Deakin Research Online

This is the published version:

Arnould, John, Dann, P. and Cullen, M. 2004, Determining the sex of Little Penguins (Eudyptula minor) in northern Bass Strait using morphometric measurements, *Emu: official organ of the Royal Australasian Ornithologists' Union*, vol. 104, no. 3, pp. 261-265.

Available from Deakin Research Online:

http://hdl.handle.net/10536/DRO/DU:30002781

Every reasonable effort has been made to ensure that permission has been obtained for items included in Deakin Research Online. If you believe that your rights have been infringed by this repository, please contact drosupport@deakin.edu.au

Copyright: 2004, Royal Australasian Ornithologists' Union.

CSIRO PUBLISHING
www.publish.csiro.au/journals/emu
Emu, 2004, **104**, 261–265

Determining the sex of Little Penguins (*Eudyptula minor*) in northern Bass Strait using morphometric measurements

J. P. Y. Arnould[A,C], *P. Dann*[B] *and the late J. M. Cullen*[B]

[A]School of Biological and Chemical Sciences, Deakin University, 221 Burwood Highway, Burwood, Vic. 3125, Australia.
[B]Phillip Island Nature Park, PO Box 97, Cowes, Vic. 3922, Australia.
[C]Corresponding author. Email: jarnould@deakin.edu.au

Abstract. In avian species with no obvious differences in plumage or body size between the sexes, such as penguins, discriminant function analysis (DFA) of morphometric measurements that display sexual dimorphism can provide a simple and rapid means of determining sex in the field. Like most other penguin species, the Little Penguin (*Eudyptula minor*) displays sexual dimorphism in bill shape and size. In the present study, discriminant functions (DFs) were developed for sexing adult Little Penguins at two colonies in northern Bass Strait, Victoria, Australia, and their accuracies were compared with those obtained previously in other parts of the species' range. Backwards stepwise DFA indicated that birds at Phillip Island can be sexed with an accuracy of 91% using a single measurement of bill depth (>13.33 mm classed as males). Similar analyses at Gibson Steps produced a DF incorporating bill length, bill depth and head length [although the model with the greatest accuracy when applied to birds from Phillip Island (91%) also contained only bill depth]. Published DFs derived in New Zealand had accuracies of 50–85% when applied to birds from Phillip Island and Gibson Steps, supporting earlier suggestions that DFs are not applicable across subspecies of the Little Penguin. In contrast, there was little difference between the accuracy of the DFs in the present study and that previously derived for the same subspecies in Tasmania when applied to birds from Phillip Island (89%) and Gibson Steps (92%). However, as the degree of variation in bill size within a subspecies is unknown it may still be prudent to derive colony-specific DFs.

Introduction

Knowledge of the sex of study animals is a fundamental requirement in most ecological studies. In mammals, the presence of external genitalia makes determining sex in field situations relatively simple. Similarly, despite the lack of external genitalia, obvious differences in plumage and/or size in many bird species enable researchers to determine easily the sex of their study animals. Determining the sex of penguins, however, is more problematic because they have no easily recognisable differences in plumage or body size (Agnew and Kerry 1995). While molecular procedures have proven to be accurate means of determining sex in some species (e.g. Magellanic Penguin (*Spheniscus magellanicus*) (Bertellotti *et al.* 2002), their use may be limited by the inability of researchers to collect genetic material in some situations or the delay in sample analysis. Fortuitously, differences in morphology between the sexes have been demonstrated in numerous species (Agnew and Kerry 1995). For example, discriminant function analyses (DFA) incorporating combinations of bill depth, bill length, head length, flipper length and flipper width have been used to determine sex accurately in Magellanic, Gentoo (*Pygoscelis papua*) and Chinstrap (*P. antarctica*) Penguins (Amat *et al.* 1993; Renner *et al.* 1998; Bertellotti *et al.* 2002).

The Little Penguin (*Eudyptula minor*) is the smallest of the penguin species, with a body mass of 0.8–1.3 kg (Marchant and Higgins 1990). Its breeding distribution stretches east–west from Penguin Island off the south-west coast of Australia to Chatham Island east of the South Island of New Zealand and north–south from Port Stephens on the mid-east coast of Australia to Stewart Island off the southern tip of New Zealand, encompassing six subspecies (*E. m. albosignata*, *E. m. iredale*, *E. m. minor*, *E. m. variabilis* in New Zealand; *E. m. novaehollandiae* in Australia; *E. m. chathamensis* on Chatham Island) (Kinsky and Falla 1976; Marchant and Higgins 1990). In Australia, the majority of birds (~60%) reside within Bass Strait (Dann *et al.* 1996), a shallow body of water (average depth <85 m) between the south-eastern tip of the Australian mainland and Tasmania. Phillip Island, located on the Victorian coast in northern central Bass Strait, is a major centre for Little Penguin research, with over 35 years of continuing studies (Newman 1992), and its researchers, in conjunction with the Penguin Study Group (Victoria), conduct studies at various colonies along the Victorian coastline.

© Royal Australasian Ornithologists Union 2004
10.1071/MU04035 0158-4197/04/030261

Several discriminant functions (DFs) have been developed for sexing Little Penguins using morphometric measurements and, while the inclusion of other variables improved the accuracy in some cases, bill depth has generally been found to be the most significant dimension for identifying sex (Gales 1988; Renner and Davis 1999; Hocken and Russell 2002). Comparisons of these DFs, however, have highlighted the need for separate functions to be determined for each subspecies. Furthermore, variation in mean bill depth and the degree of sexual dimorphism between Little Penguin colonies in south-eastern Australia have recently been documented (JPYA and PD, unpublished data). Depending on the extent of geographic variation in bill morphology within a subspecies, therefore, it may be necessary to obtain sex-determination DFs for individual study sites (Renner and Davis 1999). While Gales (1988) determined a DF for sexing Little Penguins in Tasmania and applied it to birds on Albatross Island (southern Bass Strait), its accuracy has not been ascertained for other parts of the range of *E. m. novaehollandiae*.

The aims of this study, therefore, were to determine DFs for sexing Little Penguins in northern Bass Strait using bill measurements and to compare their accuracy to those previously obtained elsewhere for the species.

Methods

Morphometric measurements were made on carcasses of Little Penguins that had been struck and killed by motor vehicles or killed by foxes (*Vulpes vulpes*) (Dann 1992). The carcasses were collected opportunistically from Gibson Steps (38°40′S, 143°06′E) on the south-west coast of Victoria (n = 103) and Summerland Peninsula (38°31′S, 145°08′E) on Phillip Island (n = 625) between 1982 and 1989. All carcasses were gathered within 12 h of death and stored frozen (–20°C) until analysis. Upon thawing in the laboratory, measurements (±0.1 mm) of bill length (length of the exposed culmen), bill depth (vertical thickness of the bill at the nostrils), bill width (lateral thickness of the bill at the nostrils) and head length (distance from occiput to tip of the bill minus bill length) were made on the carcasses using vernier callipers. The same person (PD) took all measurements. Sex was determined by inspection of the internal reproductive organs and birds were classed as adult on the basis of a reduced or obscure bursa of Fabricius (Camphuysen 1995), convolution of the oviduct, presence of a parous ovary and/or the size of the testes.

A randomly selected subsample of individuals (n = 400) from the Phillip Island birds was assigned to a *Reference* group and the remainder (n = 225) were assigned to a *Test* group. To assess the overall reliability of the DF derived from the *Reference* group, DF scores were calculated for the *Test* group individuals and their classification compared to that determined by dissection. The smaller sample size for the Gibson Steps birds prevented a similar testing of reliability and all individuals were used as a *Reference* group.

In addition to the *Test* group, the reliability of the DF derived from the Phillip Island *Reference* group was assessed by determining the DF scores for a *Wild* group. The *Wild* group consisted of 2163 breeding pairs captured together in burrows on Phillip Island between 1969 and 2003. On the assumption that a breeding pair always consisted of a male and a female, the smaller bird was nominally classified as female, and the classifications for both birds were compared to that predicted by the DF scores.

Statistical analyses were performed using the Statistica® software (Version 5.1, Statsoft Inc., Tulsa, USA). For each study site, pooled correlation matrices showed that all values were less than 0.7, indicating that there was no multicollinearity between variables (Zar 1984; Hedderson 1986). Kolmogorov–Smirnov tests were used to determine whether the sex-grouped data were normally distributed and F tests were used to confirm homogeneity of variances. Starting with the inclusion of all variables, backwards stepwise DFAs were computed using F values of 11 and 10 to *enter* and *remove*, respectively, as the criterion. Unless otherwise stated, data are presented as means ± 1 s.e. and results are considered significant at the $P < 0.05$ level.

Results

Within each of the sampling groups, there were significant differences between males and females in all of the measured variables, with males being the larger sex ($P < 0.0001$ in all cases) (Table 1). As expected, there were no significant dif-

Table 1. Mean (±s.e.) of bill and head measurements (mm) of male and female Little Penguin carcasses collected at Phillip Island and Gibson Steps, Victoria
See text for details

Group	Variable	Female	Male	t	P
Phillip Island *Reference*		n = 193	n = 207		
	Bill length	36.90 ± 0.10	39.10 ± 0.13	11.29	<0.0001
	Bill width	6.72 ± 0.05	7.54 ± 0.04	12.96	<0.0001
	Bill depth	12.24 ± 0.05	14.36 ± 0.05	28.23	<0.0001
	Head length	56.19 ± 0.23	57.97 ± 0.22	5.38	<0.0001
Phillip Island *Test*		n = 109	n = 116		
	Bill length	37.17 ± 0.19	39.14 ± 0.20	7.26	<0.0001
	Bill width	6.81 ± 0.06	7.50 ± 0.06	8.23	<0.0001
	Bill depth	12.28 ± 0.07	14.27 ± 0.07	20.38	<0.0001
	Head length	55.93 ± 0.27	58.42 ± 0.27	6.54	<0.0001
Gibson Steps *Reference*		n = 52	n = 51		
	Bill length	36.84 ± 0.21	39.15 ± 0.19	8.18	<0.0001
	Bill width	6.63 ± 0.06	7.42 ± 0.07	8.42	<0.0001
	Bill depth	12.49 ± 0.09	14.54 ± 0.13	12.95	<0.0001
	Head length	58.28 ± 0.32	61.44 ± 0.38	6.34	<0.0001

ferences in any of the measured variables between the Phillip Island *Reference* and *Test* groups ($P > 0.1$ in all cases). However, while there were no significant differences in bill length and bill width between the Phillip Island and Gibson Steps *Reference* groups, there were significant differences in bill depth for females ($t_{243} = 2.21$, $P < 0.02$) and head length for females ($t_{243} = 4.09$, $P < 0.0001$) and males ($t_{256} = 7.28$, $P < 0.0001$).

Inclusion of all variables produced the following canonical DF for the Phillip Island *Reference* group:

$$DS = 0.103 \cdot BL + 0.133 \cdot BW + 1.170 \cdot BD + 0.050 \cdot HL - 23.345 \quad (1)$$

where *DS* is the discriminant score and *BL*, *BW*, *BD* and *HL* are bill length, bill width, bill depth and head length, respectively (Wilks' $\lambda = 0.320$, $F_{4,395} = 209.5$, $P < 0.0001$). The distribution of discriminant scores for the *Reference* group derived from this function is shown in Fig. 1*a*. The overall

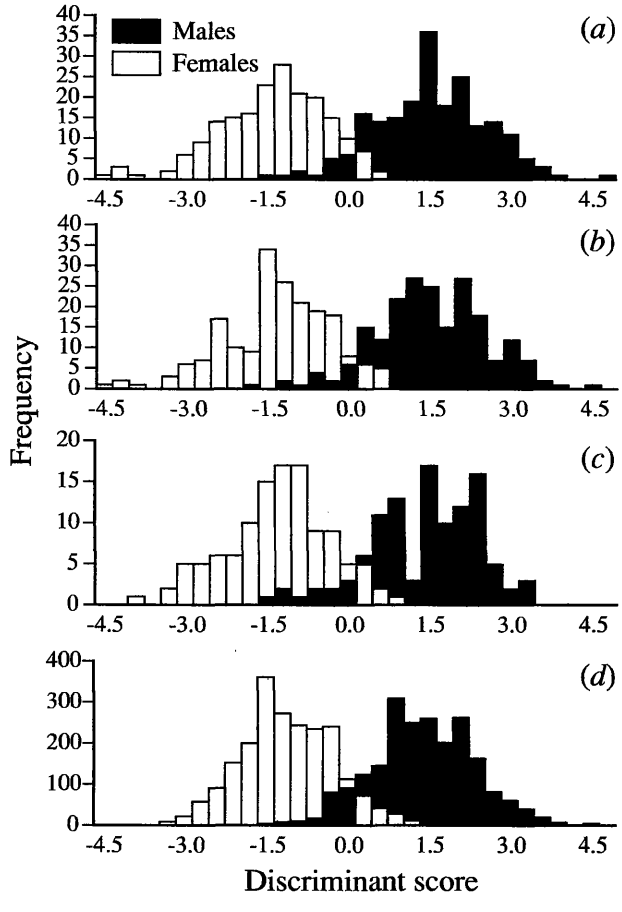

Fig. 1. Distribution of discriminant scores for the Phillip Island *Reference* group using a DF incorporating (*a*) bill length, bill width, bill depth and head length, (*b*) bill depth only, and discriminant scores for the (*c*) Phillip Island *Test* and (*d*) *Wild* groups derived using the DF for bill depth only.

reliability of this function on the *Test* group, taking all birds with *DS* values >0 as male and those <0 as females, was 92.9%, with 6 females being misclassified as males and 10 males as females. Substitution of the variables *HL* and *BL* for a combined measurement of total head length (*THL* = *HL* + *BL*) in the analyses did not improve the accuracy of the all-variable canonical DF or alter the results of the backwards stepwise exclusion. Backwards stepwise exclusion removed all variables except bill depth from the DF (Wilks' $\lambda = 0.333$, $F_{1,398} = 797.1$, $P < 0.0001$):

$$DS = 1.331 \cdot BD - 17.747 \quad (2)$$

which resulted in only a slight reduction in accuracy (91.1%), with 9 females misclassified as males and 11 males as females. The reduced DF had a similar accuracy (91.3%) in classifying the *Wild* group, with 161 females misclassified as males and 217 males as females. The distributions of discriminant scores for the *Reference*, *Test* and *Wild* groups derived using the bill-depth-only DF are presented in Fig. 1*b–d*. Applying the reduced Phillip Island DF to the Gibson Steps *Reference* group accurately sexed 90.3% of birds (equally misclassifying 5 males and 5 females).

Inclusion of all variables produced the following DF for the Gibson Steps *Reference* group:

$$DS = 0.275 \cdot BL + 0.036 \cdot BW + 0.909 \cdot BD + 0.188 \cdot HL - 34.222 \quad (3)$$

(Wilks' $\lambda = 0.287$, $F_{4,98} = 70.0$, $P < 0.0001$), which had an accuracy of 87.1% on the Phillip Island *Test* group, misclassifying 4 females as males and 29 males as females. Backwards stepwise exclusion removed only bill width (Wilks' $\lambda = 0.287$, $F_{3,99} = 82.1$, $P < 0.0001$) from the DF:

$$DS = 0.276 \cdot BL + 0.920 \cdot BD + 0.189 \cdot HL - 34.208 \quad (4)$$

with only a slight reduction in accuracy (85.3%; 3 females misclassified as males and 30 males misclassified as females). The distribution of discriminant scores for the Gibson Steps *Reference* group derived using the two DF models, and the scores when the reduced model is applied to the Phillip Island *Test* group, are presented in Fig. 2. Further reduction of the DF model to include only bill depth:

$$DS = 1.242 \cdot BD - 16.774 \quad (5)$$

resulted in increased accuracy (91.1%; 3 females misclassified as males and 17 males misclassified as females) when applied to the Phillip Island *Test* group. This is due to there being significant differences in head length between the Phillip Island and Gibson Steps birds. It should be noted, however, that while this DF had the greatest accuracy of the Gibson Steps models when applied to the Phillip Island *Test* group it may not be the most accurate for determining sex in birds from Gibson Steps.

Discussion

The lack of obvious sex differences in plumage or body size in penguins necessitates the use of alternative sexing techniques. While sexing by behavioural observations or the size of the cloaca have been shown to be reliable (Boersma and Davies 1987; Gales 1988; Scolaro *et al.* 1990), these methods are limited to the breeding period and, whereas molecular techniques are the most accurate and applicable at all times (Bertellotti *et al.* 2002), they are time consuming and expensive. Discriminant function analysis of morphometric variables remains the most practical (cheapest and fastest) method to field biologists (Amat *et al.* 1993; Renner *et al.* 1998; Bertellotti *et al.* 2002).

As found previously elsewhere throughout the species' range, the results of the present study indicate that Little Penguins at Phillip Island can be sexed using bill and head measurements with a reliability of >91% (Gales 1988; Renner and Davis 1999; Hocken and Russell 2002). Earlier studies presented DFs for Little Penguins that incorporated two or more variables whereas in the present study it was found that using only bill depth provided similar reliability. The need for only a single, and relatively simple, measurement is likely to be more efficient in field situations due to the reduced time needed, potentially fewer errors from measuring multiple variables, and providing a straightforward cut-off between males and females (i.e. 13.33 mm and 13.51 mm for Phillip Island and Gibson Steps, respectively).

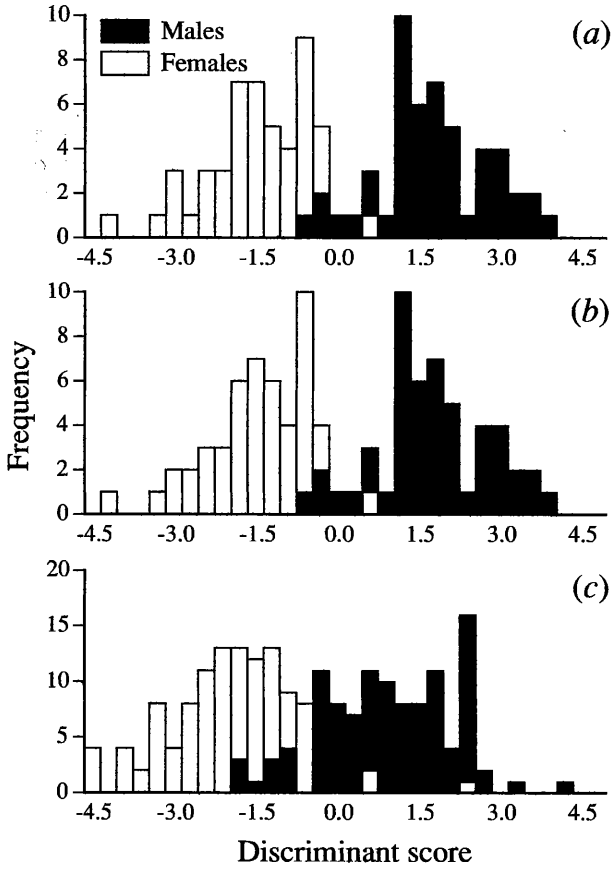

Fig. 2. Distribution of discriminant scores for the Gibson Steps *Reference* group using (*a*) a DF incorporating bill length, bill width, bill depth and head length, (*b*) the reduced DF model of bill length, bill depth and head length, and (*c*) discriminant scores for the Phillip Island *Test* group derived using the reduced Gibson Steps DF model.

The previous studies using DFs for sexing Little Penguins found a high level of misclassification when the DFs applied were derived from different subspecies (Gales 1988; Renner and Davis 1999; Hocken and Russell 2002). The results of the present study are consistent with these findings as the reliabilities of the DFs derived for *E. m. variabilis* ($DS = 1.245 \cdot BD + 0.202 \cdot BL - 26.459$: Renner and Davis 1999) and *E. m. minor* ($DS = -6.45712 + 0.208155 \cdot BD + 0.036974 \cdot HL$ and $DS = -4.59116 + 0.230657 \cdot BD + 0.034646 \cdot BL$: Hocken and Russell 2002) were 64.0%, 51.5% and 56.0%, respectively, when applied to the Phillip Island *Test* group and 68.0%, 50.5% and 84.5%, respectively, when applied to the Gibson Steps *Reference* group, with all misclassifications being males identified as females. This high degree of misclassification of males is due to the New Zealand birds of both sexes having greater mean bill depths (females 12.66–13.83 mm, males 15.10–16.91 mm) than those in northern Bass Strait (females 12.24–12.49 mm, males 14.36–14.54 mm).

While the need for DFs to be derived for individual subspecies is evident, the reliability of DFs applied to different parts of a subspecies' range has not been previously investigated. Renner and Davis (1999) noted that the geographic variation in body size of Little Penguins was as great within the Australian subspecies as between the New Zealand subspecies and cautioned that researchers should verify the applicability of DFs derived elsewhere. Furthermore, recent observations suggest that mean bill depths for each sex, and the degree of sexual dimorphism of bill depth, vary significantly between Little Penguin colonies in south-eastern Australia (JPYA and PD, unpublished data) such that separate DFs might indeed be needed for individual study colonies.

The previously published DF for sexing Australian Little Penguins ($DS = -83.10 + (10.06 \cdot \ln BL) + (17.99 \cdot \ln BD)$: Gales 1988) was derived from birds at Marion Bay (42°48′S, 147°53′E) on the east coast of Tasmania (R. Gales, personal communication). It was found to have a reliability of 94% in sexing birds from Albatross Island (40°22′S, 144°39′E) in southern Bass Strait. When applied to the Phillip Island *Test* and Gibson Steps *Reference* groups in the present study the reliability was 89.3% (equally misclassifying 12 males and 12 females) and 92.2% (5 females misclassified as males and 3 males as females), respectively. Similarly, the reliability of the Phillip Island DF was the same as Gibson Steps DF (91.1%) when applied to the Phillip Island *Test* group and only slightly lower (90.3%) when applied to the Gibson

Steps *Reference* group. Consequently, the degree of error in sexing Australian Little Penguins using DFs derived from different colonies of the subspecies appears minor compared with that when using DFs derived from different subspecies. However, there was little difference in the mean bill depths between the Little Penguin colonies investigated by Gales (1988) (females 12.4 mm, males 14.3–14.5 mm) and those in the present study whereas significant variation has been observed between other colonies in south-eastern Australia (JPYA and PD, unpublished data). Therefore, as the extent of variability in bill depth among Little Penguin colonies elsewhere in Australia is not known, applying DFs between other colonies should be done with caution and it may be prudent to derive colony-specific DFs when initiating studies at new locations.

Acknowledgments

The assistance of the many people who helped collect and dissect the penguin carcasses, in particular Tom Montague, Karl Harrigan and Ros Jessop, is gratefully acknowledged. This work was carried out under permits from the then Department of Conservation and Environment (Victoria).

References

Agnew, D. J., and Kerry, K. (1995). Sexual dimorphism in penguins. In 'The Penguins: Ecology and Management'. (Eds P. Dann, I. Norman and P. Reilly.) pp. 299–318. (Beatty and Sons: Surrey, UK.)

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

Bertellotti, M., Tella, J. L., Godoy, J. A., Blanco, G., Forero, M. G., Donazar, J. A., and Ceballos, O. (2002). Determining sex of Magellanic Penguins using molecular procedures and discriminant functions. *Waterbirds* **25**, 479–484.

Boersma, P. D., and Davies, E. M. (1987). Sexing monomorphic birds by vent measurements. *Auk* **104**, 779–783.

Camphuysen, K. (1995). Ageing guillemots and razorbills in the hand. *Sula* **9**, 1–22.

Dann, P. (1992). Distribution, population trends and factors influencing the population size of Little Penguins *Eudyptula minor* on Phillip Island, Victoria. *Emu* **91**, 263–272.

Dann, P., Cullen, J. M., and Weir, I. (1996). National review of the conservation status and management of Australian Little Penguin colonies. Final report. Australian Nature Conservation Agency, Canberra, ACT.

Gales, R. (1988). Sexing adult Blue Penguins by external measurements. *Notornis* **35**, 71–75.

Hedderson, J. (1986). 'SPSS^X Made Simple.' (Wadsworth: Belmont, CA.)

Hocken, A. G., and Russell, J. J. (2002). A method for determination of gender from bill measurements in Otago Blue Penguins (*Eudyptula minor*). *New Zealand Journal of Zoology* **29**, 63–69.

Kinsky, F. C., and Falla, R. A. (1976). A subspecific revision of the Australasian Blue Penguin (*Eudyptula minor*) in the New Zealand area. *National Museum of New Zealand Records* **1**, 107–126.

Marchant, S., and Higgins, P. J. (Eds) (1990). 'Handbook of Australian, New Zealand and Antarctic Birds. Volume 1. Ratites to Ducks.' (Oxford University Press: Melbourne.)

Newman, G. (1992). Studies on the Little Penguin *Eudyptula minor* in Victoria – an introduction. *Emu* **91**, 261–262.

Renner, M., and Davis, L. S. (1999). Sexing Little Penguins *Eudyptula minor* from Cook Strait, New Zealand, using Discriminant Function Analysis. *Emu* **99**, 74–79.

Renner, M., Valencia, J., Davis, L. S., Saez, D., and Cifuentes, O. (1998). Sexing of adult gentoo penguins in Antarctica using morphometrics. *Colonial Waterbirds* **21**, 444–449.

Scolaro, J. A., Stanganelli, Z. B., Gallelli, H., and Vergani, D. F. (1990). Sexing of adult Adelie Penguins by discriminant analysis of morphometric measurements. In 'Selected Scientific Papers of the Scientific Committee of CCAMLR'. pp. 543–549. (CCAMLR: Hobart.)

Zar, J. H. (1984). 'Biostatistical Analysis.' (Prentice-Hall Inc.: Englewood Cliffs, NJ.)

Manuscript received 28 November 2003, accepted 8 July 2004