BioOne COMPLETE

Sex Determination by Morphological Measurements of Black-browed Albatrosses (Thalassarche melanophrys) Using Discriminant Analysis

Authors: Ferrer, Miguel, Morandini, Virginia, Perry, Lynelle, and Bechard, Marc

Source: Waterbirds, 39(3) : 295-299

Published By: The Waterbird Society

URL: https://doi.org/10.1675/063.039.0309

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Waterbirds on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Sex Determination by Morphological Measurements of Black-browed Albatrosses (*Thalassarche melanophrys*) Using Discriminant Analysis

Miguel Ferrer[1,*], Virginia Morandini[1], Lynelle Perry[2] and Marc Bechard[2]

[1]Applied Ecology Group, Estación Biológica de Doñana (CSIC), AméricoVespucio s/n, 41092, Seville, Spain

[2]Raptor Research Center, Department of Biological Sciences, Boise State University, 1910 University Drive, Boise, Idaho, 83725, USA

[*]Corresponding author; E-mail: mferrer@ebd.csic.es

Abstract.—To provide an easy and reliable work tool to identify the sex of individuals, adult Black-browed Albatrosses (*Thalassarche melanophrys*) (n = 31) were weighed and measured, and the sex determined using DNA analyses. Stepwise discriminant analysis showed upper bill depth and weight to be the best predictor variables for sex determination. This model classified correctly 95.0% of the males and 81.8% of the females (overall success was 90.3%). Additionally, a single measure discriminant analysis of upper bill depth was developed that is able to separate sexes using a threshold upper bill depth of 29.87 mm, with values above this point being males and values below it being females. *Received 21 October 2015, accepted 11 March 2016.*

Key words.—Black-browed Albatross, discriminant analysis, forearm, morphometric, sea birds, sex determination, *Thalassarche melanophrys*, upper bill depth.

Waterbirds 39(3): 295-299, 2016

Easy and reliable methods to identify the sex of individuals are useful for the study of many aspects of avian biology, such as evolutionary ecology and genetics, population dynamics, behavior, dispersion, and migration and conservation genetics, but also for active management of species and populations (Clutton-Brock 1986; Sarrazin and Barbault 1996; Newton 1998). Procellariiformes generally exhibit sexual dimorphism where males are larger and heavier than females, but the degree of overlap renders sexing without behavioral or morphometric confirmation difficult (Hedd *et al.* 1998). Accurate and easy methods to determine the sex of an individual in the field is necessary for scientific studies.

Discriminant function analyses have been largely used as a tool for sexing species of birds that are sexually monochromatic but show sexual dimorphism in size (Brennan *et al.* 1991; Ferrer and de le Court 1992; Delgado and Penteriani 2004). External morphometric measurements are taken from individuals of known sex to develop discriminant functions and then test them using skins from museums and collections, individuals hunted/killed or depredated, and both captive and wild live animals (Amat *et al.* 1993; Lorentsen and Røv 1994). William (1983) and Titus *et al.* (1984) have noted the power and limitation of these discriminant techniques.

Discriminant functions have been widely used to sex birds, including albatrosses and related species (Hedd *et al.* 1998; Sagar *et al.* 1998; Copello *et al.* 2006; Polito *et al.* 2012), but no reliable criteria have been described to identify sex for the Black-browed Albatross (*Thalassarche melanophrys*), even though significant differences in morphological measurements have been found between sexes (Phillips *et al.* 2004). Therefore, an easy and accurate field method to sex this species would be helpful for studying conservation of the Black-browed Albatross. The aim of this study was to find a versatile and reliable morphometric criterion for sex determination of Black-browed Albatrosses in the field by means of discriminant models.

Methods

Study Area

The Falkland Islands, a British overseas territory, lie in the southwest region of the South Atlantic Ocean, approximately 600 km east of the mainland of South America, between latitudes 51° S and 53° S, and longitudes 57° W and 62° W. The present study was carried out in the Saunders Island. Saunders Island (51° 20′ 34″ S, 60° 10′ 50″ W) is a 12,500-ha sized island off the north coast of West Falkland Island. It is home to four species of penguins: King (*Aptenodytes patagonicus*), Gentoo

Downloaded From: https://bioone.org/journals/Waterbirds on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(*Pygoscelis papua*), Rockhopper (*Eudyptes chrysocome*), and Magellanic (*Spheniscus magellanicus*); two species of shags, Rock (*Phalacrocorax magellanicus*) and Imperial (*P. atriceps*); and several colonies of Black-browed Albatrosses (*Thalassarche melanophrys*). Upland (*Chloephaga picta*) and Ruddy-headed (*C. rubidiceps*) geese also occur on the island.

Body Measurements and Molecular Sex

During 17-26 February 2015, we trapped a total of 31 adult Black-browed Albatrosses. We weighed and measured adults when they arrived at their nests to provision their nestlings (Figs. 1, 2 and 3). Using digital calipers and a ruler, we measured: 1) bill length (to the nearest 0.1 mm); 2) upper bill depth (to the nearest 0.1 mm); and 3) diagonal tarsus and forearm length (to the nearest mm). We also used a spring balance to measure body mass (to the nearest 10 g).

We extracted DNA from feather bulb samples. Feathers (2-7 cm length) were taken from the neck or the back of the adults. The samples were placed in paper envelopes without touching their tips and then into new plastic bags containing silica gel. Silica gel absorbs moisture, which can degrade DNA. Sex identification was carried out by means of Polymerase Chain Reaction amplification of sections from CHD1 genes that are located on the avian sex chromosomes (Z in males and females and W in females only). We followed the protocol for primer sets using Griffiths *et al.* (1998), which provide the best amplification and the best fragment separation with Black-browed Albatross samples. Using this technique, we identified 11 females and 20 males from the 31 birds used in the discriminant analysis.

Statistical Analyses

Mean and standard deviation for all measurements were calculated for each sex. All five variables were normally distributed and met the conditions for homogeneity of variance. To check for overall differences in size between sex classes, we performed a one-way analysis of variance (ANOVA) of all five morphometrics. We then used forward stepwise discriminant analysis procedures that build the best explanatory discriminant model between sexes with the minimum possible number of morphometric variables. Each variable was moved into the model in successive steps, with an F to enter set to 3.84 (0.95 probability) and an F to remove set to 2.71 (0.90 probability). Wilks's Lambda statistic was derived to quantify the discriminant power of each model. Finally, we used a Jackknife procedure as posterior cross-validations of the predictive accuracy of the resulting functions (Manly 1986). We conducted analyses using STATISTICA software (StatSoft, Inc. 2007).

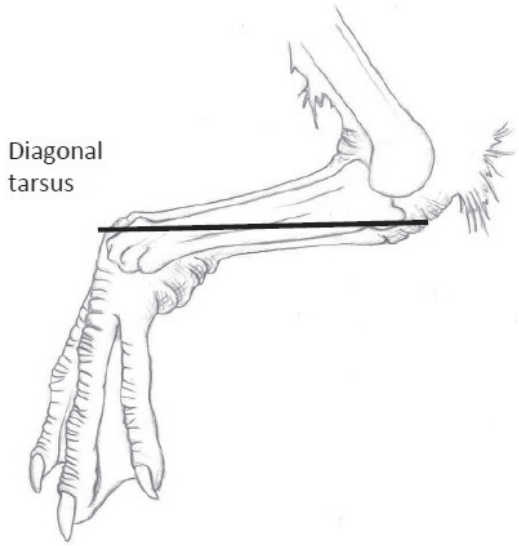


Figure 2. Measure of diagonal tarsus length (to the nearest mm).

RESULTS

There were significant difference in overall size between sex classes of adult Black-browed Albatrosses (ANOVA, $F = 8.91$, df = 5, $P < 0.001$). Results showed that females

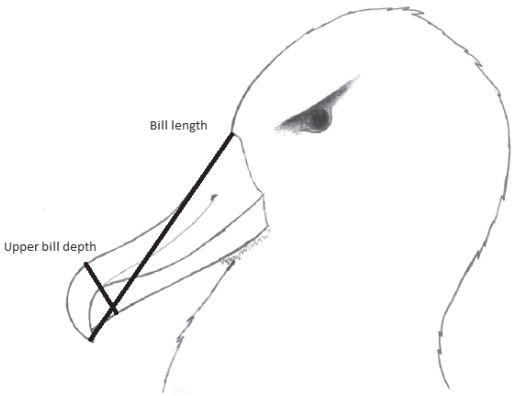


Figure 1. Measure of bill length and upper bill depth (to the nearest 0.1 mm).

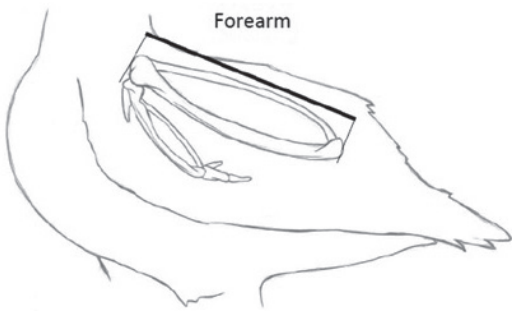


Figure 3. Measure of forearm length (to the nearest mm).

Downloaded From: https://bioone.org/journals/Waterbirds on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

were significantly smaller than males for all the morphometric measures, with upper bill depth, weight and forearm length as the most dimorphic characters (Table 1). The stepwise discriminant analysis performed retained upper bill depth and weight as the best predictor variables in the discriminant model and excluded the other variables. The discriminant function was:

D_1= 36.55 (upper bill depth) – 0.01 (weight) – 1,103.38

Values of D > 0 identified males, and values of D < 0 identified females. This model correctly classified 95.0% of the males and 81.8% of the females (overall success was 90.3%; two females and one male were misclassified) with a low value for Wilks's Lambda statistic (Wilks's Lambda = 0.388, F = 22.03, P < 0.001). The Jackknife procedure also correctly classified 90.3% of the whole sample. Because a discriminant function with only one measurement might be more useful for sex identification, when you find only a skull or a fragment of wing for instance, we conducted the analysis using only upper bill depth. This analysis correctly classified 87.5% of the birds (90% of males and 83.3% of females) with a low value of Wilks's Lambda close to the first selected model (Wilks's Lambda = 0.461, F = 35.03, P < 0.001).

Because Ferrer and de le Court (1992) demonstrated that forearm length is an easier measurement to take than diagonal tarsus length and repeated measurements taken by different observers showed less variance, we ran the discriminant function using only forearm length measurements. This model correctly classified 85% of cases, with a low value of Wilks's Lambda (Wilks's Lambda = 0.719, F = 11, 64, P < 0.001). The discriminant function D2 (upper bill depth) was

D_2 = 42.697 (upper bill depth) – 652.82

and that of the forearm, D3, was

D_3 = 4.284 (forearm) – 621.87

By solving the function for D_2 = 0, we obtained a threshold upper bill depth of 29.87 mm, with values above this point being males and values below it being females. Similarly, solving D_3 = 0, the threshold value for forearm length was 286.25 mm, with values above this point being males and values below it being females.

DISCUSSION

Our results indicate significant size dimorphism between female and male Black-browed Albatrosses, and sexes may be separated by means of external morphometric measurements in an easy and reliable way. Since there was considerable overlap in the ranges of some of these measurements, discriminant functions using morphometrics as predictor variables are more accurate tools for sex identification.

Even sexing birds using DNA amplification on sex chromosomes can falsely classify an individual due to sample contamination, experimental error and observer error (Palma *et al.* 2001). The best model obtained in our discriminant analysis showed a high level of overall correct classification of sex (95%), supported by several statistics and cross-validations. Measures like weight, wing length, tail length, length of wing feathers, and length of claws or foot pad length are widely used

Table 1. Mean and SD of morphometric measures of the Black-browed Albatross.

	Males (n = 20)		Females (n = 11)			
Parameter	Mean	SD	Mean	SD	F	P
Weight (g)	3,800.00	303.05	3,245.45	305.33	23.63	< 0.001
Forearm (mm)	290.10	9.29	278.91	5.28	13.43	< 0.001
Tarsus (mm)	89.03	2.64	85.56	3.24	10.35	0.003
Bill length (mm)	121.25	2.94	116.70	4.23	12.39	0.001
Upper bill depth (mm)	30.55	0.79	28.77	0.96	30.89	< 0.001

Downloaded From: https://bioone.org/journals/Waterbirds on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

in sex determination of birds (Amat *et al.* 1993; Hedd *et al.* 1998; Sagar *et al.* 1998; Copello *et al.* 2006), although these types of morphometric characters are quite variable and dependent on other factors. Weight has high variation, even within the same day, and is dependent on food eaten, stage of growth and environmental conditions, and wing feathers keep growing after the first flight and are subject to loss and molt. Morphometric variables derived from hard body structures like bills and bones (e.g., upper bill depth or forearm) are preferable as stable predictors (Counsilman *et al.* 1994).

Identification of sexes in dead birds (e.g., from skeletal material, specimens from museums or depredated birds) can only be determined from measurements of bones or bills. Although it has not been used traditionally in sex determination studies in birds, the forearm has become more widely used due to the accuracy that it provides (Ferrer and de le Court 1992). Furthermore, it has been shown to be an easier and more reliable measure with lower variance in measurements among different observers than has the diagonal tarsus (Ferrer and de le Court 1992). The model constructed only with this measurement provides a forearm length threshold of 286.25 mm between males and females, and the predictive power was 85%.

For a predictive model using just one measurement, the upper bill depth was found to be an easy, immediate, low-cost and accurate explanatory reference for sex determination in the Black-browed Albatross. The forearm length would be the next most valid predictor to separate sexes. These morphometric measurements should be considered standard work tools for future scientific studies and population management of this species.

ACKNOWLEDGMENTS

Fundacion Migres supported the present study. We thank Micky Reeves, N. Huin, the Falkland Government, and David and Suzan Pole-Evans for their support of this project. Procedures used in this study comply with the current laws for working in the Falklands. Permits to work in the study area and for albatross handling were granted by the Falkland Government as well as the owners of Saunders Island.

LITERATURE CITED

Amat, J. A., J. Vinuela and M. Ferrer. 1993. Sexing Chinstrap Penguins (*Pygoscelis antarctica*) by morphological measurements. Colonial Waterbirds 16: 213-215.

Brennan, A. L., J. B. Buchanan, C. T. Schick and S. G. Herman. 1991. Estimating sex ratios with discriminant function analysis: the influence of probability cutpoints and sample size. Journal of Field Ornithology 62: 357-366.

Clutton-Brock, T. H. 1986. Sex ratio variation in birds. Ibis 128: 317-329.

Copello, S., F. Quintana and G. Somoza. 2006. Sex determination and sexual size-dimorphism in Southern Giant-Petrels (*Macronectes giganteus*) from Patagonia, Argentina. Emu 106: 141-146.

Counsilman, J. J., K. Nee, A. K. Jalil and W. L. Keng. 1994. Discriminant analysis of morphometric characters as a means of sexing mynas. Journal of Field Ornithology 65: 1-7.

Delgado, M. and V. Penteriani. 2004. Gender determination of Eurasian Eagle-Owls (*Bubo bubo*) by morphology. Journal Raptor Research 38: 375-377.

Ferrer, M. and C. de le Court. 1992. Sex determination in the Spanish Imperial Eagle. Journal of Field Ornithology 62: 359-364.

Griffiths, R., M. C. Double, K. Orr and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7: 1071-1075.

Hedd, A., R. Gales, and N. Brothers. 1998. Reliability of morphometric measures for determining the sex of adult and fledgling Shy Albatrosses, *Diomedea cauta cauta*, in Australia. Wildlife Research 25: 69-79.

Lorentsen, S.-H. and N. Røv. 1994. Sex determination of Antarctic Petrels *Thalassoica antarctica* by discriminant analysis of morphometric characters. Polar Biology 14: 143-145.

Manly, B. F. J. 1986. Multivariate statistical methods: a primer. Chapman and Hall, London, U.K.

Newton, I. 1998. Population limitation in birds. Academic Press, San Diego, California.

Palma, L., S. Mira, P. Cardia, P. Beja, T. Guillemaud, N. Ferrand, M. L. Cancela and L. C. Da Fonseca. 2001. Sexing Bonelli's Eagle nestlings: morphometrics versus molecular techniques. Journal of Raptor Research 35: 187-193.

Phillips, R. A., J. R. D. Silk, B. Phalan, P. Catry and J. P. Croxall. 2004. Seasonal sexual segregation in two *Thalassarche* albatross species: competitive exclusion, reproductive role specialization or foraging niche divergence? Proceedings of the Royal Society of London B 271: 1283-1291.

Polito, M. J., G. V. Clucas, T. Hart and W. Z. Trivelpiece. 2012. A simplified method of determining

Downloaded From: https://bioone.org/journals/Waterbirds on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the sex of *pygoscelis* penguins using bill measurements. Marine Ornithology 40: 89-94.

Sagar, P. M., J. C. Stahl and J. Mollof. 1998. Sex determination and natal philopatry of Southern Buller's Mollymawks (*Diomedea bulleri bulleri*). Notornis 45: 271-278.

Sarrazin, F. and R. Barbault. 1996. Reintroduction: challenges and lessons for basic ecology. Trends in Ecology and Evolution 11: 474-477.

StatSoft, Inc. 2007. STATISTICA (data analysis software system), v. 8.0. StatSoft, Inc., Tulsa, Oklahoma. http://www.statsoft.com, accessed 12 July 2015.

Titus, K., J. A. Mosher and B. K. Williams. 1984. Chance-correct classification for use in discriminant analysis: ecological applications. American Midland Naturalist 111: 1-7.

William, B. K. 1983. Some observations on the use of discriminant analysis in ecology. Ecology 64: 1283-1291.

Downloaded From: https://bioone.org/journals/Waterbirds on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use