MORPHOMETRIC INDICES FOR SEXING ADULT ROYAL *EUDYPTES SCHLEGELI* AND ROCKHOPPER *E. CHRYSOCOME* PENGUINS AT MACQUARIE ISLAND

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SUMMARY

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Four measurements were taken from a sample of known-sex Royal *Eudyptes schlegeli* and Rockhopper *E. chrysocome* Penguins at Macquarie Island. Significant differences were found between sexes in all measurements. Bill depth and bill length were the most reliable measures for assigning sex, and when these were applied to a discriminant function analysis, accurately assessed 97% males, 97.2% females in Royal Penguins (97.1% overall), and 93.3% males, 93.0% females in Rockhopper Penguins (93.2% overall). Cross-validation using jackknife analysis accurately assigned the sex of 97% of males, 97% of females in Royal Penguins (97.1% overall), and 93% males, and 93% females in Rockhopper Penguins (93.2% overall), indicating the validity of using these measurements. The non-overlapping ranges (mm) were: in Royal Penguins bill depth, males 29.3–33.5, females 24.3–27.7; bill length, males 67.0–73.8, females 55.8–62.7; in Rockhopper Penguins bill depth, males 20.3–23.3, females 16.7–18.8; bill length: males 46.2–51.2, females 38.5–42.2. These ranges should be used to assign sex in the field. For penguins that fall outside these ranges bill depth and length should be applied to the derived discriminant formulae. Some significant morphometric differences were found between Royal Penguins in this and a previous study on Macquarie Island, indicating the difficulty of comparing studies involving different workers. Methods for overcoming these difficulties are discussed.

INTRODUCTION

Studies of the ecology of avian species often require the identification of the sexes of individuals. In penguins there are no reliable plumage differences that can be used to distinguish the sex of individuals visually (Davis & Spiers 1990, Marchant & Higgins 1990). In order to avoid destructive or invasive techniques, the use of external morphometrics to sex animals reliably is of great value. Whereas penguins are dimorphic in body mass, this measure is unreliable due to its variability across animals within and between years (Warham 1975, Davis & Spiers 1990, Groscolas 1990, C.L. Hull unpubl. data).

External morphometric indices have been used widely to assist in the sexing of penguins (Scolaro 1987, Gales 1988, Kerry *et al.* 1992, Amat *et al.* 1993, Agnew & Kerry 1995, Woehler 1995). However, there are no published data on the morphometrics of Rockhopper Penguins *Eudyptes chrysocome filholi* from Macquarie Island, and the few data for Royal Penguins *E. schlegeli* indicate a need for further statistical analysis of morphometric characters (Woehler 1995).

Sexual dimorphism has been noted in all species of penguins, with males always larger than females (Livezey 1989). The degree of dimorphism, however, varies between groups of penguins. Using skin measurements Livezey (1989) found crested penguins *Eudyptes* spp. to be the most dimorphic of penguins, but they were only moderately dimorphic when compared using skeletal measurements.

The purpose of this paper is to provide data on morphometric indices for identifying the sex of individual Royal and Rock-

hopper Penguins, and to thus determine the most reliable means of sexing birds in the field. These data are then compared to previous studies of Royal and Macaroni *E. chrysolophus* Penguins.

MATERIALS AND METHODS

The study was carried out at Macquarie Island (54°30'S, 158°57'E) during the 1993/4 and 1994/5 breeding seasons. Royal Penguins were measured at Sandy Bay, and Rockhopper Penguins at Brothers Point, at the southern end of Sandy Bay. Measurements (to 0.1 mm) were made with Vernier calipers of bill depth (at a point proximal to the tip of the triangular inter-ramal feather patch), bill width (maximum width of the culminicorn), bill length (length of exposed culmen) (as per Warham 1972, 1975) and head length (maximum length from the dorsal brain case to tip of the bill) of penguins of presumed sex as determined by breeding behaviour. Breeding behaviour included date of return to the island (males return at least one week earlier than females), incubation shift (females carry out the first incubation shift), and guard-stage foraging shifts (undertaken by females) (Warham 1963, Smith 1970, Warham 1971, Carrick 1972, Warham 1972, Marchant & Higgins 1990). As there is no published, or observed, evidence of reverse-role behaviours in these species this technique was deemed to be reliable. Individuals were marked with permanent metal flipper bands and were observed at least once per week throughout the breeding season, enabling further confirmation of the sex of an individual (Hull & Wilson 1996). During the 1993/4 season, 50 pairs of breeding Royal and 50 pairs of breeding Rockhopper Penguins were measured. Pairs

TABLE 1

Species	Sex	Bill depth	Bill width	Bill length	Head length	BSI *
Royal Penguin	male (67)	30.4 (1.57)	14.1 (1.23)	68.7 (2.85)	143.5 (4.72)	2954.3 (373.1)
	female (71)	26.8 (1.27)	13.3 (1.15)	61.1 (2.63)	133.8 (4.34)	2181.6 (246.8)
t values		14.6	4.1	16.2	12.5	14.3
% difference between sexes		88.3	94.1	88.9	93.3	73.8
Rockhopper Penguin	male (60)	21.0 (0.99)	10.8 (0.81)	46.4 (2.05)	115.6 (2.98)	1051.8 (114.6)
	female (57)	18.7 (0.85)	10.0 (0.85)	41.9 (2.13)	109.6 (3.63)	788.2 (116.4)
t values		13.8	4.9	11.6	9.8	12.3
% difference between sexes		88.7	92.9	90.3	94.7	74.9

MORPHOMETRIC INDICES IN MM (MEAN AND STANDARD DEVIATION) OF ROYAL AND ROCKHOPPER PENGUINS FROM MACOUARIE ISLAND. ALL SIGNIFICANTLY DIFFERENT, P<0.05.

* Bill Shape Index

on nests were selected haphazardly from three transects in the Royal Penguin colony and two in the Rockhopper Penguins colony (see Hull & Wilson 1996). All birds were measured while on the nest to minimise disturbance (Hull & Wilson 1996). During the subsequent season, previously unbanded breeders on the transects were measured. Therefore, a total of 138 Royal (67 males, 71 females), and 117 Rockhopper (60 males, 57 females) Penguins were measured.

Comparisons of morphometric data between the sexes were made using Student t tests. Discriminant Function Analyses (DFA) were used to determine the accuracy of assigning penguins to a sex using these morphometric data, and to determine the most reliable measurements. A jackknife analysis was then used to cross-check the accuracy of the DFA (Tabachnick & Fidell 1989). From these results, discriminant formulae were derived to assign a sex to individuals for future studies. In addition, a Bill Shape Index (BSI) was calculated from the multiplication of bill depth, bill width and bill length, and divided by 10 (see Warham 1975) for the purpose of comparisons with Woehler (1995). A Mean Dimorphism Index (MD), a Separation Index (S), and a Bill Surface Area (BSA) were also calculated for each of the characters for comparison to other studies (see Agnew & Kerry 1995). These indices were defined as follows:

MD = 200 ($x_m - x_f$) (%) where x_m is the mean of the male

character and $(x_m+x_f) x_f$ is the mean of the female character;

S = 1-p where p is the proportion of individuals that are misclassified by a single factor discriminant analysis;

BSA = prl where *l* is bill length and *r* is (=0.5 bill depth) (the formula is the shape of a cone).

RESULTS

Sexing penguins by morphometric indices

All mean measurements in this study were significantly different between the sexes in both species (*t*-tests P<0.05), with males being larger (Table 1). The mean difference ranged from 73.8% (BSI) to 94.1% (bill width) in Royal Penguins, and 74.9% (BSI) to 94.7% (head length) in Rockhopper Penguins (Table 1).

In Royal Penguins the canonical loadings from the DFA determined that bill length was the most reliable predictor of sex, and in Rockhopper Penguins, bill depth (Table 2). Lower loadings for bill width and head length in both species indicate a lesser contribution of these measurements to the accurate assignment of sex to a penguin. Using the four variables, the discriminant function analysis accurately assessed 95.5%

TABLE 2

CANONICAL LOADINGS OF MORPHOMETRIC INDICES FOR ROYAL AND ROCKHOPPER PENGUINS FROM THE DISCRIMINANT FUNCTION ANALYSIS

Variable	Royal Penguins	Rockhopper Penguins
Bill depth	0.743	-0.883
Bill width	0.207	-0.318
Bill length	0.818	-0.749
Head length	0.633	-0.637

Group Classification function coefficients:

Variable	Males	Females	Males	Females
Bill depth	46.137	34.313	76.349	53.613
Bill width	125.164	121.261	73.915	75.703
Bill length	35.303	27.955	-20.634	-27.904
Head length	59.340	58.022	101.552	101.891

of males, and 97.2% of females in Royal Penguins (96.4% overall), and 93.3% of males and 93.0% of females in Rockhopper Penguins (93.2% overall). However, removing bill width and head length increased the accuracy of the DFA to 97.0% males, 97.2% females in Royal Penguins (97.1% overall), and 93.3 of males, 93.0% of females (93.2% overall). Cross-validation of these two variables using a jackknife analysis found that 97% of males and 97% of females in Royal Penguins (97.1% overall), and 93% of males, and 93% of females in Rockhopper Penguins (93.2% overall) were accurately assigned a sex. The DFA produced the following formulae for the determination of sex in these penguins:

Royal Penguins

D = -919.9 + (13.45 BD) + (8.24 BL)

Rockhopper Penguins

D = -739.3 + (21.97 BD) + (6.86 BL)

Where BD = Bill Depth, BL = Bill Length, and D is the discriminant function. Using these formulae, individual penguins that fall above zero are male and those that fall below are female.

Removing data that overlap between the sexes in each species, the non-overlapping ranges for bill depths and lengths for each of the sexes (mm) are given in Table 3.

Inter-population comparisons

Royal Penguin measurements obtained in this study were compared with those of Woehler (1995). Bill depth of males, and female and male bill length were found to be significantly different, with bill depth being less, and bill length being greater in this study compared to Woehler's (1995) (two-tailed *t*-test, P<0.05) (Table 4). BSI differed in opposite directions for males and females. Bill depth, bill width, bill length, and BSI were also compared between Macaroni Penguins from Heard Island (Woehler 1995) and Royal Penguins (this study). Significant differences were found in all measurements, with Royal Penguins being larger in both sexes (two-tailed *t*-test, P<0.05) (Table 4).

TABLE 3

NON-OVERLAPPING RANGES FOR BILL DEPTH AND LENGTH IN ROYAL AND ROCKHOPPER PENGUINS

Species	Sex	Bill depth	Bill length	
Royal				
Penguins	Males	29.3–33.5 (77.6%)	67.0–73.8 (73.1%)	
	Females	24.3–27.7 (73.2%)	55.8–62.7 (77.5%)	
Rockhopper	Males	20.3–23.3 (75%)	46.2–51.2 (60%)	
Penguins	Females	16.7–18.8 (49%)	38.5–42.2 (63%)	

The figures in parentheses represent the proportion of animals that can be sexed using these ranges.

MD and S values for all measurements are given in Table 5. Both the S values and the canonical loadings values given in Table 2 confirm that bill depth and bill length are the most reliable measurements in both species. Because it was not possible to compare the MD and S values on BSI between this study and that of Agnew & Kerry (1995) and Woehler (1995), these were not calculated.

DISCUSSION

Sexing penguins by morphometric indices

The significant differences found in bill depth, bill width, bill length and head length between the sexes of Royal and Rockhopper Penguins on Macquarie Island are of little surprise. The dimorphic nature of penguins has been well documented (e.g. Warham 1975, Gales 1988, Livezey 1989, Davis & Spiers 1990, Murie *et al.* 1991, Agnew & Kerry 1995), although the extent of the difference in the Macquarie Island populations was previously unknown for Rockhopper Penguins, and less well known for Royal Penguins. More importantly, it is now possible to determine the sex of individual penguins of these species without invasive techniques.

TABLE 4

Measurement	Sex	Royal Penguins Macquarie Is. This study 1	Royal Penguins Macquarie Is. Woehler (1995) 2	Macaroni Penguins Heard Is. Woehler (1995) 3	1×2 Significance (% difference to 1)	1×3 Significance (% difference to 1)
Bill depth	М	30.4 (1.57)	32.9 (1.87)	27.5 (0.82)	* (92.51)	* (110.62)
	F	26.8 (1.27)	27.6 (2.42)	24.0 (1.02)	ns (97.24)	* (111.69)
Bill width	М	14.1 (1.23)	14.7 (1.06)	12.9 (0.87)	ns (95.99)	* (109.37)
	F	13.3 (1.15)	13.1 (0.85)	11.1 (0.41)	ns (101.84)	* (119.30)
Bill length	М	68.7 (2.85)	64.8 (3.78)	61.4 (1.68)	* (106.04)	* (111.90)
-	F	61.1 (2.63)	57.3 (3.34)	53.7 (2.07)	* (106.69)	* (113.68)
BSI M	М	2954.3 (373.1)	3136.6 (391.2)	2166.2 (186.5)	ns (94.19)	* (136.38)
	F	2181.6 (246.8)	2078.4 (370.0)	1439.9 (95.7)	ns (104.96)	* (151.51)
Sample size	М	67	10	10		
•	F	71	10	10		

MEAN (STANDARD DEVIATION) FOR MORPHOMETRIC INDICES OF ROYAL AND MACARONI PENGUINS AT MACQUARIE AND HEARD ISLANDS. (* P<0.05)

TABLE 5

MEAN DIMORPHISM INDEX (MD) AND SEPARATION INDEX (S) FOR ROYAL AND ROCKHOPPER PENGUINS

Species	Bill depth	Bill width	Bill length	Head length	BSA
Royal Penguin	12.47 (91.30)	6.06 (60.14)	11.67 (92.75)	6.96 (85.50)	12.07 (96.38)
Rockhopper Penguin	11.78 (92.31)	7.31 (67.52)	10.17 (88.03)	5.38 (86.33)	10.96 (93.16)

Discriminant function analyses indicated that the use of only two of the measurements is sufficient to assign accurately the sex to individuals of Royal and Rockhopper Penguins on average 97.1% and 93.2% of the time, respectively. This rate is higher than that of other species subjected to the same procedure, such as the Adélie Penguin *Pygoscelis adeliae*, in which a 85% success rate was recorded, the latter being less dimorphic than the crested penguins in these measurements (Kerry *et al.* 1992).

The results from both the DFA and S (Single factor discriminant analyses) values further confirm that the most reliable single morphometric measure for assessing sex was bill length in Royal Penguins, and bill depth in Rockhopper Penguins. The S values (Table 5) indicate the percentage of individuals which did not overlap. Bill width was the least reliable measure, with only 60.1% of measurements and 67.5% of measurements not overlapping, and BSA the most reliable at 96.4% and 93.2% of measurements not overlapping in Royal and Rockhopper Penguins, respectively. The non-overlapping ranges of bill depth or bill length given above, or preferably BSI, can therefore, be used in the field to sex these species of penguin. Measurements from individuals that fall outside the ranges given, should be applied to the discriminant formulae presented above. The derived discriminant formulae cannot be applied to chicks or juveniles, which in this and other penguin species, have smaller bills than do adults (Warham 1972, Gales 1988, Scolaro 1987, C.L. Hull pers. obs.).

Both Royal and Rockhopper Penguins from this study fall within the range of the mean dimorphism indices of the other species of crested penguins given by Agnew & Kerry (1995). Rockhopper Penguins from this study were smaller in mean bill depth and mean bill length than in the other studies listed by Agnew & Kerry (1995). It has not been possible to compare data from this and other studies further statistically. However, using these morphometric indices (but not using other skeletal measurements, Livezey 1989), Royal and Rockhopper Penguins are, like all the crested penguins, among the most dimorphic, and can be sexed reliably in the field using these characters.

Inter-population comparisons

Comparisons of the Royal Penguin data presented in this study and those in Woehler's (1995) Macquarie Island study indicate significant differences in bill depth for males, and bill length for both sexes. In this study, male bill depth, male bill length and female bill length were 92.5%, 106.0% and 106.7% the size of individuals measured in Woehler's (1995) study, respectively. Woehler's (1995) study was carried out at a different colony than this study, and whereas one cannot discount the possibility that there are morphological differences between the various colonies on the island, the most likely cause is differences in measurements taken between various workers (e.g. Barrett *et al.* 1989, Lorentsen & Rov 1994). Whereas this study and that of Woehler's (1995) followed the techniques of Warham (1975), there were subtle differences in the interpretation of precisely where on the bill measurements should be taken. This indicates that bill length, and to an extent bill depth, were more variable between workers than some other measurements, with BSI being the most reliable measure. Although BSI is a derived index, the differences between measurers of these variables deviates in different directions, resulting in a masking of inconsistencies. The results also suggest differences in all measurements between Royal (this study) and Macaroni Penguins (Woehler 1995). Due to the above difficulties these differences have to be viewed tentatively. Therefore, comparative studies with data derived by different workers should be conducted with caution, particularly studies describing inter-population variation within species. These findings confirm the work of Barrett et al. (1989) who suggest that all workers should measure the same birds to resolve differences in techniques, or a number of samples from various measurers be taken in all cases.

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