

# GENDER DETERMINATION FROM MORPHOMETRICS IN MIGRATING MAGELLANIC PENGUINS *SPHENISCUS MAGELLANICUS*

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## SUMMARY

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Magellanic Penguins *Spheniscus magellanicus* are sexually dimorphic while breeding, but in winter the difference is not always sufficiently evident for visual determination of gender. We examined published discriminant functions and produced additional discriminant functions using morphometric data from 408 beachcast Magellanic Penguins from the Centro de Recuperação de Animais Marinhos on the most southerly point of the Brazilian coast (31°15'38"S to 33°45'03"S), for which gender was determined by post mortem examination or polymerase chain reaction. The discriminant functions correctly determined the gender of 70–90% of these penguins. However, an important gender-associated bias was detected, as previously published discriminant functions developed for animals in breeding colonies were systematically misclassifying males as females. A method is proposed to adjust the functions and correct this gender bias. While other methods may be more accurate, gender determination from morphometric data still offers reasonably reliable results and is an accessible, rapid, inexpensive and non-invasive method for determining the gender of Magellanic Penguins.

Key words: biometrics, Brazil, discriminant function, Magellanic Penguin, gender, *Spheniscus magellanicus*

## INTRODUCTION

Penguins are sentinels of the marine environment; observation of and research on penguins can provide important information on the intensity of human impacts on the southern oceans (Luna *et al.* 2002, Boersma 2008). The Magellanic Penguin *Spheniscus magellanicus* inhabits Argentina, Chile and the Falkland islands, and visits the Uruguayan and Brazilian continental shelves during winter (Williams & Boersma 1995, Luna *et al.* 2002). Some of these animals are eventually beachcast on the Brazilian coast, often dead or in poor health (Petry & Fonseca 2002, García-Borboroglu *et al.* 2010). Oiling, parasites, ingestion of anthropogenic materials, lesions from interactions with fishing nets, juvenile inexperience, weather abnormalities and changes in food availability are known to be among the factors leading these animals to become beachcast, although the dynamics of interactions among these mechanisms are not yet entirely clear (Petry & Fonseca 2002, Luna *et al.* 2002, Pinto *et al.* 2006, García-Borboroglu *et al.* 2010).

Gender and reproductive status have been shown to influence feeding and migrating behaviour of penguins, which may affect the geographic pattern of migration and survival (Forero *et al.* 2002, Walker & Boersma 2003). However, because gender dimorphism in the Magellanic Penguin is subtle and cannot always be visually identified outside the breeding season (Scolaro *et al.* 1983, Williams & Boersma 1995), gender-associated effects on migration and beachcasting of penguins along the southern Atlantic coast have not been well studied.

There are a number of strategies for gender determination in penguins, including behavioural observation during breeding season (Warham 1972a, Kerry *et al.* 1993), post mortem examination of gonads (Scolaro *et al.* 1983, Scolaro 1987), laparoscopy or laparotomy (Richner 1989, Boersma & Davis 1987), ultrasonography (Hildebrandt *et al.* 1996), post-laying cloacal morphometrics (Boersma & Davis 1987), cloacal endoscopy (Samour *et al.* 1983), chromosome analysis (Seddon & Seddon 1991), radioimmunoassay for blood testosterone and estradiol (Pennington 1996), polymerase chain reaction of gender-specific sequences (Bertellotti *et al.* 2002, Constantini *et al.* 2008) and discriminant analyses of morphometrics (Ainley & Emison 1972, Warham 1972a). There are also indications that vocalizations and iris colour may be useful for gender determination purposes (Scholten 1999, Miyazaki & Waas 2003).

Of these, gender determination from morphometrics is one of the cheapest and simplest methods (Bertellotti *et al.* 2002). Morphometric gender determination has been successfully developed for Magellanic Penguins *S. magellanicus* (Scolaro *et al.* 1983, Scolaro 1987, Gandini *et al.* 1992, Bertellotti *et al.* 2002), as well as Adélie *Pygoscelis adeliae* (Ainley & Emison 1972, Kerry *et al.* 1992), Chinstrap *P. antarctica* (Amat *et al.* 1993), Erect-crested *Eudyptes sclateri* (Warham 1972a), Fiordland Crested *E. pachyrhynchus* (Murie *et al.* 1990), Gentoo *P. papua* (Renner *et al.* 1998), Humboldt *Spheniscus humboldti* (Edgington 1989, Yamazaki *et al.* 1994, Zavalga & Paredes 1997, Wallace *et al.* 2008), Little Blue *Eudyptula minor* (Gales 1988, Renner & Davis 1999, Hocken & Russel 2002, Arnould *et al.* 2004), Macaroni *Eudyptes*

*chrysolophus* (Hart *et al.* 2009), Royal *E. schlegeli* (Hull 1996), Southern Rockhopper *E. chrysocome* (Warham 1972b, Hull 1996) and Yellow-eyed Penguins *Megadyptes antipodes* (Setiawan *et al.* 2004). For Magellanic Penguins, discriminant functions have been produced for different breeding colonies on the Argentinean coast: Scolaro *et al.* (1983) at Punta Tombo (44°02'S, 65°11'W); Scolaro (1987) at Punta Clara (43°58'S, 65°16'W); Bertellotti *et al.* (2002) at San Lorenzo (42°05'S, 63°51'W), Asentamiento Oeste (42°06'S, 63°56'W), Isla Primera de Caleta Valdés (42°21'S, 63°37'W), Caleta Interna (42°27'S, 63°36'W), Punta Tombo (44°02'S, 65°11'W) and Cabo Dos Bahías (44°54'S, 65°32'W); Gandini *et al.* (1992) at Cabo Vírgenes (52°24'S, 68°16'W).

Because body measurements often overlap between genders, it is possible to accurately determine the gender of only a fraction of the animals (Brennan *et al.* 1991, Hart *et al.* 2009). The asymmetry in growth of certain body parts measured, especially in early stages of life, may be an important source of error and variation for gender discriminant functions (Forero *et al.* 2001, Bertellotti *et al.* 2002). Phenotypic variation among subpopulations of different breeding colonies and among subspecies is known to interfere and even invalidate the discriminant analyses, and subpopulation-specific discriminant functions are generally advised (Gandini *et al.* 1992, Renner *et al.* 1998). Intra- and inter-observer biases are always involved in any body measurements, and such biases can sometimes reach levels that may invalidate subsequent discriminant analyses (Hull 1996, Blackwell *et al.* 2006). Body measurements, like any other phenotypic traits, are subject to selective environmental and sexual pressures, and thus can be expected to change over time (Moreno *et al.* 1999; Forero *et al.* 2001, 2002), compromising the results of gender determination. The application of these functions to animals in captivity should be considered cautiously, as the bills of captive animals may wear differently than expected and/or nutritional problems may affect body measurements (Zavalaga & Paredes 1997).

In this paper, we examine the use of gender determination from morphometrics for Magellanic Penguins beachcast along the

southern Brazilian coast, calibrating and verifying the accuracy of different discriminant functions by comparing their results to post mortem examination and molecular methods.

## METHODS

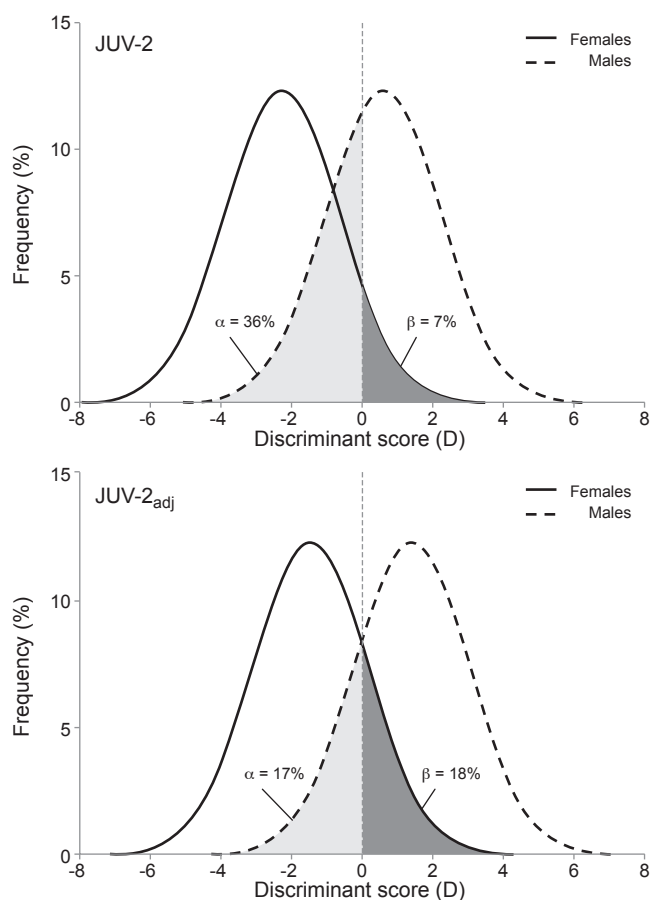
We studied 408 Magellanic Penguins from the Centro de Recuperação de Animais Marinhos of the Museu Oceanográfico Prof. Eliézer de Carvalho Rios from 2002 to 2009; these animals were found beachcast either dead or debilitated (often due to oiling) on the most southerly coast of Brazil, from Lagoa do Peixe (31°15'38"S, 50°55'5"W) to Chuí (33°45'03"S, 53°22'37"W). Gender was determined either during post mortem examination (n = 382 birds that had died during rehabilitation or were found dead on the beach) or by standard molecular methods (n = 26 birds that were beachcast alive; polymerase chain reaction of the CHD gene as described by Constantini *et al.* 2008). Most birds examined were juveniles (age < 1 year; 375 juveniles, 33 adults), as determined from feathering (Williams & Boersma 1995). Post mortem dissection identified 127 juvenile males, 225 juvenile females, 14 adult males and 16 adult females. Molecular methods identified an additional six juvenile males, 17 juvenile females and three adult females.

The following body measurements (adapted from Scolaro 1987 and Bertellotti *et al.* 2002) were taken with vernier callipers, steel rules or chords: bill depth (BD) measured dorso-ventrally at the nostrils; bill length (BL) from the dorsal edge of implantation of feathers to the tip of the bill culmen; bill width (BW) measured latero-laterally at the nostrils; total flipper length (TFL) maximum flattened chord from the scapulo-humeral joint to the tip of the flipper (dorsal side, flipper entirely extended); elbow-to-tip flipper length (EFL) maximum flattened chord from the humero-radial joint to the tip of the flipper (dorsal side, flipper entirely extended); pelvic member length (PML) maximum flattened chord from the proximal tibio-tarsus to the tip of the middle toe (plantar side, leg and foot extended); tibiotarsus length (TL) maximum flattened chord along the tibiotarsus (plantar side, leg extended); and middle-toe length

**TABLE 1**  
Discriminant functions for gender determination in fledgling and juvenile Magellanic Penguins from morphometrics, and their estimated accuracy for penguins beachcast in southern Brazil

Function	Reference	Discriminant function <sup>a</sup>	Accuracy, %		
			♂	♀	Total
FLEDG	Scolaro 1987	$D = (2.92BD) + (0.57MTL) - 91.77$	Not tested in this study		
JUV-1	Scolaro 1987	$D = (2.9BD) + (0.68TL) - 91.65$	59	98	79 (n = 140)
JUV-2	Bertellotti <i>et al.</i> 2002	$D = (0.6869BD) + (0.1976EFL) - 42.47$	64	93	79 (n = 345)
JUV-2 <sub>adj</sub>	Adapted from Bertellotti <i>et al.</i> 2002	$D = (0.6869BD) + (0.1976EFL) - 41.65$	83	82	83 (n = 345)
JUV-A	This paper	$D = (0.984BD) - 18.644$	73	79	76 (n = 355)
JUV-B	This paper	$D = (0.885BD) + (0.180EFL) - 42.835$	80	82	81 (n = 345)
JUV-C	This paper	$D = (0.834BD) + (0.148EFL) + (0.155PML) - 55.539$	83	85	84 (n = 343)
JUV-D	This paper	$D = (0.625BD) + (0.244BL) + (0.131EFL) + (0.145PML) - 61.134$	84	85	85 (n = 343)

<sup>a</sup> Body measurements (millimetres): bill depth (BD), bill length (BL), elbow-to-tip flipper length (EFL), middle toe length (MTL), pelvic member length (PML), tibiotarsus length (TL).



**Fig. 1.** Histograms of discriminant scores for adult males and females for the non-adjusted (JUV-2) and adjusted (JUV-2<sub>adj</sub>) variants of a discriminant function. If  $D > 0$ , the individual is presumed to be a male; if  $D < 0$ , the individual is presumed to be a female; and if  $D = 0$ , it is impossible to determine the gender. Note how the adjustment of the function changes the percentage of males mistakenly classified as females ( $\alpha$ ) and females mistakenly classified as males ( $\beta$ ).

(MTL) from the proximal phalanx joint to the tip of the nail of the third toe (dorsal side, foot extended). All measurements were taken or directly supervised by the same person (A.C. Adornes), minimizing observer bias and maximizing data consistency.

The previously published discriminant functions (DFs) will hereafter be referred to as FLEDG, JUV-1, JUV-2 and ADU-1 to ADU-4 (Tables 1 and 2). A procedure was developed to adjust these published DFs to reduce gender-associated systematic bias. The histogram of discriminant scores for males and females given by each published DF was plotted, and a best-fit normal curve was determined for each gender (see example in Fig. 1). The difference between the crossing point of the histogram curves of males and females and the discriminant threshold (i.e. zero) was visually estimated (in the example shown in Fig. 1, this difference was 0.820), and then added to the constant coefficient of the discriminant function. In the example in Fig. 1, the original function  $D = (0.6869BD) + (0.1976EFL) - 42.47$  was adjusted to  $D = (0.6869BD) + (0.1976EFL) - 41.65$ . The adjusted functions resulting from this procedure were named JUV-2<sub>adj</sub>, ADU-1<sub>adj</sub>, ADU-2<sub>adj</sub> and ADU-4<sub>adj</sub>; no attempt was made to adjust the functions FLEDG, JUV-1 and ADU-3 as the sample size of specimens with the required body measurement data was too small.

Binary logistic regression analyses were used to produce new DFs that would best fit our data. Models with one to four predictors were tested with the parameters BD, BL, EFL and PML. Only discriminant functions for which (1) an “All slopes equal zero” test was significant and (2) all predictors had coefficients that were significantly different from zero were further analysed. The best functions for each number of predictors were selected based on the significance level of their predictors and on the percentage of concordant pairs. Finally, the constant coefficient of the functions was adjusted using the procedure previously described. The resulting DFs were named JUV-A to JUV-D and ADU-A and ADU-B.

Anderson–Darling tests were used to verify normal distribution of data. Student’s *t*-tests were used to determine whether body measurements were different between genders within an age class.

**TABLE 2**  
Discriminant functions for gender determination in adult Magellanic Penguins from morphometrics, and their estimated accuracy for penguins beachcast in southern Brazil

Function	Reference	Discriminant function <sup>a</sup>	Accuracy, %		
			♂	♀	Total
ADU-1	Scolaro <i>et al.</i> 1983	$D = (2.247BD) - 52.3$	21	100	61 (n = 32)
ADU-1 <sub>adj</sub>	Adapted from Scolaro <i>et al.</i> 1983	$D = (2.247BD) - 47.1$	64	78	71 (n = 32)
ADU-2	Scolaro <i>et al.</i> 1983	$D = (2.217BD) + (0.773BL) - 95.4$	43	100	72 (n = 32)
ADU-2 <sub>adj</sub>	Adapted from Scolaro <i>et al.</i> 1983	$D = (2.217BD) + (0.773BL) - 90.5$	86	83	85 (n = 32)
ADU-3	Scolaro <i>et al.</i> 1983	$D = (2.072BD) + (0.746BL) + (0.21TFL) - 130.4$	60	100	80 (n = 20)
ADU-4	Bertellotti <i>et al.</i> 2002	$D = (2.4267BD) + (0.5653BL) - 85.7425$	57	100	79 (n = 32)
ADU-4 <sub>adj</sub>	Adapted from Bertellotti <i>et al.</i> 2002	$D = (2.4267BD) + (0.5653BL) - 83.0225$	86	83	85 (n = 32)
ADU-5	Gandini <i>et al.</i> 1992	$D = BW - 2.23$	Not tested in this study		
ADU-A	This paper	$D = (0.573BL) - 32.779$	79	83	81 (n = 32)
ADU-B	This paper	$D = (0.617BL) + (0.277EFL) - 76.721$	93	82	88 (n = 31)

<sup>a</sup> Body measurements (millimetres): bill depth (BD), bill length (BL), bill width (BW), elbow-to-tip flipper length (EFL), total flipper length (TFL).

Gender determined by each function was compared with known gender from post mortem examination or PCR; accuracy was defined as correct gender determinations per animals examined, and was calculated separately for males, females and total (average between males and females). Significance level was 0.05 for all tests.

## RESULTS

Comparisons of the examined published DFs and of those obtained in this study are presented in Tables 1 and 2. Table 3 presents the morphometrics of the examined birds; all measurements were significantly different between genders within age groups (all  $P < 0.002$ ), except TL among adults ( $P = 0.650$ , likely due to the small sample size).

## DISCUSSION

Published DFs, originally described for Magellanic Penguins in breeding colonies, performed poorly when applied to the migrating birds examined here. Bertellotti *et al.* (2002) reported an accuracy of 78% for juveniles ( $n = 226$ ; 78% for males and 78% for females) and 97% for adults ( $n = 331$ ; 95% for males and 99% for females). Sclaro (1987) and Sclaro *et al.* (1983) reported 95.7% accuracy for juveniles ( $n = 140$ ; 93.3% for males and 96.2% for females), and 92.8–95.9% for adults ( $n = 98$ ; accuracy for males and females not reported). When tested against our data from beachcast animals along the Brazilian coast, the same functions achieved accuracies of 76–85% for juveniles and 61–88% for adults.

Body measurements are known to differ between penguin breeding colonies, and thus it is expected that DFs perform poorly when applied to different populations than those they were developed for. Gandini *et al.* (1992) pointed out significant morphometric differences among the Magellanic Penguins from the Cabo Vírgenes and Punta Tombo breeding colonies in Argentina, limiting extrapolation of discriminant functions among regions. Bertellotti *et al.* (2002) propose that, despite differences in body measurements among Magellanic Penguin breeding colonies, their discriminant functions yielded significantly consistent results when applied among colonies. Because little is known of the winter migrations of Magellanic Penguins (Sick 1997, Pütz *et al.* 2000, 2007; García-Borboroglu *et al.* 2010), it is difficult to determine from which breeding colony (or, most likely, from which range of colonies) the birds examined here came from. Their body measurements and the poor results obtained

by the published discriminant functions suggest that the animals examined probably came from several colonies, possibly not those for which the published DFs had been developed.

More importantly, however, when applied to the examined population, the DFs tested produced an important bias towards females, as 93–98% of juvenile females and 100% of adult females would be correctly classified as females, but up to 36–41% of juvenile males and 40–69% of adult males would be mistakenly classified as females as well. As a result, any gender ratio investigation of the studied population using these DFs would produce a bias towards females, with an even gender ratio of 1♂:1♀ being mistakenly calculated as from 1♂:1.8♀ (JUV-2) up to 1♂:2.3♀ (JUV-1) for juveniles, and from 1♂:2.5♀ (ADU-4) up to 1♂:8.5♀ (ADU-1) for adults. These gender-associated biases result from a displacement of the discriminant score histogram curves to the left (i.e. both males and females had generally lower values for the discriminant score) as a result of phenotypic variations in those body measurements for the studied population. This displacement could be corrected by an adjustment of the discriminant function's constant, as provided in the "adj" functions in Tables 1 and 2; this procedure does not necessarily augment the overall accuracy but should provide more reliable results by reducing systematically biased misclassifications.

It should also be noted that we chose to classify all birds other than those for which the discriminant score was zero. Higher accuracy and more reliable results would be obtained, even for small sample sizes, by discarding from the analysis a small percentage of animals for which discriminant scores are close to zero (Brennan *et al.* 1991, Kerry *et al.* 1992, Renner *et al.* 1998). Brennan *et al.* (1991) concluded that if 20–30% of the birds closest to zero were excluded from the analysis a much more accurate gender ratio could be estimated. We recommend that this method should be considered whenever the functions discussed in this paper are applied to investigate gender ratio, to improve the reliability of the results.

We propose that the DFs described for penguins in breeding colonies might be transposable to specimens obtained outside breeding areas, but that verifying gender bias and fine-tuning the DF will improve the reliability of the gender determination. Accuracy rates of around 80–90% may not be suitable for some research purposes but are reasonable for husbandry and medical purposes and for coarse gender ratio assessments, particularly when there are time constraints (for

TABLE 3  
Body measurements of Magellanic Penguins beachcast in southern Brazil

Body measurement	Measure, mm, mean $\pm$ SD					
	Juvenile ♂	Juvenile ♀	Juvenile ♂+♀	Adult ♂	Adult ♀	Adult ♂+♀
Bill depth	19.98 $\pm$ 1.54 (n = 123)	17.86 $\pm$ 1.30 (n = 232)	18.60 $\pm$ 1.71 (n = 355)	21.98 $\pm$ 1.79 (n = 14)	19.70 $\pm$ 1.33 (n = 18)	20.70 $\pm$ 1.91 (n = 32)
Bill length	55.94 $\pm$ 2.81 (n = 123)	52.23 $\pm$ 2.70 (n = 232)	53.52 $\pm$ 3.25 (n = 355)	59.84 $\pm$ 2.94 (n = 14)	54.52 $\pm$ 2.90 (n = 18)	56.85 $\pm$ 3.93 (n = 32)
Pelvic member length	121.21 $\pm$ 3.83 (n = 123)	115.31 $\pm$ 5.89 (n = 232)	117.35 $\pm$ 5.97 (n = 355)	122.71 $\pm$ 3.54 (n = 14)	115.61 $\pm$ 4.34 (n = 18)	118.72 $\pm$ 5.33 (n = 32)
Tibiotarsus length	47.29 $\pm$ 2.00 (n = 62)	44.83 $\pm$ 2.22 (n = 132)	45.62 $\pm$ 2.44 (n = 194)	47.78 $\pm$ 3.44 (n = 6)	46.90 $\pm$ 2.45 (n = 4)	47.43 $\pm$ 2.96 (n = 10)
Elbow-to-tip flipper length	148.89 $\pm$ 5.95 (n = 116)	141.43 $\pm$ 6.06 (n = 231)	143.92 $\pm$ 6.97 (n = 347)	153.21 $\pm$ 5.32 (n = 14)	145.22 $\pm$ 4.71 (n = 18)	148.72 $\pm$ 6.35 (n = 32)

example, emergency veterinary procedures or oil spill responses) or for retrospective studies where available morphometric data provide the only means of gender determination.

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