base**	**Tarsus length**	**Wing**
South American	Male	357.8 ± 28.6	81.2 ± 6.8	106.1 ± 7.2	149.2 ± 7	30.8 ± 2.4	36.6 ± 4.9	404 ± 16.1
	Female	238.7 ± 26.7	65.9 ± 15.1	84.0 ± 7.9	122.9 ± 8.4	25.2 ± 1.8	34.2 ± 6.4	366 ± 13.2
Difference between the sexes (%)		33.3	18.8	20.8	17.6	18.2	6.7	9.4
Amazonian	Male	365.7 ± 10.4	97 ± 13.3	108.1 ± 17.3	157.7 ± 18.3	32.5 ± 3.7	35 ± 6.8	399.5 ± 21.7
	Female	228.5 ± 21.2	66.2 ± 9.4	82.3 ± 17	134 ± 20.6	24.7 ± 2.2	30.6 ± 1.9	362.5 ± 27.8
Difference between the sexes (%)		37.5	31.7	23.9	15.1	24	12.6	9.3

Table 2. Number of Black Skimmer museum specimens sexed based on label information, visual determination, and biometric measurements, given separately for each sub-species. Discordance between methods indicates how many times one method disagreed with the other two.

	Label Information		**Visual Observation**		**Biometric Measurement**		**Discordance between methods**
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	
South American Black Skimmers	11	12	11	12	11	12	0
Amazonian Black Skimmers	11	12	11	12	11	12	0

Figure 1. Females (circle) and males (square) of Amazonian (A) and South American (B) Black Skimmer sub-species partitioned according to linear discriminant functions using head + bill length and bill depth. The triangle represents the mean value for each group.

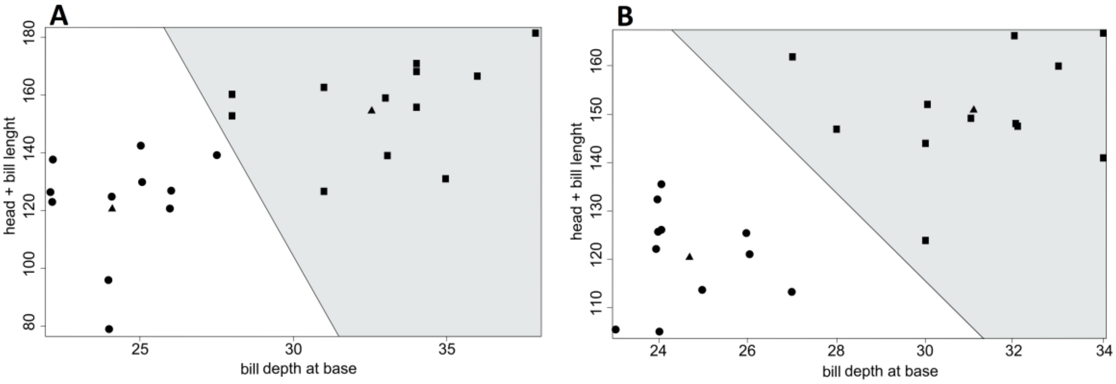
A
head + bill lenght
80
100
120
140
160
180
25
30
35
bill depth at base
B
head + bill lenght
110
120
130
140
150
160
24
26
28
30
32
34
bill depth at base

Figure 1.