Sexual size dimorphism in the extreme SW breeding population of the European Storm Petrel *Hydrobates pelagicus* (Aves: Procellariiformes)

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Abstract

We used molecular sexing and morphological analysis to characterise sexual size dimorphism (SSD) in the European Storm Petrel *Hydrobates pelagicus* (LINNAEUS, 1758) in the southernmost and westernmost breeding colony (El Hierro, Canary Islands). We analysed SSD in 79 individuals (46 males, 33 females). We performed discriminant analysis on 9 morphometric variables and correctly classified 70.5% of the original cases. By binary logistic regression, the accuracy in sexing birds previously sexed by DNA analysis was 70.5%. Females were larger than males in wing and tarsus length but there were no differences for bill size and shape. There was a large biometric overlap between sexes in this Canarian breeding colony of *H. pelagicus*. However, a moderate female-biased dimorphism in flight-relevant variables such as tail and wing traits could be of interest in trophic segregation of sexes and optimization of resource use in these pelagic-feeding birds.

Key words

Introduction

Sexual size dimorphism (SSD) is a widespread feature in many bird species (ANDERSON 1994, LEZANA *et al*. 2000, CAMPOS *et al*. 2005). In most bird taxa, males are of the same size, slightly larger, or significantly larger than females (PRICE 1984) and it is usually more pronounced in aggressive and predatory species (THIOLLAY 1994). In raptors, females are larger and in extreme cases may have twice the weight of the males (THIOLLAY 1994). By contrast, in some terrestrial, cursorial birds such as bustards, females are much lighter and smaller (COLLAR 1996). The evolution of SSD has probably occurred through sexual selection together with promiscuous mating (LACK 1971), reflecting differences in sex-specific reproductive roles and responses to environmental factors (BLONDEL *et al*. 2002, GONZÁLEZ-SOLÍS 2004), feeding behaviour (DE JUANA *et al*. 2004), trophic niche segregation of sexes and sexual selection (GUTIÉRREZ-CORCHERO *et al*. 2007, NAVARRO *et al*. 2009).

Pelagic seabirds exhibit SSD. Among the Procellariidae, females are generally slightly smaller and more lightly built than males (RISTOW & WINK 1980, CARBONERAS 1992, GONZÁLEZ-SOLIS *et al*. 2000). However, Storm pet-

rels (Hydrobatidae) show female-biased SSD (Warham 1990), along with Sulidae and Fregatidae, whereas Phaetontidae show no apparent SSD (Serrano-Meneses & Székely 2006).

Several methods have been used to sex bird species which are monomorphic in plumage. Molecular sexing consists of a non-intrusive technique and the most popular, requiring only a small amount of DNA from blood or a single feather (Taberlet & Bouvet 1991, Ristow & Wink 2004, Sutherland *et al*. 2004, Infante & Peris 2004). For many species of birds including Procellariiformes, sexing has been achieved by discriminant analysis using morphological measurements (Ristow & Wink 1980, Coulter 1986, Lo Valvo 2001, Butler & Gosler 2004, Alarcos *et al*. 2007).

The European Storm Petrel *Hydrobates pelagicus* (Linnaeus, 1758) is a small pelagic seabird species distributed in the NE Atlantic from S Iceland to NW France, N Spain, Salvages and Canary Islands; it is also present in the Mediterranean from N Spain to Greece (Del Hoyo *et al*. 1992). *Hydrobates pelagicus* has been considered a monotypic species although some authors suggest that the Mediterranean population must be re-established as a valid subspecies (*H. p. melitensis* Schembri, 1843). This distinction has been supported not only by biometrics (Hemery & d'Elbee 1985, Massa & Catalisano 1986, Catalisano *et al*. 1988, Lalanne *et al*. 2001) but also by genetics (Cagnon *et al*. 2004). The Canary Islands correspond to the southern limit of the European Storm Petrel distribution range and represent the only known nesting place among the Macaronesian archipelagos. On the Canary Islands, a minimum of 1,000 breeding pairs of *H. pelagicus* have been estimated occupying small islets and rocks such as Alegranza (N of Lanzarote) and Roque Grande de Salmor, a rocky islet off El Hierro (Nogales *et al*. 1993). Like other pelagic seabird species, *H. pelagicus* is a colonial breeder nesting in caves or small crevices, under heaps of boulders or under collapses of coastal cliffs (Delgado *et al*. 1985, Nogales *et al*. 1993).

Geographical variation in morphology and ethology has great importance in species-specific recognition (Bretagnolle 1989). Variation underlying speciation processes is methodologically difficult to delimit in these marine birds (Bolton & Thomas 2001, Cagnon *et al*. 2004 and references there in). Morphological differences have been found for *H. pelagicus* even within the Mediterranean Basin (Lalanne *et al*. 2001), where there is, however, low genetic variation. Genetic differentiation is higher among the Atlantic populations. In addition, there is strong evidence of a lack of genetic exchange between Mediterranean and Atlantic populations.

Among the Atlantic Ocean populations, movements of Canarian *H. pelagicus* are poorly known. There have been records of birds reaching South Africa, and the islands would receive European migrants (Martín & Lorenzo 2001). Despite the wide distribution of *H. pelagicus*, the large population size of this species (130,000−290,000 pairs after Carboneras 1992; 130,000−400,000 pairs after Franco *et al*. 2004), and the morphological variation throughout the distribution range, biometric data characterizing live specimens are scarce. There is to our knowledge no available biometric work from the breeding colonies of the Canary Islands. Moreover, because of *post mortem* shrinkage of museum skins (Kuczynski *et al*. 2003) our data from live birds become especially relevant. Here we assess SSD through molecular sexing and biometry, and analysed external morphological variation between sexes of the European Storm Petrel from its southern- and westernmost known breeding colony.

Study site

During the summer of 2007, field work was performed in Roque Grande de Salmor, a rocky islet of 0.03 km^2 (NW El Hierro island; 27° 49′ N 17° 59′ W, Canary Islands; Fig. 1). This is one of the largest colonies in the Canaries and the largest of El Hierro, with up to 200−300 pairs (Nogales *et al*. 1993, Martín & Lorenzo 2001).

Methods

Biometrics

A total of 79 individuals of *H. pelagicus* (46 males and 33 females) were caught by mist nets. Birds were ringed with conventional metal rings and no tape lures were used. All the biometric measurements were taken by G. Delgado Castro.

Wing length (maximum chord) was taken using a stopped rule (Svensson 1992) to 1 mm. Tail length was measured using an unstopped rule to the nearest millimetre. Following Hemery & d'Elbee (1985), five morphological variables of bill were measured to 0.1 mm using digital callipers: bill length (along the dorsal mid-line from the edge of the feathers at the base of the culmen to the most distant part of the hook), bill depth 1 (BD1, mandibles + narines), bill depth 2 (BD2, mandibles at level of the opening of narines), narine width (BW1), bill width (BW2, mandibles at level of the opening of narines) and "maximum" tarsus length (strictly, tarsometatarsus length; Sutherland *et al.* 2004). Body mass was estimated using a 50 g Pesola balance to 0.1 g.

Molecular sexing methods

Blood samples were collected by venipuncture of the brachial vein. Total DNA was isolated from 100 µl of
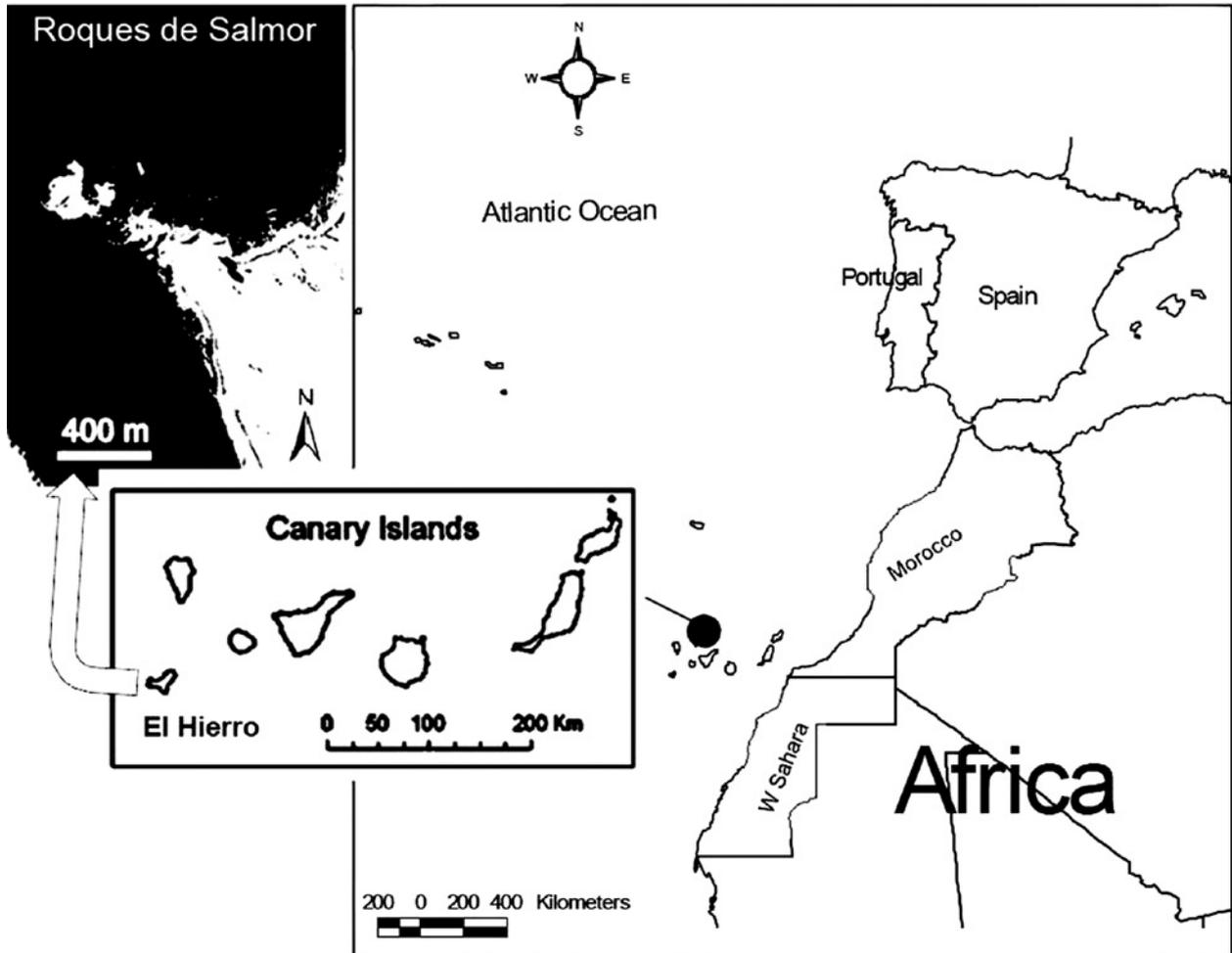
Fig 1. Study area. Location of the breeding colony of *Hydrobates pelagicus* studied in El Hierro, Canary Islands.

blood using standard Proteinase K (Merck, Darmstadt) and phenol/chloroform procedures (SAMBROOK *et al.* 1989). Sex identification was conducted following KAHN *et al.* (1998). PCR was performed with 30–60 ng of template DNA in 25 μl reaction volume containing 8 pmol of the primer H1272 and 9 pmol of the primer L1237, 0.1 mM of dGTP, dCTP, and dTTP, 0.045 mM dATP, 1 μCi [α-33P]-dATP (AmershamBiosciences), 0.6 units of *Taq*-Polymerase (Pharmacia Biotech, Freiburg) and 2.5 μl of 10× amplification buffer [10 mM Tris-Hcl (pH 8.5), 50 mM KCl and 1.5 mM MgCl$_2$]. Each reaction was overlaid with two drops of mineral oil. Thermo cycling was performed with a Trio Thermo block TB1 (Biometra, Göttingen). Following the initial 5 min denaturation at 94°C, the program consisted of 31 cycles of 30 s at 94°C, 40 s at 56°C, 40 s at 72°C and 5 min at 72°C for final elongation. DNA fragments were separated by vertical PAGE (Polyacrylamide Gel Electrophoresis; length 40 cm) for 2 h at 65 W using a Base Acer Sequencer (Stratagene). After drying, the denaturing gels were exposed for 24 h to X-ray films (BioMax MR Film, Kodak). The bands were analyzed visually. The presence of two bands was scored as female and one band as male (KAHN *et al.* 1998).

Data analysis

A Kolmogorov-Smirnoff test was used to check for normality of variables. We used a Student t-test to analyse biometric differences between the sexes. For non-normal variables (wing length, tail length and mass) we used a non-parametric Mann-Whitney test. A Principal Component Analysis (PCA) was carried out in order to find a non-redundant pool of explanatory variables for biometrics of *Hydrobates pelagicus*. We applied the varimax rotation to achieve a better interpretation of the data pool. Discriminant analysis (SOKAL & ROLF 1995) was used to generate a function which is a linear combination of the morphometric variables measured from individuals of known sex, and to maximize the variance between groups (i.e. sexes). The method applied for calculating the discriminant function was Wilk's Lambda, and the probability for including a predictor variable in the model was set to 0.05 (0.1 for exclusion). Finally, a binary logistic regression function was calculated. All statistical analyses were performed using SPSS 12.0.

Table 1. Population biometrics. Biometrics for the European Storm Petrel at Roque Grande de Salmor (NE El Hierro). Data are: means ± standard deviation (range), sample size: n. All data in mm except mass (g).

	Wing length	Bill length	Bill depth 1	Bill depth 2	Bill width 1	Bill width 2	Tail length	Tarsus length	Mass
Females	118.9 ± 2.17 (113 – 123) n = 33	11.43 ± 0.54 (10.23 – 12.6) n = 33	5.16 ± 0.24 (4.76 – 5.73) n = 33	3.70 ± 0.18 (3.32 – 4.16) n = 33	2.51 ± 0.18 (2.06 – 2.78) n = 33	2.25 ± 0.19 (1.78 – 2.63) n = 33	55.86 ± 1.92 (53.5 – 61.5) n = 33	24.19 ± 0.76 (22.1 – 25.6) n = 33	24.3 ± 2.74 (20 – 30.5) n = 33
Males	116.7 ± 2.16 (110 – 120) n = 46	11.26 ± 0.56 (9.58 – 12.46) n = 46	5.15 ± 0.27 (4.57 – 5.78) n=46	3.76 ± 0.18 (3.39 – 4.2) n=46	2.41 ± 0.22 (2.0 – 2.97) n=46	2.26 ± 0.21 (1.96 – 2.98) n = 46	54.41 ± 2.20 (49.5 – 58.5) n = 45	24.09 ± 0.81 (22.0 – 25.8) n = 46	23.1 ± 1.87 (20.0 – 28.5) n = 46
All	117.6 ± 2.77 (110 – 123) n = 79	11.33 ± 0.56 (9.58 – 12.60) n = 79	5.16 ± 0.26 (4.57 – 5.78) n = 79	3.74 ± 0.19 (3.32 – 4.2) n = 79	2.47 ± 0.22 (2.0 – 2.97) n = 79	2.26 ± 0.21 (1.78 – 2.98) n = 79	55.03 ± 2.20 (49.5 – 61.5) n = 78	24.13 ± 0.8 (22.0 – 25.8) n = 79	23.6 ± 2.35 (20.0 – 30.5) n = 79

Table 2. Biometrics for live European Storm Petrels from different breeding colonies including Roque Grande de Salmor (Canary Islands). **a** = James (1983); **b** = Hemery & d' Elbee (1985); **c** = Lalanne *et al.* (2001); **d** = present study. All data in mm except mass (g). **x** = mean; **s.d.** = standard deviation. Sample size: n. See Methods: Biometrics for explanation of codes.

	Great Britain (a)						Atlantic (b)			Biarritz (c)			Corse (c)			Salmor (d)		
	♂♂			♀♀			All sexes			All sexes			All sexes			All sexes		
	x	s.d.	n	x	s.d.	n	x	s.d.	n	x	s.d.	n	x	s.d.	n	x	s.d.	n
Wing	120.4	2.5	26	123.7	2.7	20	122.3		19	122.5	2.82	19	123.58	2.98		117.6	2.41	79
BL	12.0	0.4	26	12.1	0.7	20							11.99	0.71		11.33	0.56	79
BD1							5.28		19	5.29	0.29	19	5.56	0.22		5.16	0.26	79
BD2							3.86		19	3.87	0.42	19	4.07	0.17		3.74	0.19	79
BW							3.03		19							2.47	0.22	79
BW2							2.15		19							2.26	0.21	79
Tarsus	22.6	0.8	26	22.9	0.7	20										24.13	0.80	79
Tail	54.2	1.6	26	56.5	1.8	20	53.28		19							55.03	2.2	78
Mass							28.39		19	28.32	1.70	19	28.94	2.97		23.62	2.35	79

Results

Biometrics

The placement of males and females in the ordination space showed no clear separation of sexes (Fig. 2). Sex ratio in adults differed significantly from unity ($\chi^2_1 = 9.0$, P<0.01). Mean values for confirmed males and females are presented in Table 1. Between the sexes, significant differences were found for wing length (U = 340.5, P<0.001) and tail length (U = 478.5, P=0.007). No significant differences were observed for the other biometric measurements (i.e. bill length: t = –1.44, df = 77, P=0.166; BD1: t=0.055, df=77, P=0.956; BD2 t=1.224, df=77, P=0.225; BW1: t=–1.650, df=77, P=0.103; BW2: t=0.118, df=77, P=0.986; tarsus length: t=–0.661, df=77, P=0.511; mass: U=563.0, P=0.05). Female wing was, on average, 2.2 mm longer than that of males, and females also showed longer tails, 1.45 mm on average, than did males. Further information is summarized in Table 2 where data of James (1983), Hemery & d' Elbee (1985), and Lalanne *et al.* (2001) from other Atlantic and Mediterranean (Corsican) colonies are included.

The PCA analysis shows that a set of three factors accounted for 48.82% of the total variation (Fig. 2; Table 3). The first factor (PC1) was related to bill width 1 (BW1), bill length and tarsus. The second factor (PC2) involved the wing length, tail length and weight, and the third (PC3) was related to bill depth 1 (BD1) and bill depth 2 (BD2). The placement of males and females in the ordination space showed no clear separation of sexes (Fig. 2).

Sexing

The discriminant linear model based on the original combination of 9 morphometric variables was highly significant (Wilk's Lambda = 0.752; F = 12.361, P<0.001) and included wing length and tail length as the best discriminators (Tables 4 and 5). The discriminant analysis correctly classified 70.5% of the original cases.

A binary logistic regression function was calculated to predict sex from a full set of variables (n = 79 individu-

Table 3. PCA on biometric variables. Results of the Principal Component Analysis (PCA) on biometric measurements of *Hydrobates pelagicus* in Roque de Salmor, El Hierro (Canary Islands). Minor scores for each component and each variable have not been included. See Methods: Biometrics for explanation of codes.

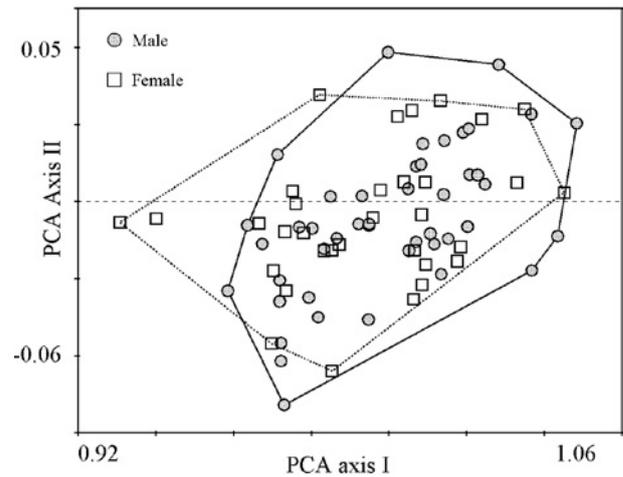|	PC1	PC2	PC3
BW1	0.675		
Bill length	0.610		
Tarsus	0.575		
Winglength		0.722	
Tail		0.680	
BW2		−0.502	
Weight		0.410	
BD1			0.735
BD2			0.730
Eigenvalues	1.744	1.392	1.259
% variance	19.382	15.462	13.985
Cumulative % variance	19.382	34.844	48.829

Fig. 2. PCA biometrics diagram. Biplot of a PCA run on the measured set of biometrical variables, with samples (individuals) separated by sex.

Table 5. Correlations between variables and functions. Correlations between the discriminant variables and the canonical discriminant function.* Variables not included in the analysis; correlations of higher order are shown in bold.

Variable	Correlation coefficient
Wing length	0.879
Tail length	−0.606
Tarsus	0.134
Weight	0.132
An1*	0.122
An2*	−0.105
LTP*	−0.063
AL1*	−0.042
Al2*	0.021

Table 4. Discriminant-functions coefficients. Coefficients of the discriminant functions for both sexes and of the final canonical discriminant functions for the model (CDF).

	Sex		CDF
	Male	Female	
Wing length	34.61	35.04	0.37
Tail length	661.21	658.55	−2.31
Constant	−10751.40	−10732.02	17.26

als). Backwards deletion of the variables was used so that only those elements that were significant ($P < 0.05$) were retained. This generated a significant binary logistic regression function (Chi-square$= 23.27$, df$= 2$, Nagelkerke $R^2 = 0.347$, $P < 0.001$), including only wing and tail length as significant parameters:

X = 0.49 (wing length) – 2.87 (tail length) + 18.07.

Discussion

We found that sexing *H. pelagicus* from the Canary Islands by using simple morphometric measurements renders a low degree of accuracy. There is a high degree of overlap between sexes in evolutionarily relevant biometrics such as bill size and shape. Also, as in the few studies published, we found a moderate female-biased sexual size dimorphism. Our results suggest the existence of large geographical variation in biometric parameters of *H. pelagicus*. Mean wing length of individuals caught at different North Atlantic breeding colonies have been reported to range between 116.7 and 123.7 mm (Table 2). Data regarding weight show similar trends (FURNESS & BAILLIE 1981, and references therein). Nevertheless, FURNESS & BAILLIE (1981) showed that storm petrel breeding on St. Kilda (Scotland) presented longer wings than wanderer birds, an observation which is also supported by FOWLER *et al.* (1986) at Yell (Shetland), where statistical differences were found between breeding and non-breeding birds. Although a proportion of birds at Roque Grande de Salmor may be non-breeding birds (at least 70% of the birds caught regurgitated food on nets), these were clearly lighter (Table 1). The Mediterranean birds are the larger ones with a wing length range of 129.25 – 123.58 mm. The tail length is quite similar between Atlantic and Mediterranean populations (Table 2).

Accuracies in sex assignation vary widely in the literature (Appendix 1). In studies using discriminant analysis for larger species, similar degrees of accuracy have been either enough (i.e. Tundra Swan, 74.1%; MILLER *et al.* 1988) or inadequate (i.e. Tengmalm's Owl, 70%; HIPKISS 2007) depending on season, bird development stage, age and the methods, among other factors (see Appendix 1).

Our values are also smaller than those above 92% found in *Larus argentatus* (Fox *et al.* 1981) and *Puffinus tenuirostris* (Einoder *et al*. 2008). Lo Valvo (2001) found that bill depth and body mass were the best discriminants (92 and 84% of correctness) for *Calonectris diomedea* in Sicily. The values presented by our *Hydrobates* population were lower than those of James (1983), where a discriminant function based on the same variables accounted for 88.5% and 80.0% for males and females, respectively. We achieved the best separation between sexes by using the wing and tail discriminant functions.

Our results on SSD in the European Storm Petrel support findings of previous works with biometrics from live birds (James 1983). Tail and wing were larger in females than in males in this El Hierro breeding colony. Other biometric variables showed no statistically significant differences. For example, bill size and shape did not differ between sexes in this population of *H. pelagicus*. Bill size and shape is one of the most relevant sexually dimorphic variables with repercussions in foraging, sexual selection and speciation in seabirds (e.g. Navarro *et al.* 2009).In this population, however, *H. pelagicus* showed a slight departure from monomorphy (the degree to which sexes are of equal size) in tail and wing size, with probable effects in resource partition between sexes and intersexual competition (Figuerola 1999). In *H. pelagicus*, the variables involved in dimorphism are directly related to flight performance, which might be relevant for foraging optimization in these pelagic-feeding birds. Furthermore, the observed intersexual variation may have repercussions in the optimization of intraspecific resource use (Navarro *et al*. 2009). The influence of low SSD in aspects of evolutionary biology and conservation of *H. pelagicus* such as assortative mating and mating success, phylopatry, foraging strategies, namely differential niche exploitation, especially when sea productivity fluctuates due to drastic environmental change, or sexual selection, deserves further research.

Acknowledgements

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APPENDIX

Examples of sex determination in birds and degrees of accuracy by Determinant Function Analysis (DFA) based on various measurements.

Bird species	% classification accuracy	Reference	Measures
Aegolius funereus	70	Hipkiss 2007	Wing length
Buteo jamaicensis calurus (*adults*)	98	Donohue & Dufty 2006	Wing chord and body mass
Buteo jamaicensis calurus (*yearlings*)	97	Donohue & Dufty 2006	Body mass, wing chord, hallux and culmen
Cyanopica cyanus cooki (*adults*)	90	Alarcos et al. 2007	Tail length, head plus bill length and wing length
Cyanopica cyanus cooki (*yearlings*)	90	Alarcos et al. 2007	Tail length and head plus bill length
Cygnus columbianus (*adult*)	74.1–91.3	Miller et al. 1988	Several inernal and external traits
Cygnus columbianus (*immature*)	27.3–52.4	Miller et al. 1988	Several inernal and external traits
Eudyptes chrysocome	93.2	Hull 1996	Bill depth and bill length
Eudyptes schlegeli	97.1	Hull 1996	Bill depth and bill length
Fulmarus glacialis	88–90	Mallory & Forbes 2005	Head, bill and tarsus
Hirundo rustica	90.1–91.9	Hermosell et al. 2007	Length of outermost tail feathers, inner tail feathers and keel
Phalacrocorax carbo	92.6–95.1	Liordos & Gouttner 2008	Wing length, culmen length and tarsus length
Puffinus yelkouan	87.2	Bourgeois et al. 2007	Bill depth at gonys, nalospi and tarsus height
Puffinus yelkouan	100	Bourgeois et al. 2007	Four acoustic parameters