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A method for determination of gender from bill measurements in Otago blue penguins (*Eudyptula minor*)

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Abstract Ability to distinguish the gender of individuals of any animal population is a useful field tool. Blue penguins (*Eudyptula minor*) exhibit a sexual dimorphism in bill size which has not been confirmed for any New Zealand population solely by internal anatomy. In this study, bill and head dimensions were taken from birds sexed by dissection. Measurements taken from a reference group of morphologically adult birds were used to construct equations by discriminant analysis. Those equations were applied to the bill measurements of a separate, opportunistically collected test sample, including juveniles, the gender of which was known from necropsy. We found a 15% inaccuracy in the test group, mostly derived from errors in identifying juvenile males. Removal of those individuals reduced the error to 10%. Application of the equation to 47 pairs of living birds of a monitored breeding population determined the gender wrongly for only two of 94 birds. Except for juvenile males, this method is useful for identifying gender in unselected populations of blue penguins in the field. The principle is readily applicable to other New Zealand populations.

Keywords blue penguin; *E. minor*; gender; bill size

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INTRODUCTION

It is important to many aspects of field work to be able to recognise easily the gender of individual animals. In blue penguins sexual dimorphism has been considered by Richdale (1940), O'Brien (1940), Kinsky & Falla (1976), Gales (1988) and Renner & Davis (1999) using bill dimensions of living birds, as well as body weight, foot and flipper lengths. The data of Kinsky & Falla (1976), Gales (1988) and Renner & Davis (1999) show a sex-governed bimodal distribution of bill size. From the various formulae presented, bill depth is the most influential discriminating factor (Gales 1988; Renner & Davis 1999). Both of the latter authors used discriminant factor analysis in allocating gender. However, Gales (1988) demonstrated that her equation was applicable only to Australian blue (little) penguins (*E. m. novaehollandiae*) and the equation of Renner & Davis (1999) was devised for a Cook Strait population of birds (*E. m. variabilis*), a group which Kinsky & Falla (1976) identified as morphologically distinct from Otago birds.

To date there has been no confirmation of gender allocation of New Zealand blue penguins from internal anatomy of all birds, although Gales (1988) dissected some birds; see Marchant & Higgins (1990) and Renner & Davis (1999). Our present work aims to provide a reliable field tool for gender identification by the application of discriminatory analysis to a sample representative of *E. m. minor* (Kinsky & Falla 1976).

METHODS

Birds picked up dead, or dying in captivity (Hocken 2000) were stored frozen and later dissected. To minimise freezer distortion and dehydration, carcasses were heat-sealed into plastic bags for storage awaiting necropsy, although Kinsky & Falla (1976) demonstrated that desiccation did not induce distortion of the bill. Weight and external measurements were recorded. Measurements of bill depth and width were taken at mid nares. Bill length was measured as the chord from the skin at the base to the tip of the maxillary horn. Head length was measured from the occiput to the tip of the bill (Warham 1975; Kinsky & Falla 1976; Darby & Seddon 1990). Measurement of the bill was to ±0.1 mm and of the head to ±1.0 mm using a Vernier micrometer. Four study groups were defined as follows:

Group A

The reference sample (60 male, 68 female) comprised adult blue penguins of Otago breeding stock, selected from necropsies carried out up to December 1998. The date was arbitrarily selected as a time when sufficient material had accumulated. Birds were classed as adult from bill morphology (Kinsky & Falla 1976), a reduced or obscure bursa of Fabricius (Camphuysen 1995) and/or a parous ovary. Data from the reference sample were used to construct regression analyses. We used the four dimensions of bill depth, bill width and bill length or head length as *x* values and constructed a dummy *y* variable to identify group membership:

$n_2/(n_1 + n_2)$ for males and $-n_1/(n_1 + n_2)$ for females,

where n_1 was the male sample size and n_2 the female sample size. This manoeuvre created an average of zero for the dummy variable over the whole data set, allowing the allocation of gender according to whether the discriminatory factor (*d*) is negative (female) or positive (male) (Gales 1988). This application in taxonomy was suggested by Fisher (1936) and Everitt & Dunn (1991).

Group B

The first 100 dead birds opportunistically collected from coastal Otago and examined after 1 January 1999, served as a (necropsy) trial sample, to which the equations derived from Group A were applied. Excluded from this second sample were birds that were clearly chicks (carrying down) or presumed to be recently fledged from their bill shape and plumage colouration (Kinsky & Falla 1976; Gales 1987; Meredith & Sin 1988a).

Group C

Both birds, of 47 banded currently breeding pairs, in monitored nest sites in the established population of blue penguins at the Oamaru Blue Penguin Colony (Houston & Russell 2001), were examined for body weight, bill depth and length and head length. Each of the pairs had eggs or chicks, confirming the presence of a male/female couple at each site.

Group D

A sample of 30 dissected fledged juveniles (16 females), made up partly (15 of 30) by those excluded by definition from Group A and the rest from Group B, were considered separately.

Table 1 Bill dimensions and head length of blue penguin study groups, Groups A, C, and D. For Group B, see Table 2.

	Males		Females		Difference between means	
Dimensions (mm)	Mean ± SE (*n*)	Range	Mean ± SE (*n*)	Range	*t*	*P*
Group A 128 adult blue penguins; (necropsy) Reference Group						
Depth	14.86 ± 0.13 (60)	11.7 – 16.4	12.99 ± 0.11 (68)	10.8 – 15.5	10.99	<0.0001
Length	39.47 ± 0.28 (60)	32.6 – 45.6	36.96 ± 0.30 (68)	32.0 – 46.1	6.10	<0.0001
Width	8.08 ± 0.10 (60)	6.2 – 9.9	7.31 ± 0.08 (68)	6.0 – 9.1	6.25	<0.0001
Head	99.2 ± 0.48 (60)	92.0 – 117.0	94.29 ± 0.40 (68)	88.0 – 102.0	7.81	<0.0001
Group C 47 breeding pairs of blue penguins; Field Trial Group						
Depth	15.71 ± 0.12 (47)	14.6 – 19.0	13.42 ± 0.09 (47)	12.3 – 14.8	15.03	<0.0001
Length	38.31 ± 0.27 (47)	34.0 – 42.4	35.72 ± 0.31 (47)	26.5 – 39.1	6.34	<0.0002
Head	99.13 ± 0.42 (47)	93.0 – 105.0	95.31 ± 0.51 (46)	87.0 – 103.0	5.78	<0.0003
Group D 30 post-fledging blue penguins; (necropsy) Juveniles						
Depth	11.62 ± 0.19 (14)	10.6 – 12.7	11.37 ± 0.24 (16)	10.0 – 13.5	0.84	0.41
Length	33.49 ± 0.58 (14)	30.2 – 37.0	32.92 ± 0.89 (16)	27.0 – 38.6	0.54	0.60
Width	6.79 ± 0.23 (14)	5.5 – 8.1	6.55 ± 0.14 (16)	5.7 – 7.6	0.86	0.40
Head	87.57 ± 1.01 (14)	81.5 – 93.0	87.9 ± 1.43 (16)	77.5 – 96.0	0.19	0.85

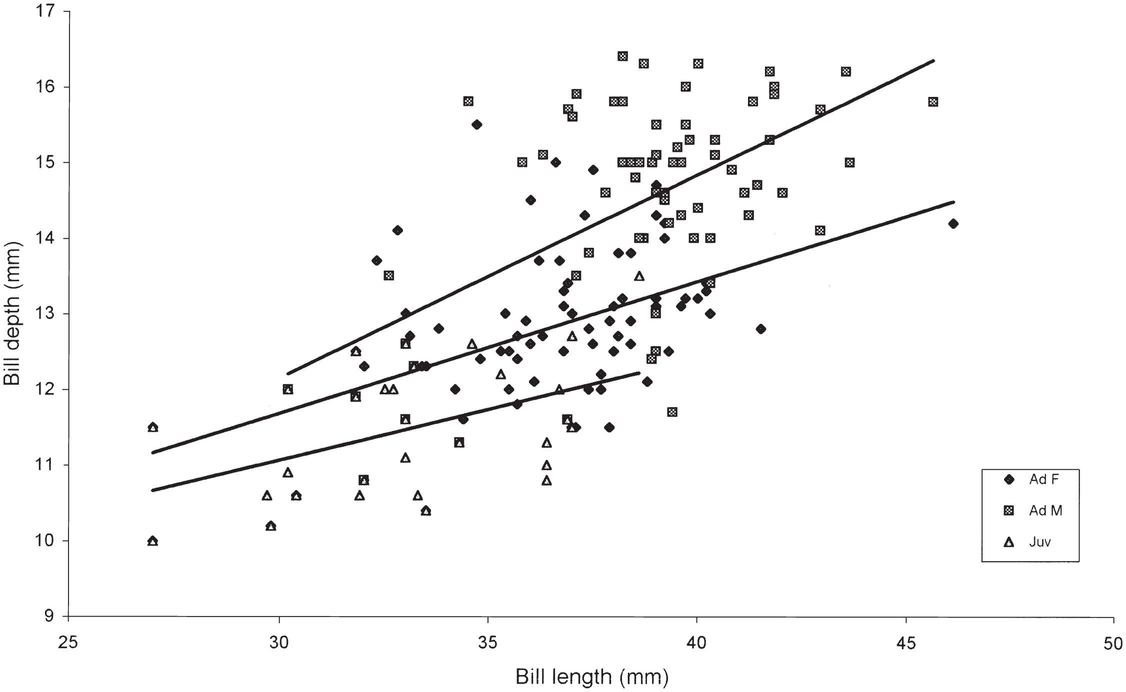

Fig. 1 Plot of bill length against bill depth for the 60 adult males, 68 adult females of the (necropsy) Group B and 30 juvenile birds Group D. Note that most bill depths 12 mm belong to the juvenile birds. The graphic similarity of plots of bill depth against the other dimensions, i.e., bill width and head length, to Fig. 1 is such that their reproduction is unnecessary. The juveniles provide a (third) separate population. Note: the "regression line" is inserted only to clarify identification of the three samples, male, female, and juvenile.

RESULTS

Table 1 shows that, for Group A all dimensions are larger in males than in females, $P < 0.0001$. For juveniles, Group D, the males' and females' dimensions are not significantly different, (*P* 0.4 for all dimensions) indicating that, for juveniles, unlike the adults, differentiation is not possible. Fig. 1 plots bill depth against length for Group A (males and females) and Group D. A "regression line" is drawn for each group only to clarify their distinction. A bill depth of 12.0 mm is found in juveniles of either gender.

The equations derived from the dimensions Group A were:

$$d\,(\mathrm{H}) = -6.45712 + 0.208155(\mathrm{D}) + 0.036974(\mathrm{H})$$
$$d\,(\mathrm{L}) = -4.59116 + 0.230657(\mathrm{D}) + 0.034646(\mathrm{L})$$

where D, L and H represent bill depth, length and head length, respectively. Exploration of equations in the prediction of gender established the relative influences, D > H or L. Bill width (W) exercised insignificant influence upon the predictability value of the equations for this species.

Bill measurements of Group B are described in Table 2, and the difference between means was applied (*P* 0.0001); the influence of juvenile males is illustrated. Application of the two equations derived from Group A to Group B revealed 14 and 16 errors respectively, representing an 86% [*d*(H)] and 84% [*d*(L)] accuracy of prediction of gender as confirmed by dissection. Six of the errors referred to the same juvenile males, which were the only juvenile males in the sample (Fig. 2 and 3).

Bill dimensions of Group C are described in Table 1, confirming sexual dimorphism in all three parameters measured, $P < 0.0001$. Application of the equation *d*(L) to the 94 breeding birds of Group C provided 45 pairs and one each of an apparent "pair" of males and females. This result represents 95.7% correct allocation of one male and one female to each established breeding pair. Assuming that in the two "same sex" pairs only one bird was misidentified, then the correct gender was allocated to 92/94 (97.8%) of birds when the equation was applied (Fig. 4). However, significant difficulty was experienced in measuring head length in live birds in the field and

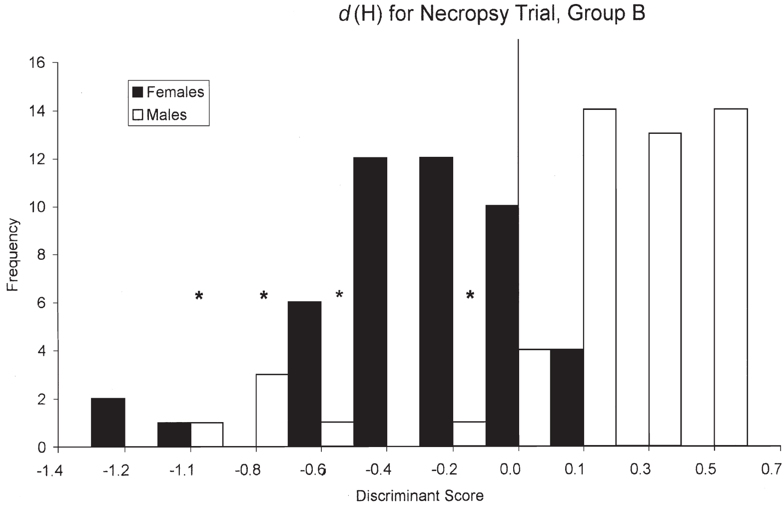

Fig. 2 Distribution of the discriminatory factor using head length and bill depth [*d*(H)] among blue penguins of the (necropsy) Group B. The distribution is bimodal, with females (solid bars) occupying the peak to the left of the vertical zero line; open bars represent male distribution. The six individuals marked * are the six juvenile males contained within this sample of 100 opportunistically collected blue penguins.

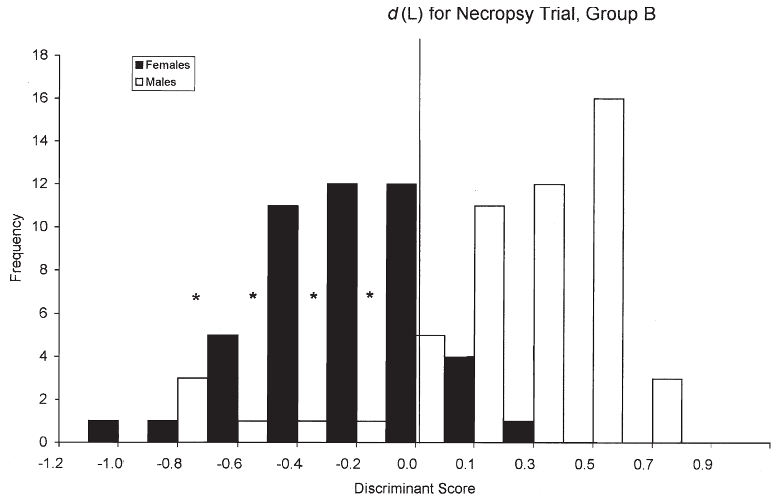

Fig. 3 Distribution of the discriminatory factor using bill length and depth [*d*(L)] among blue penguins of the (necropsy) Group B. The distribution is bimodal, with females (solid bars) occupying the peak to the left of the vertical zero line; open bars represent male distribution. The six individuals marked * are the six juvenile males contained within this sample of 100 opportunistically collected blue penguins.

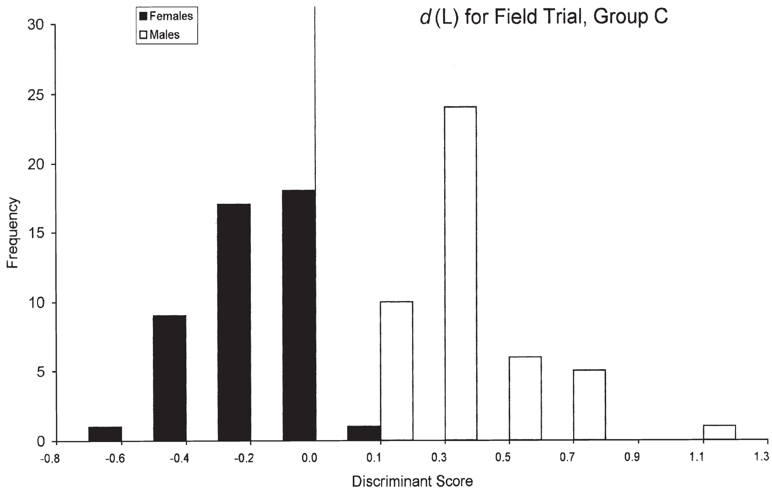

Fig. 4 Distribution of the discriminatory factor using bill length and depth among 94 breeding adult blue penguins of the (field trial) Group C [*d*(L)]. The bimodal distribution is about the zero vertical line, with females represented by solid bars, males by open bars.

Table 2 Bill dimensions and head lengths of 100 dissected blue penguins; (necropsy trial) Group B. Comparison of descriptive statistics of total males with those of adult males, with juveniles' data extracted.

Group B	Total males		Males excl. juveniles		Females		Difference between means	
							Total males *v* females	
Dimensions (mm)	Mean ± SE (*n*)	Range	Mean ± SE (*n*)	Range	Mean ± SE (*n*)	Range	*t*	*P*
Depth	14.69 ± 0.21 (53)	10.6 – 16.7	15.1 ± 0.14 (47)	13.3 – 16.7	12.66 ± 0.13 (47)	10.6 – 14.7	8.37	<0.0001
Length	38.34 ± 0.36 (53)	30.2 – 43.6	38.88 ± 0.31 (47)	34.2 – 43.6	36.51 ± 0.31 (47)	29.7 – 40.0	3.82	0.0001
Width	8.16 ± 0.12 (53)	5.5 – 9.8	8.35 ± 0.1 (47)	6.8 – 9.8	7.35 ± 0.08 (47)	5.9 – 8.2	5.62	<0.0001
Head	95.77 ± 0.67 (53)	82.0 – 104.0	97.03 ± 0.48 (47)	83.0 – 104.0	93.42 ± 0.52 (46)	81.0 – 99.0	2.91	0.0023

it is not surprising that application of the equation *d*(H) to the field trial group resulted in 15/47 same sex pairs, a 32% error.

DISCUSSION

Sexual dimorphism in penguins was reviewed by Agnew & Kerry (1995), who discussed, among other things, the use of body weight and bill size. These features were also considered by authors previously cited and reviewed by Marchant & Higgins (1990). However, no method for use in field work on New Zealand penguins has been described, except that described for use on Cook Strait birds by Renner & Davis (1999). It is clear from all previous work that bill depth is the most significant dimension for identifying gender in blue penguins (O'Brien 1940; Kinsky & Falla 1976; Gales 1988; Renner & Davis 1999). The use of bill depth and length to create a discriminant function score provided an expression with 92% reliability for identifying gender in Australian blue penguins (Gales 1988). That expression was "less reliable" for "southern" New Zealand birds and the author concluded that "discriminant formulae derived from one subspecies cannot be used reliably to sex other subspecies". Gales (1988) did not describe the derivation of her mathematics, an omission repaired in this paper.

In our study, we used two trial groups (B, dissected and C, live birds) in order firstly, to explore the application of the equations to a random sample of fledged birds (Group B was unselected for age and would thus include immature and subadult birds). Secondly, it was necessary to demonstrate the application of the equations in the field upon a known adult (breeding) population (Group C) where the gender could be safely inferred.

Working with blue penguins from Cook Strait, Renner & Davis (1999) measured 43 birds. They used cloacal size to identify 25 females and deduced that their co-habitants must be males. A discriminant function expression was evolved, using bill depth and length, which provided a 94.8% prediction reliability. They did not test their results on a subsequent trial sample. Our experience with Group C would suggest that the discriminatory accuracy of Renner & Davis' (1999) method would be improved by using a selected reference sample of breeding adult birds, back-trialled, so eliminating juveniles and subadults that appear in random field work and are hard to identify. The application of Renner and Davis' (1999) formula to our own Group C yielded

15/47 female:female couples. This 32% error exemplifies the need for care in the application of quantitative data between sub-populations of the greater New Zealand blue penguin population. Such an outcome is to be expected in the light of the range of bill morphology described by Kinsky & Falla (1976). We also consider that the demarcation score defined by Renner & Davis (1999) as distinguishing males (>0.311) is less easy to work with than our zero point of demarcation between the genders (Gales 1988).

Head length has been used previously for sexing yellow-eyed penguins (Darby & Seddon 1990). Head length had some advantage over bill length for allocating gender for our Group B. However, the blue penguin is a pugnacious species and Meredith & Sin (1988b) advocated the use of rubber bands on the bill, ostensibly to minimise the birds' self-injury. The practical difficulties of measuring the head length of live penguins in the field have invalidated the *d*(H) formula until a technique for reliable measurement can be found.

However, recognition of juvenile males will remain difficult. Renner & Davis (1999) considered the possibility of sexing chicks but did not find a bimodal distribution in either bill or foot size, and neither did we in Group D. At present the age at which the bill depth is complete is unknown (Renner & Davis 1999). Indeed, in herring gulls (*Larus argentatus*) for example, Coulson et al. (1981) found bill growth to continue for 9 years. Such an extended change could be invoked to explain the merely 90% accuracy of gender identification in Group B (which would have included subadults), which is less accurate than that achieved in the mature, i.e., breeding, population of Group C and in the birds examined by Renner & Davis (1999). Our data (Fig. 1, Table 1) suggest that where the bill depth is at or below 12.0 mm, gender identification becomes unreliable, because juvenile males fall into the range (*d*(L)   zero) applicable to adult females.

CONCLUSION

An equation employing bill depth and length is a useful field tool for gender determination in southern populations of blue penguins, providing 85% accuracy for all fledged birds of an unselected sample. However, there is an inescapable error in identifying young males, so interpretation of birds with bill depth of 12.0 mm or less must be applied with caution. In a randomly collected population, the gender of those birds with a bill depth >12.0 mm can be identified with an accuracy of 90%. When applied to known adult (breeding age) birds, the equation *d*(L) will provide more than 97% accuracy, at least in the Otago population.

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