Sex determination of black petrels (*Procellaria parkinsoni*) using morphometric measurements and discriminant function analysis

CLAUDIA P. MISCHLER*
Wildlife Management International Limited, PO Box 607, Blenheim 7240, New Zealand

ELIZABETH A. BELL
Wildlife Management International Limited, PO Box 607, Blenheim 7240, New Zealand

TODD J. LANDERS
Auckland Council, Private Bag 92300, Auckland 1142, New Zealand

TODD E. DENNIS
School of Biological Sciences, University of Auckland, Auckland PB 92019, New Zealand

Abstract Discriminant function analysis (DFA) is widely used to determine sex in the field from morphological measurements of bird species with monomorphic plumage. Sexual dimorphism was examined in black petrels (*Procellaria parkinsoni*) using 7 external measurements of adult birds breeding on Great Barrier Island, New Zealand. Males were significantly larger than females in absolute values of all measurements except for tarsus. Two stepwise DFA models were developed. The first used all 7 parameters, while the second model used only 6 parameters in order to increase sample size. Model one and two showed an 88 and 82% classification success, respectively, most likely due to the high overlap in measurements between males and females. These canonical functions were not accurate enough for field surveys, but may be improved using a larger and more representative sample size.

Mischler, C.P.; Bell, E.A.; Landers, T.J.; Dennis, T.E. 2015. Sex determination of black petrels (*Procellaria parkinsoni*) using morphometric measurements and discriminant function analysis. *Notornis 62 (2):* 57-62.

Keywords field measurements; sexing; conservation; population dynamics; black petrel

INTRODUCTION
The ability to determine sex in the field for monomorphic bird species using a reliable, inexpensive, and efficient method can help in designing protocols for the study of breeding biology, behaviour, demography, and time budgets (Mallory & Forbes 2005). Although sexual-size dimorphism generally exists in petrels (Procellariiformes) for at least some body measurements (Guicking *et al.* 2004; Einoder *et al.* 2008; Bugoni & Furness 2009; Landers

et al. 2011), differences often are not overtly obvious (Genevois & Bretagnolle 1995). Consequently, sex frequently needs to be determined using a variety of other techniques, such as cloacal inspection, vocalisations, molecular analyses, or statistical approaches such as discriminant function analysis (Genevois & Bretagnolle 1995; Weidinger & van Franeker 1998; Bertellotti *et al.* 2002; Mallory & Forbes 2005; Bourgeois *et al.* 2007). Cloacal inspection can provide a means of identifying sex as the cloacae of females are larger than those of males due to the egg passage, but this technique is limited to the period during egg laying for breeding

Table 1. Morphometrics of breeding adult black petrels measured at Great Barrier Island, New Zealand. Levels of significance are shown for unpaired *t*-tests. Gender was confirmed by DNA (*n* = 38), egg in belly (*n* = 4), necropsy (*n* = 2), and long-term partners of DNA birds (*n* = 24).

		Male	Female	*t*	*df*	*P*
Head and bill	Mean ± SD	95.9 ± 2.1	93.2 ± 2.1			
	Range	91.5 - 102.6	88.9 - 98.5	5.46	66	< 0.001
	n	34	34			
Skull width	Mean ± SD	35.2 ± 1.2	33.2 ± 1.9			
	Range	33.2 - 37.1	29.8 - 36.7	3.74	32	0.001
	n	16	18			
Culmen	Mean ± SD	42.5 ± 1.2	40.9 ± 1.2			
	Range	39.5 - 45.7	38.3 - 43.1	5.51	66	< 0.001
	n	34	34			
Culmen depth at base	Mean ± SD	18.6 ± 0.9	18.0 ± 0.9			
	Range	17.3 - 21.1	15.9 - 19.6	2.43	52	0.019
	n	29	25			
Culmen width at base	Mean ± SD	18.0 ± 0.7	17.2 ± 0.5			
	Range	16.2 - 19.2	16.1 - 18.2	5.35	63	< 0.001
	n	32	33			
Minimum bill depth	Mean ± SD	12.7 ± 0.6	12.0 ± 0.6			
	Range	11.7 - 14.0	10.5 - 13.0	5.47	64	< 0.001
	n	33	33			
Right tarsus length	Mean ± SD	55.8 ± 1.3	55.4 ± 1.7			
	Range	52.9 - 58.3	52.9 - 60.1	0.91	66	0.37
	n	34	34			

pairs (Boersma & Davies 1987; O'Dwyer *et al.* 2006). Differences in vocalisation patterns have been used to determine sex in some shearwater species (Cure *et al.* 2009), but this too relies on birds calling and cannot be used at times of the year when birds are silent. As a result, morphological differences assessed by discriminant function analysis (DFA) have been widely used to determine sex for a variety of bird species (Bertelloti *et al.* 2002; Mallory & Forbes 2005; Einoder *et al.* 2008; Liordos & Goutner 2008; Landers *et al.* 2011). This analytical technique identifies those morphological characters which best discriminate males and females, and the resulting canonical classification function may be used to classify sex (McGarigal *et al.* 2000). Results from the DFA allows one to estimate the proportion of correctly identified individuals in a sample of

birds using the classification function (Dechaume-Moncharmont *et al.* 2011).

The black petrel (*Procellaria parkinsoni*), also known as Parkinson's petrel, is endemic to New Zealand, and currently only breeds on 2 islands, Great Barrier (GBI) and Little Barrier Island (Marchant & Higgins 1990). It is a medium-sized procellariid, and breeds during the austral summer from October to July (Imber 1987). Black petrels nest in burrows in forested areas, and are active at night (Spear *et al.* 2005). The species is listed as Vulnerable on the IUCN Red List of Threatened Species (BirdLife International 2012), and as Nationally Vulnerable by the Department of Conservation Threat Classification System, using the criteria that the species has a moderate population that is in decline (Robertson *et al.*

Fig. 1. Probability of correct classification of being male as a function of discriminant score, based on model 1 where minimum bill depth, skull width, and culmen width at base of bill were the chosen parameters. An overlap in measurements between males and females is reflected in the discriminant score.
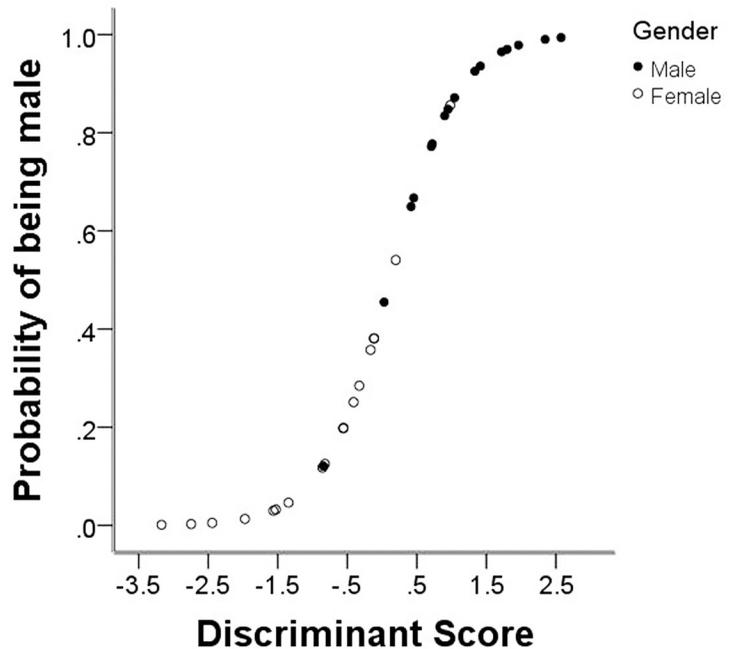
2012). Currently, the population is estimated to be approximately 5,000 individuals, with the majority breeding on GBI and only about 100 pairs breeding on Little Barrier Island (ACAP 2009). Scofield (1989) found an 84.3% success in sexing individuals with a DFA using GBI breeding bird measurements (*n* = 37; 21 males, 16 females) taken between 1987-89. No additional studies have been undertaken since then to further examine sexual-size dimorphism in black petrels. A quick and inexpensive method for sex differentiation could provide useful insights into population demographics and behaviour at the breeding grounds. A DFA for the closely related Westland petrel (*Procellaria westlandica*) correctly assigned sex for 95% of cases (Landers *et al.* 2011).

The aims of our study were to determine the extent of morphological differences between the sexes of black petrels, and to develop a canonical discriminant function that could be used in the field to accurately and quickly predict sex.

METHODS
Since 1996, annual monitoring of black petrels has been carried out in a 35 ha study area on Mount Hobson/Hirakimata, GBI (-36° 11′ 11.9″, 175° 25′ 7.3″). The status of marked burrows are checked 3 times a year – at early incubation, at hatching, and then shortly before chicks fledge. All birds in marked burrows are identified with metal leg bands.

Between November 2004 and February 2014, a total of 68 breeding birds were measured (34 males, 34 females). All birds were adults caught in their study burrows during the breeding season, between November to February, on GBI. The sex of each study bird was confirmed by: (i) DNA analyses (*n* = 38: 19 males, 19 females); (ii) presence of an egg in the abdomen (*n* = 4); (iii) necropsy (*n* = 2, both males); or (iv) long-term partners (8.7 ± 0.9 years; range 3-18 years) of birds whose sex was confirmed by molecular analyses of DNA (*n* = 24: 13 males, 11 females). For DNA samples, 2 to 3 feathers were pulled from the chest and placed in a plastic bag labeled with each bird's metal band number, burrow, and date. The feather samples were sent to the Equine Parentage and Animal Genetic Services Centre, Massey University, New Zealand for molecular analysis of the CHD gene of the avian sex chromosomes. Morphological measurements were taken with vernier calipers (± 0.1 mm), and included the following (see Fraser 2005): (1) head and bill, from supraoccipital to the front curve of the bill; (2) skull width, narrowest width of the head behind the eye sockets; (3) culmen, from base of feathers to front curve of the bill; (4) culmen depth at base, the depth at the base of the bill at the edge of the feathers; (5) culmen width at base, the width at the base of the bill at the edge of the feathers; (6) minimum bill depth, narrowest point of bill excluding nostrils; and (7) right tarsus, from middle of midtarsal joint to distal end of tarsometatarsus with foot towards tail. Not
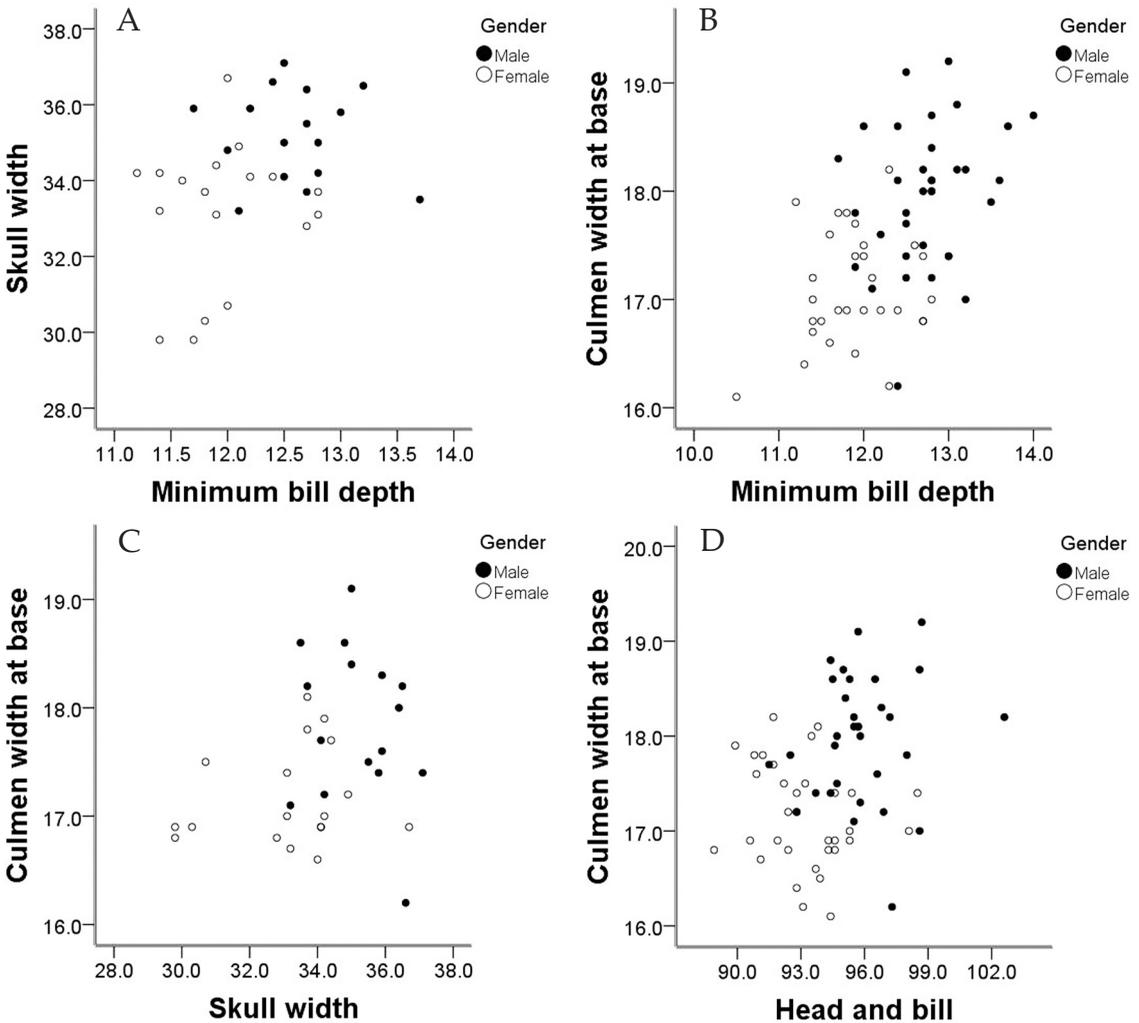
Fig. 2. Plots of measurement parameters used in 2 DFA models for predicting gender in black petrels. Model 1 used minimum bill depth, skull width, and culmen width at base of bill as parameters (a, b, c), and model 2 used head and bill and culmen width at base of bill as parameters (d). Skull width was excluded in model 2 due to small sample size. Males were significantly larger than females in these measurements ($P < 0.01$); however, a large overlap is seen.

all measurements were taken for all birds (Table 1). Each bird was measured prior to knowing its sex by one of the same 3 researchers. As all researchers used the same methods, it is assumed there was no significant observer bias in measurements.

A multivariate analysis of variance (MANOVA) was carried out to determine the overall differences between the sexes on morphometric characters, using SPSS v. 21 (IBM, Armonk, NY). Following this, an unpaired *t*-test was used to determine differences in each of the morphometric measurements between males and females. A forward stepwise DFA was used to determine how well sex could be differentiated by the morphometric characteristics. Cross-validation using a jackknife procedure, where each bird was classified using a function created from all birds except the one being classified, was performed to avoid reporting an exaggerated success of the DFA model (Bertellotti *et al.* 2002; Shealer & Cleary 2007). When a parameter for which few measurements were available (*e.g.*, skull width) was selected by the DFA procedure, another DFA was run, excluding that parameter.

RESULTS
Means, standard deviations, and ranges of all measurements taken are reported in Table 1. Sex was a significant determinant of morphometric differences (MANOVA; $F_{7,26} = 4.808$, $P < 0.01$). All

Table 2. Discriminant function models developed to predict gender of black petrels using morphological measurements. Model 2 was created excluding skull width as a variable. Discriminant variables: MBD = minimum bill depth, SW = skull width, CWB = culmen width at base of bill, HB = head and bill. Cutting score = mean of group centroids for males and females; D > cutting score = male, D ≤ cutting score = female.

Model	Discriminant Function [Cutting Score]	Percent correct classification		
		Male	Female	Total
1	0.551MBD + 0.6SW + 0.495CWB [0.0605]	87.5	88.9	88.2
2	0.703HB + 0.693CWB [-0.0545]	78.1	84.8	81.5

measures differed significantly between the sexes ($P < 0.05$) except for right tarsus ($P = 0.37$; Table 1).

A stepwise DFA (model 1) selected skull width, culmen width at base, and minimum bill depth as the most sexually dimorphic parameters (Table 2). The model was developed using a limited sample size, due to a low number of measurements of skull width ($n = 34$: 16 males, 18 females). There was a large overlap in the discriminant scores and corresponding probabilities of correct classification of the model (Fig. 1), as well as in the chosen parameters between males and females (Fig. 2). Nevertheless, the model correctly classified 14 of the 16 males, and 16 of the 18 females (Table 2) with an overall correct classification success of 88.2%.

To mitigate the problem of small sample size, another stepwise DFA was run, excluding skull width as a variable (model 2). This model selected head and bill and culmen width at base as the most discriminatory parameters (Table 2). This doubled the sample size for the model ($n = 65$: 32 males, 33 females), but reduced the classification success for both sexes. The large overlap in the selected morphometric parameters between males and females again was evident (Fig. 2). Following the jackknife procedure, 25 out of 32 males and 28 out of 33 females were classified correctly, with an overall success of 81.5%.

DISCUSSION

Males were significantly larger than females in all 7 measurements except for tarsus thereby confirming that sexual dimorphism is present in black petrels. The difference in size between males and females could be indicative of different foraging strategies and diets of the sexes, as seen in northern giant petrels (*Macronectes halli*) (González-Solís *et al.* 2000). Although some tracking studies have been done on black petrels, none have focused on differences in strategies between the sexes (Freeman *et al.* 2010). Reproductive behaviour could also have selective pressures on sexual dimorphism, where

males are larger than females to defend territories or dig burrows (Shine 1989). Similar results to our study were found for the closely related Westland petrel (*Procellaria westlandica*), where significant differences in head length, minimum bill depth, and body mass were revealed between the sexes (Landers *et al.* 2011).

Scofield (1989) reported that culmen length, culmen width, culmen depth, wing length, tarsus length, and weight all differed significantly between male and female black petrels. The DFA developed by Scofield (1989) did not employ a stepwise elimination procedure, and therefore all measurements were used in the model. However, it showed an 84.3% success rate, which is similar to the jackknife output in both our model 1 and 2.

The small sample size used for building model 1 likely had a large effect on classification success, thereby making it potentially inaccurate. A study on black terns (*Chlidonias niger*) found that functions generated from approximately 5% of the population were highly unreliable, and that a sample of 10% of the population was required to produce a satisfactory model (Shealer & Cleary 2007). These proportions are not directly applicable to the black petrel population; however, results of the Shealer & Cleary study suggest that sample size plays an important role in DFAs. Dechaume-Moncharmont *et al*. (2011) advised that caution should be exercised when using models constructed from small sample sizes ($n < 60$), as classification success could be high simply due to chance. There also is a strong interaction between sample size and number of variables – too many variables and too few individuals commonly leads to poor classification ability, referred to as 'overfitting' (Dechaume-Moncharmont *et al*. 2011).

Overall, the DFA models we describe in this paper do not appear to be sufficiently accurate on their own to reliably distinguish between sex in the field. This could be a result of too large of an overlap in morphometric measurements between males and females. Perhaps accuracy would increase

if DFA models were built with larger and more representative sample sizes than what was used in this study, particularly for skull width. Models also may improve if additional measurements, such as wing length, were added.

LITERATURE CITED
Agreement on the Conservation of Albatrosses and Petrels (ACAP). 2009. ACAP species assessment: black petrel *Procellaria parkinsoni*. Downloaded from www.acap.aq on 22 July 2014.

Bertellotti, M.; Tella, J.L.; Godoy, J.A.; Blanco, G.; Forero, M.G.; Donazar, J.A.; Ceballos, O. 2002. Determining sex of magellanic penguins using molecular procedures and discriminant functions. *Waterbirds* 25: 479-484.

BirdLife International. 2012. *Procellaria parkinsoni*. The IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Downloaded on 22 July 2014.

Boersma, P.D.; Davies, E.M. 1987. Sexing monomorphic birds by vent measurements. *Auk* 104: 779-783.

Bourgeois, K.; Curé, C.; Legrand, J.; Gómez-Díaz, E.; Vidal, E.; Aubin, T.; Mathevon, N. 2007. Morphological versus acoustic analysis: what is the most efficient method for sexing yelkouan shearwaters *Puffinus yelkouan*? *Journal of Ornithology* 148: 261-269.

Bretagnolle, V. 1996. Acoustic communication in a group of nonpasserine birds, the petrels. *Ecology and evolution of acoustic communication in birds.* Ithaca: Cornell University Press.

Bugoni, L.; Furness, R.W. 2009. Age composition and sexual size dimorphism of albatrosses and petrels off Brazil. *Marine Ornithology* 37: 253-260.

Cure, C.; Aubin, T.; Mathevon, N. 2009. Acoustic convergence and divergence in two sympatric burrowing nocturnal seabirds. *Biological Journal of the Linnean Society* 96: 115-134.

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

Einoder, L.D.; Page, B.; Goldsworthy, S.D. 2008. Sexual size dimorphism and assortive mating in the short-tailed shearwater *Puffinus tenuirostris*. *Marine Ornithology* 36: 167-173.

Fraser, M.J. 2005. Characteristics of white-chinned petrels *Procellaria aequinoctialis* Linnaeus in New Zealand waters. Unpubl. MSc thesis, Massey University, Palmerston North, New Zealand.

Freeman, R.; Dennis, T.; Landers, T.; Thompson, D.; Bell, E.; Walker, M.; Guilford, T. 2010. Black petrels (*Procellaria parkinsoni*) patrol the ocean shelf-break: GPS tracking of a vulnerable Procellariiform seabird. *PloS ONE 5*: e9236.

Genevois, F.; Bretagnolle, V. 1995. Sexual dimorphism of voice and morphology in the thin-billed prion (*Pachyptila belcheri*). *Notornis* 42: 1-10.

González-Solís, J.; Croxall, J.P.; Wood, A.G. 2000. Sexual dimorphism and sexual segregation in foraging strategies of northern giant petrels, *Macronectes halli*, during incubation. *Oikos* 90: 390-398.

Guicking, D.; Fiedler, W.; Leuther, C.; Schlatter, R.; Becker, P.H. 2004. Morphometrics of the pink-footed shearwater (*Puffinus creatopus*): influence of sex and breeding site. *Journal of Ornithology* 145: 64-68.

Imber, M.J. 1987. Breeding ecology and conservation of the black petrel (*Procellaria parkinsoni*). *Notornis 34*: 19-39.

Landers, T.J.; Dennis, T.E.; Hauber, M.E. 2011. Gender assignment of Westland petrels (*Procellaria westlandica*) using linear discriminant function analysis. *Wilson Journal of Ornithology* 123: 720-725.

Liordos, V.; Goutner, V. 2008. Sex determination of great cormorants (*Phalacrocorax carbo sinensis*) using morphometric measurements. *Waterbirds* 31: 203-210.

Mallory, M.L.; Forbes, M.R. 2005. Sex discrimination and measurement bias in northern fulmars *Fulmarus glacialis* from the Canadian Arctic. *Ardea* 93: 25-36.

Marchant, S.; Higgins, P.J. 1990. *Handbook of Australian, New Zealand & Antarctic birds*. Melbourne: Oxford University Press.

McGarigal, K.; Cushman, S.; Stafford, S. 2000. Multivariate *statistics for wildlife and ecology research*. New York: Springer.

O'Dwyer, T.W.; Priddel, D.; Carlile, N.; Bartle, J.A.; Buttemer, W.A. 2006. An evaluation of three field techniques for sexing Gould's petrels (*Pterodroma leucoptera*) (Procellariidae). *Emu* 106: 245-252.

Robertson, H.A.; Dowding, J.E.; Elliott, G.P.; Hitchmough, R.A.; Miskelly, C.M.; O'Donnell, C.F.J.; Powlesland, R.G.; Sagar, P.M.; Scofield, R.P.; Taylor, G.A. 2012. Conservation status of New Zealand birds, 2012. *New Zealand Threat Classification Series 4*: 1-22.

Scofield, R.P. 1989. Breeding biology and conservation of the black petrel (*Procellaria parkinsoni*) on Great Barrier Island. Unpubl. MSc thesis, University of Auckland, Auckland, New Zealand.

Shealer, D.A.; Cleary, C.M. 2007. Sex determination of adult black terns by DNA and morphometrics: tests of sample size, temporal stability and geographic specificity in the classification accuracy of discriminant function models. *Waterbirds* 30: 180-188.

Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Quarterly Review of Biology* 64: 419-461.

Spear, L.B.; Ainley, D.G.; Webb, S.W. 2005. Distribution, abundance, habitat use and behaviour of three *Procellaria* petrels off South America. *Notornis* 52: 88-105.

Warham, J. 1988. Vocalisations of *Procellaria* petrels. *Notornis* 35: 169-183.

Weidinger, K.; van Franeker, J.A. 1998. Applicability of external measurements to sexing of the cape petrel (*Daption capensi*) at within-pair, within-population and between-population scales. *Journal of Zoology, London* 245: 473-482.