Sexing Cape Vulture *Gyps coprotheres* based on head morphometrics

Margaret T. Hirschauer[1], Tapiwa Zimunya[1,2], Kerri Wolter[1*], and Ara Monadjem[3,4]

*Corresponding author: kerri.wolter@gmail.com
[1] VulPro NPC, PO Box 285, Skeerpoort, South Africa
[2] Percy FitzPatrick Institute of African Ornithology, University of Cape Town
[3] All Out Africa Research Unit, Department of Biological Sciences, University of Swaziland, Kuwaluseni, Swaziland
[4] Mammal Research Institute, Department of Zoology & Entomology, University of Pretoria, Private Bag 20, Hatfield, Pretoria, 0028 South Africa

Abstract
The Cape Vulture, *Gyps coprotheres*, is considered sexually monomorphic in the literature, however visual differences in head shape between the sexes have been observed. Furthermore, head morphometrics of other *Gyps* species show statistically significant variation between the sexes. We show that head morphometrics can be used to determine the sex of Cape Vultures. Males generally have wider and shorter heads, and larger bill depths than females. Discriminant function analysis with data from 63 individuals identified the three most predictive variables in sex determination to be head width, head length, and bill depth. We also provide an equation which can be used in conjunction with head measurements as a method to determine the sex of Cape Vultures in the field with an overall accuracy of 84% (92% accuracy for females and 72% for males).

Keywords
Cape Vulture, sexual dimorphism, head morphometrics

Sexing of individuals in wild or captive populations is important in studies of avian biology (Xirouchakis and Poulakakis 2008). Furthermore, sexual determination has serious conservation implications as it is fundamental in calculating effective population size, as well as playing a role in understanding population dynamics, population structure, habitat use, behaviour and mating systems (Hughes 1998). A wide range of ecological or behavioural studies have used data obtained from body characteristics to determine gender (Hughes 1998). However, vultures of the genus *Gyps* appear to lack plumage characteristics or external features to distinguish between the sexes (Xirouchakis and Poulakakis 2008), with Cape Vultures *Gyps coprotheres* having been reported to be sexually monomorphic in plumage (Mundy et al. 1992).

Several sex determination approaches in birds, including vultures, have been used including observations of copulation and courtship behaviour (Catry et al. 1999), cloacal examination (Boersma and Davies 1987, Gray and Hamer 2001), brood patches during the breeding season (Cowley 1999), or the analysis of steroid hormones and DNA analysis (Fry 1983, Richner 1989, Griffiths et al. 1998, Wink et al. 1998, Ito et al. 2003). But such approaches are only limited to narrow time periods (e.g. the breeding season), require trained researchers and specialized equipment, or are expensive and time consuming (Jodice et al. 2000; Palma et al. 2001). Some authors have suggested the use of morphometric traits in determining sex by either measuring a single feature (Coulson et al. 1983) or a discriminant multivariate analysis (Van Franeker and Ter Braak 1993). Multivariate analysis of morphological measurements has been regarded as a successful approach in sexing many bird species (Donohue and Dufty 2006; Svagelj and Quintana 2007; Reynolds et al. 2008), including vultures of the genus *Gyps* (Xirouchakis and Poulakakis 2008). Other studies in southern Africa have discussed sex differentiation in Cape Vultures according to general morphological characteristics of the head, highlighting that females have a narrow head (as seen from the lateral canthus of the eyes) with an egg-shaped dome on top, while the males have a more triangular, flattened head with more prominent eye sockets (Mundy et al. 1992, Naidoo et al. 2011, Nsikani et al. 2015, Hirschauer 2016). As this method has not been statistically validated, it therefore remains important to test the applicability of such morphological characteristics of the head as a method to determine sex in this species. The main objective of this study was to determine if head shape of Cape Vultures can be used as a sexing tool.

The study was conducted over the course of 18 months at VulPro, a vulture rehabilitation and breeding centre located outside Hartbeespoort in North West Province, South Africa (25˚ 42' 7" S; 27˚ 57' 2" E). Measurements were taken opportunistically from 63 Cape Vultures of all ages (6 months and older) and backgrounds, i.e. birds kept for rehabilitation, captive bred individuals, or dead vultures.

Biometric data were taken using Vernier callipers following the standard measurements as described for vultures (Mendelssohn et al. 1989, Mundy et al. 1992): head length (HL), from the supraoccipital bone to the tip of the bill (Figure 1a); head width (HW), distance between the widest points in the auricular patches behind the eyes (Figure 1b); bill length (BL), from the tip of the culmen to its junction with the cere; bill plus cere length (BC), from the bill tip to the edge of implantation of feathers; and bill depth (BD), dorso-ventral distance at the nostrils (Figure 1c). However, some parameters were not measured for all subjects (HL n=38, BD n=44, the remaining features had n = 63). Morphometric measurements were made on Cape Vultures while being handled according to VulPro's protocols without any anaesthesia (see Wolter et al. 2014). A small blood sample was collected from each vulture with a 23-gauge needle and syringe from the tarsal vein. DNA sexing analysis was conducted by the National Zoological Gardens of South Africa following the methods described by Griffiths et al. (1998) based on two conserved CHD (chromohelicase-DNA-binding) genes located on the sex chromosomes of all birds.

Figure 1: Vernier callipers are used to measure Cape Vulture (a) head length, (b) head width, and (c) bill depth.


(a)


(b)


(c)

A one-way analysis of variance (ANOVA) was used to test whether measured head characteristics varied with sex in Cape Vultures, after testing for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). The sexual dimorphism index for each variable was also calculated following Weidinger and Van Franeker (1998). The percentage of sexual dimorphism in each measurement was expressed as $\bar{x}_m - \bar{x}_f / \bar{x}_m$ where $\bar{x}_m$ and $\bar{x}_f$ are mean values for males and females, respectively. MANOVA analyses were conducted with head measurements as the dependent variables to identify and remove those features which may be influenced by either age or body condition. Subsequently, discriminant function analysis (DFA) was conducted to obtain canonical values which were then used to produce an equation to estimate the sex of any individual. All statistical analyses were conducted in R version 3.2.0 (R Core Team 2013).

The means and standard deviations of the morphometric measurements recorded for Cape Vultures in this study are summarized in Table 1. Only HW, HL, BC, and BD differed significantly with sex ($p < 0.05$, test statistic values are presented in Table 1); BL did not differ significantly ($p > 0.05$). BC and HL for males were generally shorter than those of females, while BD and HW were larger for males. However, there was a large amount of overlap between the sexes in all these features (Table 1).

Table 1: The mean ± standard deviation, and range of male and female Cape Vulture head measurements. Morphometric measurements in bold denote a significant difference based on sex (p < 0.05).

Morphometric measurements	Males (mm)	Females (mm)	% sexual dimorphism	F- value	*P*
Bill depth (BD)	**36.4 ± 2.2 (n= 28) 32.5 to 42.0**	**35.0 ± 1.8 (n= 16) 32.5 to 38.9**	**3.88**	**4.653**	**0.037**
Bill length (BL)	53.7 ± 1.8 (n= 35) 50.9 to 57.1	54.4 ± 2.2 (n= 28) 50.4 to 59.5	-1.22	1.709	0.196
Bill and cere (BC)	**72.9 ± 11.6 (n= 35) 70.5 to 80.0**	**73.8 ± 13.2 (n= 28) 72.5 to 83.5**	**-1.18**	**4.657**	**0.035**
Head width (HW)	**56.7 ± 2.2 (n= 35) 52.1 to 61.5**	**54.5 ± 2.2 (n= 28) 50.5 to 58.5**	**3.93**	**16.05**	**0.000**
Head length (HL)	**151.1 ± 2.9 (n= 25) 147.0 to 157.5**	**153.3 ± 1.8 (n= 13) 148.5 to 155.4**	**-1.45**	**6.165**	**0.018**

MANOVA analyses using all four significant variables showed that age contributed a small but significant contribution to determining head morphometrics in Cape Vultures (Wilks' lambda = 0.255, F = 42.62, p = 0.012). However, when BC was removed, age became non-significant (Wilks' lambda = 0.509, F = 41.52, p = 0.189) with sex being highly significant (Wilks' lambda = 0.365, F = 17.00, p = 0.001). Therefore, the three head measurements that provided the most predictive power in determining sex in DFA analyses were HW, HL, and BD.

Canonical values produced from the DFA using the three variables that significantly differed by sex (HW, HL, and BD) produced an equation that can be used to estimate the sex of any individual, based solely on these measurements. Values ≥ 24 indicate a male and values ≤ 24 a female.

$$X = (0.411 * HW) - (0.354 * HL) + (0.199 * BD)$$

Cross-validation of the DFA resulted in the successful identification of 84% of individuals to the correct sex. By applying the equation given above, 92% of the females and 72% of the males were correctly assigned.

Although our sample sizes were sufficient to enable sexing of Cape Vultures, cross validation in the discriminant function analysis can be influenced by small sample sizes (Dechaume-Moncharmont et al. 2011). We therefore recommend that this analysis be repeated with more birds, although we note that obtaining large samples of Cape Vultures for sexing and measuring may be challenging. Several other potentially influential variables were not measured in this study and may be worth investigating in the future; for example, our sample size was insufficient to consider the effect of age and we did not include body mass in our analyses. Based on the measurement of a large number of birds, Mundy (1982) concluded that there were no sexual differences in mass. However, it may be worth revisiting the relationship of both age and mass with sex. Within pair (i.e. a nesting male and female bird) comparisons were also not possible in our study. Such comparisons have been previously used in the field (Fletcher and Hamer 2003) and may be worth investigating in the future. Another worthwhile line of inquiry for future studies may be to investigate the adaptive significance of sexual differences in head shape and size that we have demonstrated in this study.

We have shown that morphological characteristics of the head can be successfully used to determine the sex of Cape Vultures, with head width, head length, and bill depth being the most important features to measure. Males generally have shorter and wider heads, and larger bill depths than females. While our results are in agreement with those of Xirouchakis and Poulakakis (2008) who concluded that morphological differences in head width, head length, and bill plus cere length are effective measurements for gender determination of Griffon Vultures (*Gyps fulvus)*, some sex differences observed in Griffon Vultures show the reverse trend compared to Cape Vultures. Specifically, HL, BL, and BC were all larger in male Griffon Vultures, compared to females. Additionally, BL was shown to be highly significant in analysis of Griffon Vulture morphometrics, but not Cape Vultures.

The method that we outline here can be readily and rapidly employed in the field to sex Cape Vultures and is cheaper than using molecular techniques.

Acknowledgements

We would like to thank the National Zoological Gardens of South Africa, notably Dr. Antoinette Koetze, Dr. Desire Dalton, and Kim Labuschagne, for conducting the genetic sexing analysis. We would also like to acknowledge the following VulPro sponsors which contributed directly to the transport or housing of birds, facilitating data collection: Boehringer-Ingelheim, Cheyenne Mountain Zoo, Columbus Zoo, Colchester Zoo, Dallas Zoo, DHL Supply Chain, Different.org, Gauntlet Conservation Trust, Hans Hoheisen Charitable Trust, Lomas Wildlife Protection Trust, LUSH, Prince Bernhard Nature Fund, Rand Merchant Bank, and The Tusk Trust. We would also like to thank two anonymous reviewers who made useful comments on an earlier draft of the manuscript.

References

Boersma PD, Davies EM. 1987. Sexing monomorphic birds by vent measurements. *The Auk* 104: 779–783.

Bosè M, Le Gouar P, Arthur C, Lambourdière J, Choisy JP, Henriquet S, Lecuyer P, Richard M, Tessier M, Sarrazin F. 2007. Does sex matter in reintroduction of griffon vultures *Gyps fulvus*? *Oryx* 41: 503-508.

Catry P, Ruxton GD, Ratcliffe N, Hamer KC, Furness RW. 1999. Short-lived repeatabilities on long-lived Great Skuas: implications for the study of individual quality. *Oikos* 84: 473–479.

Coulson JC, Thomas CS, Butterfield JEL, Duncan N, Monaghan P, Shedden C. 1983. The use of head and bill length to sex live gulls Laridae. *Ibis* 125: 549–557.

Cowley E. 1999. Sand Martin *Riparia riparia* – male or female? *Ringing Migration* 19: 205–209.

Dechaume-Moncharmont F-X, Monceau K, Cezilly F. 2011. Sexing birds using a discriminant function analysis: a critical appraisal. *The Auk* 128: 78-86.

Donohue KC, Dufty AM. 2006. Sex determination of Red-tailed Hawks (*Buteo jamaicanensis calurus*) using DNA analysis and morphometrics. *Journal of Field Ornithology* 77: 74–79.

Fletcher KL, Hamer KC. 2003. Sexing terns using biometrics: the advantage of within-pair comparisons. *Bird Study* 50: 78-83.

Fry DM. 1983. Techniques for sexing monomorphic vultures. In: Wilbur SR and Jackson JA (eds) *Vulture Biology and Management*. University of California Press, Berkeley, pp. 356–374.

Gray CM, Hamer KC. 2001. Food provisioning behaviour of male and female Manx Shearwaters *Puffinus puffinus*. *Animal Behaviour* 62: 117–121.

Griffiths R, Double MC, Orr K, Dawson RJG. 1998. DNA test to sex most birds. *Molecular Ecology* 7: 1071–1075.

Hirschauer MT. 2016. Release success of captive bred Cape Vultures (*Gyps coprotheres*) in the Magaliesberg Mountains, South Africa. MSc Thesis. Rhodes University, South Africa.

Hughes C. 1998. Integrating molecular techniques with field methods in studies of social behaviour: a revolution results. *Ecology* 79: 383–399.

Ito H, Sudo-Yamaji A, Abe M, Murase T, Tugboat T. 2003. Sex identification by alternative polymerase chain reaction methods in Falconiformes. *Zoological Science* 20: 339–344.

Jodice PGR, Lanctot RB, Gill VA, Roby DD, Hatch SA. 2000. Sexing adult Black-legged Kittiwakes by DNA, behaviour, and morphology. *Waterbirds* 23: 405–415.

Mendelssohn JM, Kemp AC, Biggs HC, Biggs R, Brown CJ. 1989. Wing areas, wing loadings and wing spans of 66 species of African raptors. *Ostrich* 60: 35–42.

Mundy P. 1982. The comparative biology of southern African vultures. Vulture Study Group, Johannesburg, South Africa.Mundy P, Butchart D, Ledger J, Piper S. 1992. *The Vultures of Africa*. Academic Press, London.

Naidoo V, Wolter K, Espie I, Kotze A. 2011. Vulture rescue and rehabilitation in South Africa: An urban perspective. *Journal of South African Veterinary Association* 82:24- 31.

Nsikani MM, Mundava J, Mundy PJ. 2015. Size of black patches and spots on the upperwing and underwing greater secondary coverts in selected feathers are not good indicators of age and sex in Cape Vultures *Gyps coprotheres*. *Vulture News* 69.

Palma L, Mira S, Cardia P, Beja P, Guillemaud T, Ferrand N, Cancela ML, da Fonseca L. 2001. Sexing Bonelli's eagle nestlings: morphometrics versus molecular techniques. *Journal of Raptor Research* 35: 187–193.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>

Reynolds SJ, Martin GR, Wallace LL, Wearn CP, Hughes BJ. 2008. Sexing sooty terns on Ascension Island from morphometric measurements. *Journal of Zoology* 274: 2–8.

Richner H. 1989. Avian laparoscopy as a field technique for sexing birds and an assessment of its effects on wild birds. *Journal of Field Ornithology* 60: 137–142.

Svagelj WS, Quintana F. 2007. Sexual size dimorphism and sex determination by morphometric measurements in breeding imperial shags (*Phalacrocorax atriceps*). *Waterbirds* 30: 97–102.

Van Franeker JA, Ter Braak CJF. 1993. A generalized discriminant for sexing fulmarine petrels from external measurements. *The Auk:* 492-502.

Weidinger K, Van Franeker JA. 1998. Applicability of external measurements for sexing of the Cape Petrel *Daption capense* at within-pair, within-population and between-population scales. *Journal of Zoology* 245: 473–482.

Wink M, Sauer-Gürth H, Martinez F, Doval G, Blanco G, Hatzofe O. 1998. The use of (GACA) PCR to sex Old World vultures (Aves: Accipitridae). *Molecular Ecology* 7: 779–782.

Wolter K, Neser W, and Hirschauer MT. 2014. Protocols for mass capturing, handling, and fitting tracking devices on vultures. *AFRING News* 43: 9-10.

Xirouchakis S and Poulakakis N. 2008. Biometrics, sexual dimorphism and gender determination of Griffon Vultures *Gyps fulvus* from Crete. *Ardea* 96: 91–98.