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Studies on Neotropical Fauna and Environment

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/nnfe20>

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Available online: 21 Oct 2011

To cite this article: Cecilia Perez Calabuig, Andy J. Green, Miguel Ferrer, Roberto Muriel & Heden Moreira (2011): Sexual size dimorphism and sex determination by morphometric measurements in the Coscoroba Swan, *Studies on Neotropical Fauna and Environment*, DOI:10.1080/01650521.2011.617545

To link to this article: <http://dx.doi.org/10.1080/01650521.2011.617545>



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ORIGINAL ARTICLE

Sexual size dimorphism and sex determination by morphometric measurements in the Coscoroba Swan

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(Received 19 August 2010; accepted 23 August 2011)

The accuracy of morphological sexing and the occurrence of sexual dimorphism were analyzed in mature and immature Coscoroba Swans (*Coscoroba coscoroba*, Anatidae) near the Estação Ecológica do Taim, southern Brazil. On the basis of weight and 10 linear measurements of external morphology, multivariate analysis of variance showed that males were consistently larger than females (sex confirmed via genetic markers) and mature birds were consistently larger than immatures. Overall, 38% of immatures and 14% of mature birds were sexed incorrectly by cloacal examination when compared to genetic data. Therefore, we performed a discriminant function analysis of different age classes based on morphometric measurements. Mature birds were sexed with 96% accuracy using head and tarsus lengths as predictor variables, whereas immatures were sexed with 90% accuracy based on head and forearm lengths. Method validation conducted with data for additional mature sampled in a different year showed that the use of head length alone was as accurate for sexing (92% correct classification) than discriminant functions based on two characteristics (91%).

Keywords: Brazil; cloacal examination; Coscoroba Swan; genetic sexing; morphological sexing; sexual size dimorphism

Introduction

Accurate and easy methods to determine the sex of individuals are valuable for studies of avian evolutionary ecology and genetics, population dynamics, behavior, migration and conservation management of species and populations (Clutton-Brock 1986; Newton 1998). In species lacking obvious sexual dimorphism, cloacal sexing is often used but can be unreliable (Brown et al. 2003; Odwyer et al. 2006; Volodin et al. 2009). Molecular sexing, based on the amplification of the chromo-helicase-DNA-binding 1 (CHD1) gene, is the most reliable method (Ellegren & Sheldon 1997; Griffiths et al. 1998), but is relatively expensive. However, molecular sexing can complement methods based on discriminant function analysis (DFA) in species with weak size dimorphism (Dumbell et al. 1988; Ackerman et al. 2008; Hart et al. 2009). In waterbirds, DFA has often been used to combine the discriminatory power of single characters into one formula that best discriminates between sexes (e.g. Weidinger & van Franeker 1998; Svalgelj & Quintana 2007; Ackerman et al. 2008; Quintana et al. 2008; Hart et al. 2009). DFA can be a reliable, fast and inexpensive method for discriminating the sex of individuals

during non-breeding seasons when sexually dimorphic characters are not expressed (e.g. Zavalaga & Paredes 1997; Bourgeois et al. 2007).

The Coscoroba (*Coscoroba coscoroba*) is an unusual member of the Anatidae found in South America, from the Falkland Islands and Tierra del Fuego, north through Chile and Argentina, Uruguay and southern Brazil, and as far north as Paraguay (Kear 2005). In Brazil, *C. coscoroba* occurs year-round in Rio Grande do Sul (Belton 2000) with irregular records in other states (Bornschein et al. 1997). It is traditionally considered to be a swan, but molecular studies suggest it is more closely related to the aberrant Cape Barren Goose *Cereopsis novaehollandiae* (Donne-Goussé et al. 2002). Very little is known about its ecology (see Kear 2005 for a review). It is a monochromatic species, and previous attempts to separate sexes based on morphology have been unsuccessful (Nascimento et al. 2001). DFA has previously been applied in some other swan species (e.g. Miller et al. 1988; Brown et al. 2003). Other studies of swans have compared different size measures between sexes without applying DFA (e.g. Scott 1972; Mathiasson 1981; 2005; Limpert et al. 1987).

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The present study describes sexual dimorphism of immature and mature Coscoroba in Brazil, and compares the accuracy of alternative sexing methods. The main objectives were to: (a) determine the accuracy of cloacal examination, by comparing the results with those from molecular sexing; (b) present typical morphometric measures of Coscoroba, and determine differences between the sexes and two age classes identifiable by plumage characteristics; (c) develop discriminant models to facilitate the sexing of birds based on morphometry; and (d) compare the accuracy of DFA with that of sexing by cloacal examination.

Materials and methods

Study area

Rio Grande do Sul State in the southern tip of Brazil has a big lagoon complex in the southern portion of its coastal plain, formed by Patos, Mirim, and Manguieira Lagoons and other smaller lakes. All birds were captured near the “Estação Ecológica do Taim”, an area located in the southern coastal plain of RS, between the counties of Rio Grande and Santa Vitória do Palmar (~32°23' W, 52°32' W). This area was chosen because it is within the most important area for breeding and molting of Coscoroba in Brazil (Seijas 2001).

Capture of birds and sampling

Birds were captured by hand from a boat during flightless wing molt between August and January (Nascimento et al. 2001; Seijas 2001) in three consecutive seasons (2005, 2006 and 2007). Birds were divided into two groups: immatures (fledged but less than two years old) and matures (>2 years old) according to plumage characteristics (Johnsgard 1978). Coscoroba Swans have red eyes, legs, feet and bill from immature to mature age classes but there are differences in the plumage pattern. The adult's definitive plumage is totally white with the exception of a black tip on the first six external primaries.

Although plumage is mainly white, immature Coscoroba Swans have brownish down feathers and brightish brown plumes all over the body and have grayish brown stains on the tail, back and wings (in primaries, secondaries and upper wing coverts). Mature swans have gray down feathers only on the back and under the wings (axillaries). They usually display more than six primaries with grayish brown fleckings but very rarely on the secondaries (Calabuig et al. 2010).

The immature group consisted of 41 individuals (19, 11 and 11 for 2005, 2006 and 2007, respectively).

The mature group consisted of 345 individuals (120, 123 and 102 for 2005, 2006 and 2007, respectively).

Morphometric measurements

Birds were weighed with a balance (to the nearest 20 g) and 10 biometric measures were taken by the same person (CPC). A digital caliper was used to measure the following (to the nearest 0.1 mm): head length (length of head to the occipital-tip of the bill), bill height, bill depth (maximum width of the bill), nostril (distal edge of a nostril to the end of the bill), total culmen and tarsus length (on the left side of the body). A ruler was used to measure the following (to the nearest 1 mm): tail (from the preen gland), wing length without feathers (metacarpophalangeal articulation), forearm (Ferrer & De le Court 1992) and neck length. Before releasing, all swans were banded with a numbered metal CEMAVE-IBAMA ring. Recaptures were readily identified, and data were only used for each individual on the first capture.

Cloacal and genetic sex determination

Genital identification

Cloacal examination was carried out by CPC, identifying “males” by the presence of a visible erectile groove penis on the ventral wall of the cloaca (Proctor & Lynch 1993; Brown et al. 2003; Mathiasson 2005).

DNA identification

Blood (3 ml) was taken from each bird from the wing vein and samples were stored in Vacutainer tubes with EDTA and kept cool in ice, until processing. Blood samples were analyzed in the Biotechnology Center (CenBiot), Federal University of Pelotas, Brazil. DNA extraction was performed according to the procedures of Lahiri and Nurnberger (1991) and DNA samples were amplified using primers described by He et al. (2005) for the Tundra Swan (*Cygnus atratus*). The PCR amplifications were carried out in the reaction mixture of Ito et al. (2003). After amplification, the PCR products were separated on a 1% agarose gel (synthesized by Sangon Co., Shanghai, China), stained with ethidium bromide (Sangon Co.) and visualized under UV light.

Data analyses

We used multivariate analysis of variance (MANOVA) to compare mean differences between age and sex groups for morphological measurements and body mass. Also, one-way analysis of variance was used to

determine whether individual measures varied with sex in each age group (Sokal & Rohlf 1995). For both analyses, we used only the 225 mature Coscorobas sampled during the 2006 and 2007 seasons (those used for DFA, see below). Differences were considered significant at $p \leq 0.05$. All data satisfied Lilliefors and Levene tests of normality.

For all variables, we calculated a sexual size dimorphism index as: $SSD = \{(\bar{x}_m - \bar{x}_f)/\bar{x}_f\} \times 100$ (Weidinger & van Franeker 1998; Svalgelj & Quintana 2007); where \bar{x}_m and \bar{x}_f are the mean values of different age males and females, respectively. The coefficient of variation ($CV = (SD/\bar{x}) \times 100$) was calculated for each sex and averaged between them (Fletcher & Hamer 2003) to indicate the degree of variability of each measurement (Sokal & Rohlf 1995).

DFAs were developed separately for immature and mature birds. We excluded body mass from these analyses since it varies greatly over time (Croxall 1995; Svalgelj & Quintana 2007). The performance of each single measurement as a discriminating variable (univariate DFA) was evaluated. Forward DFAs were applied to obtain combinations of characteristics (discriminant functions) that best distinguished the sexes (see Tabachnick & Fidell 1996; Phillips & Furness 1997).

For immature birds, the DFA was applied to all individuals. For mature birds, the DFA was applied to 225 Coscorobas sampled in 2006 and 2007. For both age classes, the effectiveness of the analyses was assessed, first in terms of the proportion of birds of known sex that were classified correctly, and second by jackknife validation. Correct classification rates tend to be overestimated when DFAs are validated with the same sample used to generate them (Tabachnick & Fidell 1996). The jackknife validation is a process in which each individual case is classified using a function obtained from the total sample, excluding the individual case to be classified (Tabachnick & Fidell 1996). Furthermore, the accuracy of DFAs for mature birds was confirmed by applying the resulting functions to a novel dataset, composed of 120 birds captured in the 2005 season.

Results

Cloacal and genetic sex determination

Genetic sexing showed that there were 14 males and 27 females among young birds, and 189 males and 156 females among mature birds. Some birds were incorrectly classified by cloacal examination. Although genetic females were rarely misclassified as "males", males were often wrongly identified as "female". Cloacal inspection was particularly unreliable for

immature birds; overall, 38% of immature Coscoroba were sexed incorrectly. Among mature birds, overall, 14% of mature Coscoroba were sexed incorrectly.

Morphometric differences according to sex and age

Analysis of the whole dataset showed that mature Coscoroba were larger than immatures and males were bigger than females, with no age \times sex interaction (MANOVA: age: $F_{11, 242} = 4.6, p < 0.01, Wilks = 0.82$; sex: $F = 29, p < 0.01, Wilks = 0.43$; and age \times sex: $F_{11, 354} = 1.5, p = 0.1, Wilks = 0.93$). Thus, in each age cohort, males were larger than females in all measurements, except in tail length for immature birds (Tables 1 and 2).

In immature birds, characteristics that showed the highest sexual size dimorphism were mass and tail, whereas bill height, wing and tail were less dimorphic. Mass showed the highest within-sex variation whilst head length had the lowest (Table 1). In mature birds, mass, tarsus and neck length showed the highest sexual size dimorphisms whereas bill depth and tail were less dimorphic. Mass and neck length showed the highest within-sex variation whilst bill depth and head length had the lowest (Table 2).

Table 1. Body measurements (mean \pm SD and range, in mm), coefficients of variation (CV) and sexual size dimorphism index (SSD) for immature male and female Coscoroba Swans (*Coscoroba coscoroba*) from southern Rio Grande do Sul, Brazil. All measured characteristics differed between the sexes ($p < 0.05$) except tail length. CV was first calculated for each sex and then averaged.

Body measurement	Male	Female	One-way ANOVA	(%)	
	$n = 14$	$n = 27$	$F_{1,39}$	CV	SSD
Mass (g)	3760 \pm 460 2650–4400	3230 \pm 400 2250–4100	14.4	14.4	16.1
Total culmen	70.6 \pm 4.6 62.5–79.2	65.4 \pm 2.4 62–73	22.7	6.1	8.0
Nostril	52.7 \pm 2.8 48.6–56.6	48.7 \pm 2.6 41.3–56.3	21.4	6.6	8.2
Bill height	25.7 \pm 1.0 23.6–27	24.5 \pm 1.5 22.2–28.8	6.7	6	4.9
Bill depth	30.2 \pm 1.4 27.2–32.7	28.5 \pm 1.3 24–30.1	13.7	5.2	6.0
Head length	138 \pm 4.9 129–146.3	129.6 \pm 3.1 123.1–135	46.6	4.2	6.5
Tarsus	101.6 \pm 5.6 87.8–108.2	95.4 \pm 4.4 85–104	14.6	5.7	6.5
Wing	173.8 \pm 7.1 162–185	164.1 \pm 9.7 145–185	10.9	6	5.9
Forearm	213.1 \pm 8.3 200–225	195.9 \pm 8.8 175–215	37.7	5.9	8.8
Tail	183.1 \pm 14.7 161–205	173.2 \pm 21.9 107–220	2.5	11.4	5.7
Neck length	345.4 \pm 24.7 305–390	315 \pm 21.8 260–370	16.1	8.3	9.6

Table 2. Body measurements (mean \pm SD and range, in mm), coefficients of variation (CV) and sexual size dimorphism index (SSD) for mature male and female Coscoroba Swans (*Coscoroba coscoroba*) sampled in 2006 and 2007 in southern Rio Grande do Sul, Brazil. All measured characteristics differed between the sexes ($p < 0.05$). CV was first calculated for each sex and then averaged.

Body measurement	Males	Females	One-way ANOVA	(%)	
				CV	SSD
2006–2007	$n = 125$	$n = 100$	$F_{1,223}$		
Mass (g)	4000 \pm 335 3050–5140	3340 \pm 360 2400–4500	197.2	12.9	19.8
Total culmen	70.6 \pm 2.6 62.8–78.8	65.5 \pm 2.3 59.8–71.8	241.5	5.1	7.8
Nostril	52.8 \pm 1.6 48.4–58.8	49 \pm 2.2 40–59.2	221.7	5.3	7.8
Bill height	26.3 \pm 1.1 23.7–29	24.6 \pm 1.1 22.2–27.5	136.3	5.5	7.1
Bill depth	30.7 \pm 0.8 29–33.3	29.2 \pm 0.9 26.4–31.3	152.2	3.7	4.9
Head length	140.1 \pm 2.8 132.2–147	131.2 \pm 2.8 123.8–138.8	548.9	3.9	6.8
Tarsus	105.8 \pm 3.8 93.3–117.2	96.1 \pm 3.4 84.6–104.5	397.3	6	10.1
Wing	178.7 \pm 7.5 152–200	165 \pm 8.6 130–186	162.8	6.1	8.3
Forearm	212.6 \pm 8 177.0–230	196.4 \pm 7.7 168–217	235.3	5.5	8.2
Tail	175.4 \pm 6.9 145–190	166.4 \pm 8.5 141–187	76.2	5.1	5.4
Neck length	355.9 \pm 18.6 275–400	317.3 \pm 20.2 265–365	221.1	8	12.2

Discriminant analysis

Immature swans

Head length was the most accurate single indicator of sex, correctly classifying 88% of birds (Table 3). Coscorobas with head lengths longer than 135 mm were classified as males. Jackknife validation provided exactly the same classifications as those produced by DFAs for all single measurements. DFA retained head length and forearm as the best predictor variables and excluded others. This model correctly classified 79% of females and 96% of males (Table 3). Only three females and one male were misclassified. The discriminant function obtained for immature Coscorobas was: $DF1 = 0.43$ (head length) + 0.13 (forearm) – 84.6 . Values of $DF1 > 0$ identified males and values of $DF1 < 0$ identified females.

Mature swans

Head length was the most accurate single indicator of sex, correctly classifying 93% of Coscorobas (Table 4). Jackknife validation provided exactly the same classifications as those produced by DFAs for all single measurements except for nostril length, where

Table 3. Accuracy of sexing of immature Coscoroba Swans using single measurements or combinations in a discriminant function ($DF1 = \text{head length and forearm}$). Identical results were obtained by the jackknife method (see text).

Variables	Wilks' lambda	Original sample ($n = 41$) (% correct classification)			Cut-off point (mm)
		Males	Females	Overall	
Total culmen	0.63	64.3	92.6	83	69.3
Nostril	0.65	71.4	89	83	51.9
Bill height	0.85	64.3	74.1	70.7	26.2
Bill depth	0.74	78.6	70.4	73.2	30.1
Head length	0.45	86	89	88	135.0
Tarsus	0.73	78.6	66.7	70.7	101.0
Wing	0.78	78.6	70.4	73.2	173.8
Forearm	0.51	78.6	81.5	80.5	207.1
Tail	0.94	64.3	66.7	66	202.4
Neck length	0.74	78.6	70.4	73.2	339.0
DF1	0.42	78.6	96.3	90.2	

only two Coscorobas differed between classifications (changing the correct classification to 86% for males and 85% overall). Validation with a novel sample of birds provided slightly different classifications when compared with DFA, decreasing the accuracy for all measures except for bill height and bill depth (Table 4). DFA retained head length and tarsus as the best predictors. This model correctly classified 96% of females and 95% of males (overall success, 96%, three females and four males misclassified) with a low value for Wilks' lambda (Table 4). The $DF1$ obtained for mature Coscorobas was: $DF1 = 0.84$ (head length) + 0.38 (tarsus) – 152.6 . This model was represented in Figure 1 where mature Coscorobas were classified according to head length and tarsus measurements. Misclassifications were unusually large females misclassified as males.

A DFA with only head length as predictor variable classified 93% of cases correctly, with a value of Wilks' lambda close to the best model with head length and tarsus together. This alternative discriminant function ($DF2$) was: $DF2 = 1.1$ (head length) – 150 . $DF2$ performed slightly better than $DF1$ when validated against a new sample of birds (Table 4), with 92% of individuals sexed correctly.

Discussion

Sex determination by cloacal inspection

Although cloacal examination is a widely used method for sexing waterfowl (e.g. Green 2000; Gray & Hamer 2001; Nascimento et al. 2001), it has several disadvantages. This method requires observers with considerable experience, is only possible during the breeding

Table 4. Accuracy of sexing of adult Coscoroba Swans using single measurements and a discriminant function (DF1 = head length and tarsus) for 2006 and 2007 (original sample included in discriminant analyses) and 2005 (used for validation, see text). A jackknife method produced identical results to those shown here for the original sample, with the exception of nostril (see text).

Variables	Original sample (<i>n</i> = 225) (% correct classification)			New sample cross-validation (<i>n</i> = 120)			Cut-off point (mm)
	Wilks' lambda	Males	Females	Overall	Males	Females	
Total culmen	0.48	88.8	87.9	88.4	92.2	75	67.8
Nostril	0.5	88	83.8	86.2	98.4	54	48.8
Bill height	0.62	75	81	77.7	67.2	90.2	25.3
Bill depth	0.59	80	77	78.7	100	78.4	19.4
Head length	0.29	94.4	92	93.3	91	93	136.3
Tarsus	0.36	90.4	89.9	90.1	86	87.5	100.4
Wing	0.58	83.2	78	80.9	87.5	60.3	173.3
Forearm	0.49	87.9	84.8	86.5	97	66.1	203.4
Tail	0.74	76.6	69.8	73.6	98.4	48	169.2
Neck length	0.5	87.2	82	84.9	73.5	80.4	330.8
DF1	0.26	95.2	95.9	95.5	91	91.1	

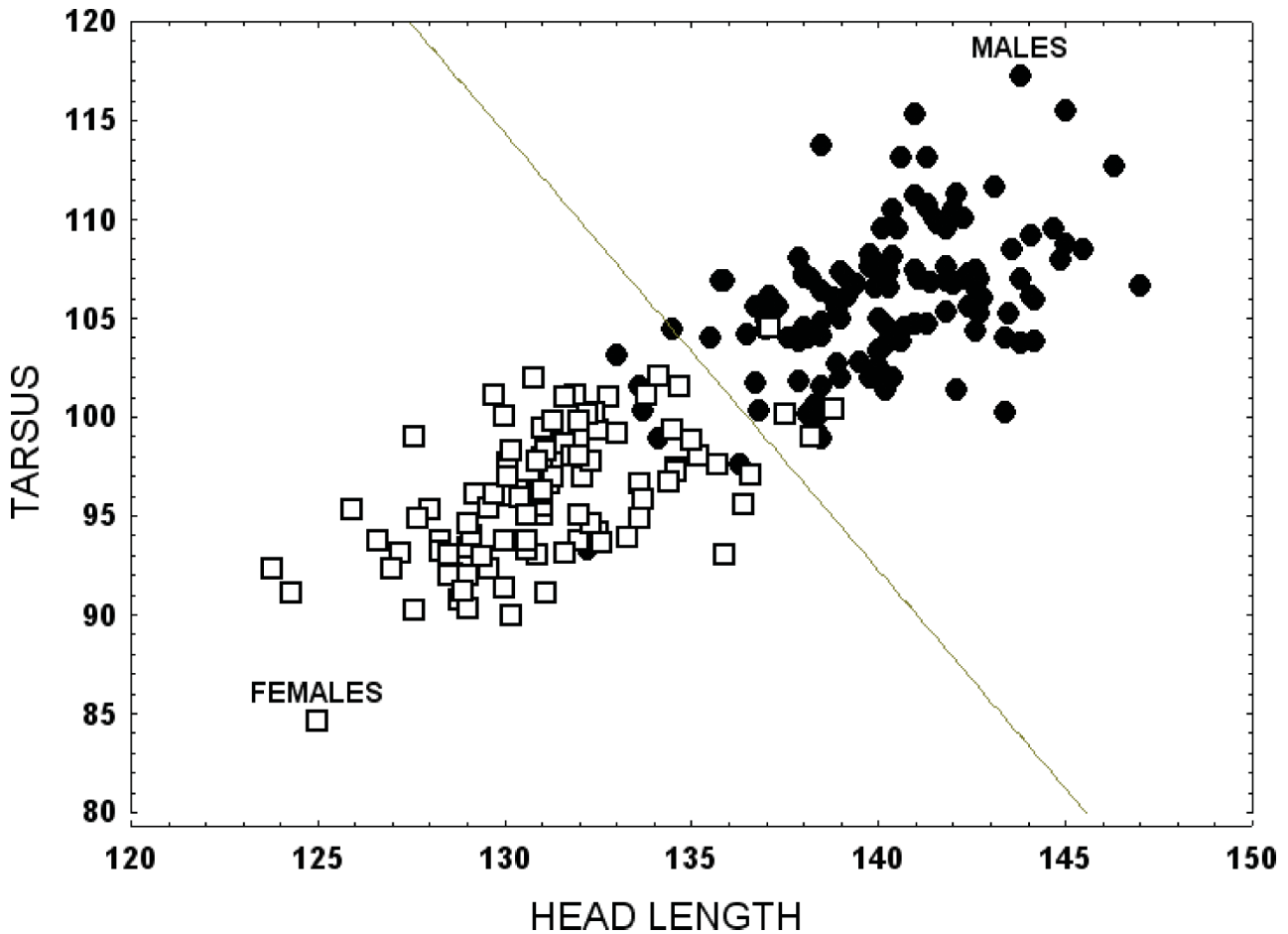


Figure 1. Plot of 329 mature Coscoroba Swans from southern Rio Grande do Sul, Brazil, according to head length and forearm length. Males and females were identified by genetic sexing. The straight line represents 50% probability of sex classification according to the discriminant function.

period for some species, and can cause internal damage to the bird due to the pressure applied during penis visualization (e.g. Sax & Hoi 1998; Lombardo 2001; Oliveira et al. 2004). We found it to be an unreliable method for the Coscoroba, owing to the difficulties of observing the penis, especially in young birds where penis development is incomplete (Odwyer et al. 2006). Similar levels of inaccuracy have been recorded in petrels (Odwyer et al. 2006) and in whistling ducks (genus *Dendrocygna*, Volodin et al. 2009). We do not believe that the error in sexing can be attributed to an observer effect in our study because the person who sexed the swans was an experienced specialist who has been ringing waterbirds for 20 years. Previous studies relying exclusively on cloacal inspection to sex Anatidae (e.g. Green 2000) may also contain errors.

Sexual size dimorphism and age effects

Few previous data were available on the morphometrics of Coscoroba (Kear 2005). Our results indicate significant size dimorphism, with male Coscoroba generally being larger than females, both for immature and mature birds. In general, large size may be advantageous in male swans and geese because they are responsible for nest protection (Scott 1972; Veselovsky 1973; Hawkins 1986; Whitehead & Tschirner 1990) or may need to defend females against males seeking extra-pair copulations (Mineau & Cooke 1979; McKinney et al. 1983; Welsh & Sedinger 1990; Gauthier & Tardif 1991; Choinière & Gauthier 1995). As in the Cape Barren Goose, both Coscoroba sexes care for their cygnets and maintain long-term pair bonds, but the male is primarily a guardian and helps with nest building but not incubation.

Mass and neck length showed the highest degree of sexual size dimorphism in Coscoroba, but head length was the most useful single variable to distinguish between males and females, because it had an intermediate level of dimorphism combined with particularly low CV. In most bird species, intraspecific variation is markedly lower in the bill and other body parts associated with food intake than in the rest of the body, perhaps as a result of adaptation to particular foraging behavior and diet (Miller et al. 1988). However, different CVs between measures can also reflect differences in measurement error (Yezerinac et al. 1992), and neck length is likely to have had a relatively high measurement error in our study. Our findings were consistent with previous studies that showed that head (e.g. Veselovsky 1973; Miller et al. 1988; Brown et al. 2003), tarsus (e.g. Veselovsky 1973; Brown et al. 2003; Mathiasson 2005) and forearm (e.g. Mathiasson 2005) lengths are key characteristics to differentiate sexes in Anseriformes.

Coscoroba Swans reach maturity when they are two years old and start breeding at three to four years old (Wilmore 1979; Silva-García & Brewer 2007). Although birds are generally assumed to have determinate growth, our results showed that mature Coscoroba differed significantly in size from swans less than two years of age, implying that growth continues in this species for an extended period prior to reaching sexual maturity (Kirkwood et al. 1989; Carrier & Auriemma 1992; Tumarkin-Deratzian et al. 2006). ANOVAs of individual measures showed that mass, bill depth, head length, tarsus, tail and neck length were all significant greater in mature birds (results not shown). Growth rates of large, precocious birds such as swans are particularly slow (Ricklefs 1973; Carrier & Auriemma 1992).

Sex determination by discriminant function analysis

Discriminant functions were developed using only two morphometric variables: head and forearm lengths for immature birds, and head and tarsus lengths for mature birds, resulting in 90% and 96% of correct sexual classification, respectively. These classification rates are much higher than those based on cloacal inspection. However, cross-validation with a new sample of mature swans suggested that a single measure (head length) was the most reliable sexing method, with 92% correct classification. While jackknife validation reveals influential observations that can bias DFA, cross-validation is a more rigorous validation process and should ideally involve a new sample of individuals measured at different times, locations and by different observers (Tabachnick & Fidell 1996). We suspect that discriminant functions would provide a better alternative to cloacal sexing in many other bird species.

More research is required to calibrate the use of our discriminant functions in other Coscoroba populations with other observers. Given the results of the validation with novel data, sex determination based on head length is likely to be the most robust discriminant function method. When cloacal inspection is carried out without genetic sexing, these data can be plotted together with head length data to establish the best cut-off point for sex determination based on morphometrics. For example, in a population in which Coscoroba were physically smaller, the cut-off points of Tables 3 and 4 for head length might be too high.

Acknowledgments

We would like to thank S. Scherer for his valuable assistance in the field. We thank Dr. B. Vaz, R. Tavares, J. Camacho and L. Bassini from the Department of

Genetics and Zoology, Federal University of Pelotas, RS, Brazil for technical help in carrying out DNA extraction.

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