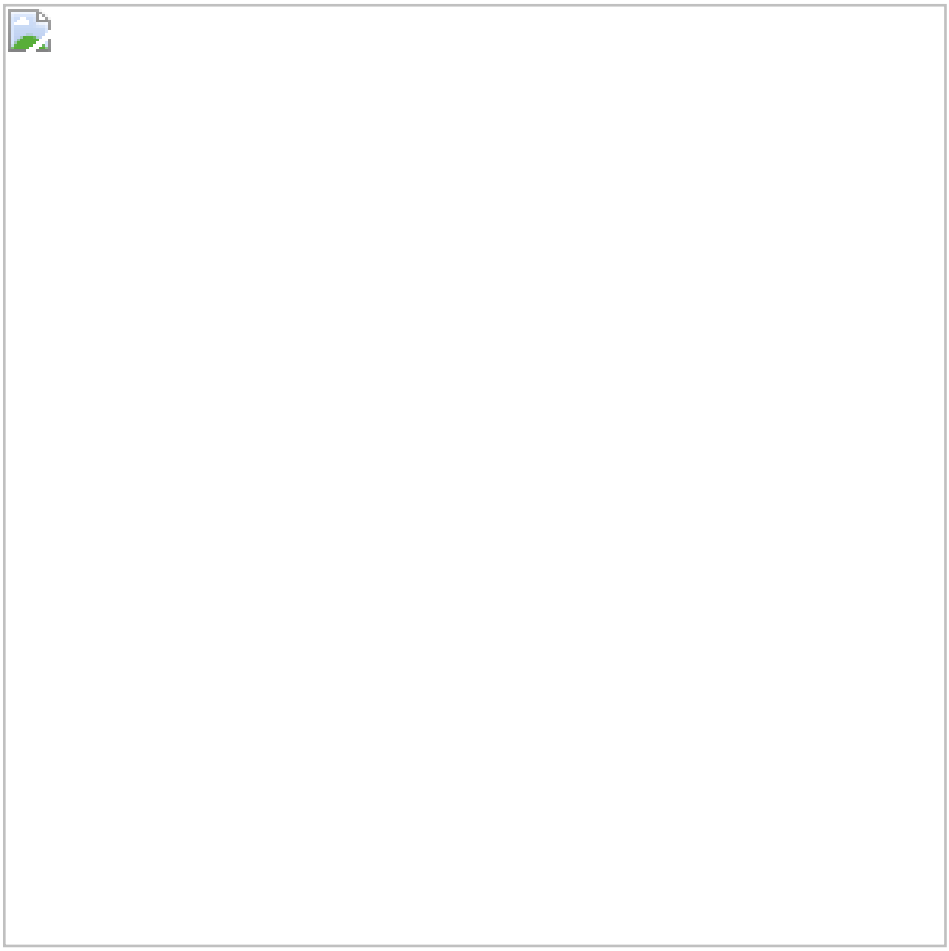

Sexual Dimorphism and use of Morphological Measurements to Sex Adults, Immatures and Chicks of Rockhopper Penguins

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Sexual dimorphism and use of morphological measurements to sex adults, immatures and chicks of Rockhopper Penguins

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Following recent phylogenetic work, Rockhopper Penguins were suggested to consist of two or three species. For the Southern Rockhopper Penguin *Eudyptes c. chrysocome*, sexual dimorphism has not been studied in detail, and only a few previous studies on penguins have investigated sexual dimorphism in immatures and chicks. Using data in the literature, we examined whether the sexual dimorphism of adults varies among the three taxa of Rockhopper Penguins and then we investigated the most reliable measurements to sex adult Rockhopper Penguins. We observed that bill length is the most useful measurement to separate males from females. To allow for sex discrimination in the field, we also examined a large dataset of Southern Rockhopper Penguins from New Island, Falkland Islands, including adults sexed via observation of behaviour, and immatures and chicks sexed genetically. We found that male adults and immatures were larger than females in bill length and bill depth and, to a lesser degree, in flipper lengths. We thus derived discriminant functions from bill length and bill depth and correctly sexed 96.2% of adults and 91.8% of immatures. In newly hatched chicks, males had a longer bill than females, but sexing was only successful for 63.5% of hatchlings. Just before the crèche age (18 and 19 days) and after the pre-fledging moult (55 days and older), all morphological measurements of chicks were significantly different between sexes, and sex determination was successful for 68.2% and 84.3% of chicks in these age groups, respectively. Consistently among age groups, bill length was the most dimorphic character in this population.

Key words: Rockhopper Penguin, sex determination, sexual dimorphism, discriminant function analysis, growth curve, age class

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Penguin species exhibit monomorphic plumage, with males and females looking similar in appearance. Nevertheless, all species exhibit some subtle sexual dimorphism, with males generally being larger and heavier than females (Agnew & Kerry 1995). These small morphological differences can be used to determine the sex of penguins for demographic, behavioural or other studies. However, the extent of this dimorphism may vary between species and subspecies (Warham 1972, Agnew & Kerry 1995, Arnould *et al.* 2004), and also between age categories within the same species (Scolaro 1987, Setiawan et al. 2004). Due to this natural variation, it may be necessary to use an independent reference dataset per species, per subspecies and per age class to obtain the best accuracy in sex determination.

Numerous studies have been interested in the use of morphological measurements for the determination of sex in adult penguins (see for examples Ainley & Emison 1972, Scolaro *et al.* 1983, Gales 1988, Murie *et al.* 1991, Kerry *et al.* 1992, Amat *et al.* 1993, Agnew & Kerry 1995, Setiawan *et al.* 2004) but very few studies have concerned immatures and penguin chicks (Scolaro 1987, Bertellotti *et al.* 2002, Hocken & Russell 2002, Setiawan *et al.* 2004). To our knowledge only

two studies have made reference to Rockhopper Penguin *Eudyptes chrysocome* (Warham 1972, Hull 1996), but only one (Hull 1996) proposed a formula to sex the adults and none addressed the question for the sub-species *chrysocome*. Yet, the measurements of only ten specimens of each sex showed the existence of a sexual dimorphism in morphological measurements for this taxon as well (Strange 1982). Moreover, there are now strong indications that rockhopper species should even be divided into two or three different species (Banks *et al.* 2006, Jouventin *et al.* 2006, de Dinechin *et al.* 2009). Indeed, in 2007, BirdLife International formally separated the Northern Rockhopper Penguin *E. moseleyi* from the Southern Rockhopper Penguin *E. chrysocome* including the sub-species *chrysocome* and *filholi*. Applying the formula given by Hull (1996) for *E. c. filholi* to a population of *E. c. chrysocome* from the Falkland Islands, we correctly sexed 100% of the 149 females and only 74.3% of the 144 males already sexed by behavioural observations (see Methods). This contrasted result could be explained (1) by a difference of morphology between populations, (2) by a difference of sexual dimorphism between populations or (3) by a difference in the measurement methods between individual scientists. Indeed, comparative studies with data derived by different individual scientists should be conducted with caution because of subtle differences in the interpretation of the measurement techniques (Hull 1996). Consequently, it is not possible to test for the first and third hypotheses from data provided in the literature. However, the sexual dimorphism being a ratio between male and female parameters, the individual scientist effect should be avoided and the second hypothesis should be tested.

The aims of this paper are to determine whether the sexual dimorphism of adults varies among the three taxa of Rockhopper Penguins. We determine the most reliable measurements to provide a simple method to identify the sex of adults, immatures and chicks of Southern Rockhopper Penguins using morphological measurements directly in the field without performing long behavioural observations or using invasive methods.

METHODS

Birds and study site

The study was carried out at the 'Settlement colony' on New Island, Falkland Islands (51°43'S, 61°17'W) during two consecutive breeding seasons (from early October to late February in 2006/07 and 2007/08). This colony has approximately 5000 pairs of breeding Southern Rockhopper Penguins. Rockhopper Penguins lay two eggs, the second egg (B-egg) is larger and hatches faster than the first egg (A-egg). The chick-rearing period is divided into discrete stages. After hatching, males guard the chicks while females forage at sea returning to feed the chicks almost every day. After three weeks, the chicks are left in crèches while both parents perform provisioning trips to feed them. During this time, chicks moult before their departure to sea (Williams 1995). In the study population, we observed that males left chicks 20–26 days after hatching and that moult started at 39–40 days of age to finish at 56–57 days of age (Strange 1982 and unpubl. data).

During the two breeding seasons, we captured 293 different adults and 85 different immatures. The first time we captured an adult, we implanted 23-mm glass-encapsulated electronic transponders (TIRIS, Texas Instruments, USA) and marked the birds with coloured and numbered Tesa adhesive tape around the base of the flippers. Immatures were easily identified by their short crests and their light-coloured throat. Although their exact age could not be assessed, they were approximately one or two years old (Williams 1995). As the capture of immatures took place over a period of 20 days in 2006/07 and 3 days in 2007/08, we temporarily marked them with a colour wax for animal marking to avoid catching the same bird twice during the capture period. Chick measurements came from monitoring of the marked chicks which were followed from hatching to fledging (see Poisbleau *et al.* 2008 for more details). We followed 75 individual chicks in 2006/07 and 142 individual chicks in 2007/08.

Measurements

After covering the bird's head to minimize stress, we measured bill length (exposed culmen) and bill depth (at junction of gonys and inter-ramal region) to the nearest 0.1 mm using callipers (following Warham 1972). We measured flipper length (extended from axilla) to the nearest mm with a ruler. As the body mass changes dramatically during the breeding period, we did not include it in the analyses (Williams 1995). The same observer (LD) carried out all measurements to minimize observer biases. When an adult was captured again during the same or the following breeding season, we measured it once more. As the average difference between two measurements was 1.4% (SD 1.3), we used the average for each measurement in the analyses. We did not record the bill depth for chicks when the shape of their bill was not regular, i.e. especially when they were very young.

Sex determination

We determined the sex of adults using the incubation and attendance pattern. The incubation is divided into three separate shifts, the first one is shared, the second one is only undertaken by the female while the male goes feeding at sea, while the opposite is observed for the third one (Marchant & Higgins 1990, Williams 1995).

We successfully confirmed the sex of 10 of the adults and determined the sex of all immatures and chicks using DNA-analyses. We extracted DNA by adding 20 μl of blood cells stored in 70% ethanol to 100 μl QuickExtract DNA extraction solution (Epicentre). We adapted the times in the manufacturers protocol to a first heating step at 65°C for 15 min and a second heating step at 98°C for 3 min. Extracted DNA was stored at –20°C. We performed the PCR with primers 2945F, cfR, 3224R according to Ellegren (1996). All samples were run on a 1.5% agarose gel and checked for a single (male) or double (female) band.

Comparison between populations

In order to use the data from the literature, Agnew & Kerry (1995) created an index of dimorphism that is an expression of the percentage difference between the male and female means. Nevertheless, this index based on differences is sometimes difficult to interpret. Therefore, we propose to directly use the proportion of female measures in relation to male measures (given as percentage value). We selected the studies with the most similar methods of measurements and the best sample size. The studies combining different populations were not retained as it was not possible to assess average measurements for each population.

Statistical analysis

The chick hatching from the B-egg is much more likely to survive than its sibling so it is unusual for parents to rear two chicks. However, A- and B-chicks have similar growth rates when they are alone (Poisbleau *et al.* 2008). In the present study, most of the chicks were also alone within the first days after hatching (see Poisbleau *et al.* 2008). We, therefore, combined both egg categories for statistical analyses on chick data.

For each age category, we compared morphological measurements between the sexes using Student independent-sample t-tests. We performed a Discriminant Function Analysis (DFA) to determine the accuracy of assigning a sex using these measurements, and to determine the most reliable method. Individuals with positive scores in discriminant function equations (D) were classified as males while those with negative scores were classified as females. We, therefore, used a cross-validation technique (jacknife procedure) to verify the accuracy of the DFA. As all the morphological parameters were normally distributed ($P > 0.05$ in all Kolmogorov-Smirnov one-sample tests), we verified the homoscedasticity of all data used in the model (variances homogeneous between and within samples) using Box's M tests before performing each DFA. We reported results of Box's M tests only for data that were not homoscedastic. All statistical analyses were performed using SPSS 16.0 (SPSS 2007). Values are presented as means ± SD.

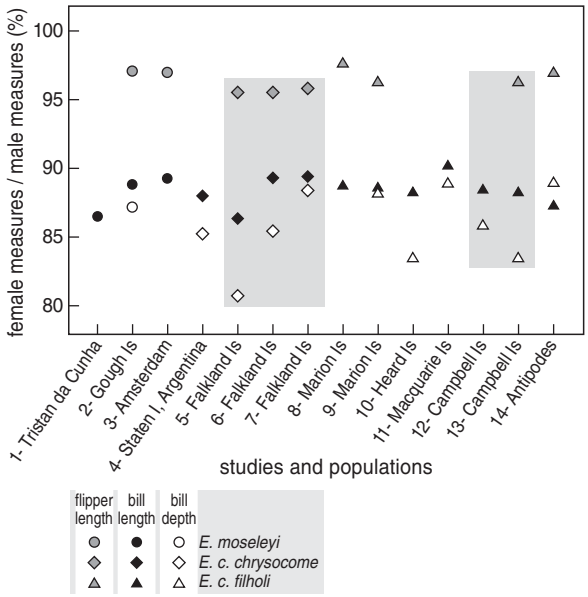
RESULTS

Comparison between populations

From the three measurements retained (Fig. 1), the sexual dimorphism of the flipper length appears to be very consistent between studies (only 2.3% of variation). However, this dimorphism is also very low (females being 96.5% of the males) hence it is not very useful as a tool to sex Rockhopper Penguins. To test which characters are less variable between studies, it is helpful to compare results within the same population. In our dataset, the studies at Falkland and Campbell islands show that the dimorphism for bill depth is more variable than for bill length (Fig. 1). Therefore, the use of bill length seems more suitable for comparison between studies than the use of bill depth. For *E. moseleyi, E. c. chrysocome* and *E. c. filholi*, the bill length of females represents respectively 88.1, 88.3 and 88.7% of the bill length of males. Although the data are too scarce to perform statistical analysis, it is highly improbable that these percentages could be significantly different. The three taxa look similar with regard to their sexual dimorphism.

Sex of adults

All adult morphological measurements were significantly different between sexes, males being larger than females (Table 1). Since flipper length had a very low standardized canonical discriminant function coefficient (0.099 compared to 0.706 for bill length and 0.437 for bill depth) when the three measurements were included in the DFA, we removed this variable from the analysis. We, therefore, correctly sexed 96.2% (96.2% after cross-validation) of all birds with the following discriminant function (DF): DF = 0.456 Bill length + 0.503 Bill depth – 29.512, where all measurements are in mm. Birds with positive scores were classified as male and those with negative scores as female (see Fig. 2A).



If we removed bill depth from the analysis and retained only bill length, which is the easiest measurement to perform, we correctly sexed 94.2% (94.2% after cross-validation) of all birds with: DF = 0.622 Bill length – 27.350. Therefore, we obtained a cut-off point of 44.0 mm for bill length, above and below which birds are classified as males and females respectively.

Sex of immatures

Immature males were significantly larger than immature females for all morphological measurements (Table 1). Again flipper length had a very low discriminant coefficient (0.027 compared to 0.852 and 0.360) with the three measurements in the DFA. We, therefore, removed this variable from the analysis and correctly sexed 91.8% (90.6% after cross-validation) of all birds with the following discriminant function (see Fig. 2B): DF = 0.599 Bill length + 0.114 Bill depth – 27.258.

Figure 1. Proportion of female measures in relation to male measures (given as percentage value) for flipper length, bill length and bill depth of *E. moseleyi*, *E. c. chrysocome* and *E. c. filholi*. Sources are: 1- Hagen in Segonzac (1972), 2- Williams (1980), 3- Duroselle & Tollu *in* Marchant & Higgins (1990), 4- Pütz *et al.* (2006), 5- Strange (1982), 6- Agnew & Kerry (1995), 7- This study, 8- Williams in Marchant & Higgins (1990), 9- Cooper *et al.* in Agnew & Kerry (1995), 10- Woehler in Marchant & Higgins (1990), 11- Hull (1996), 12- Warham (1972), 13- Moors & Cunningham in Marchant & Higgins (1990), 14- Warham (1972). Results for the Falkland and Campbell Islands are highlighted by a rectangle (see text).

Table 1. Morphological measurements (mm) between males and females for adults, immatures and chicks (at different stages) of Southern Rockhopper Penguins. Differences between males and females were tested with Student independent-sample *t*-tests. Means ± SD are given, with sample sizes in brackets.

	Male	Female	*t*–value	*P*
Adults				
Bill length	46.6 ± 1.6 (144)	41.4 ± 1.6 (149)	27.45	< 0.001
Bill depth	20.0 ± 0.9 (144)	17.7 ± 0.9 (149)	22.26	< 0.001
Flipper length	176.2 ± 4.3 (144)	169.4 ± 4.5 (149)	13.32	< 0.001
Immatures				
Bill length	44.5 ± 1.6 (41)	40.4 ± 1.5 (44)	12.04	< 0.001
Bill depth	17.4 ± 0.9 (41)	16.0 ± 0.8 (44)	7.36	< 0.001
Flipper length	175.0 ± 4.7 (41)	168.5 ± 4.1 (44)	6.78	< 0.001
Newly hatched chicks (0 and 1 day)				
Bill length	14.5 ± 0.6 (119)	14.1 ± 0.6 (119)	4.80	< 0.001
Bill depth	7.3 ± 0.3 (49)	7.4 ± 0.3 (37)	–0.46	0.65
Flipper length	34.9 ± 1.8 (119)	34.8 ± 1.7 (119)	0.44	0.66
Pre-crèche chicks (18 and 19 days)				
Bill length	23.5 ± 1.4 (111)	22.0 ± 1.5 (112)	7.23	< 0.001
Bill depth	10.7 ± 0.5 (43)	10.3 ± 0.7 (32)	2.97	0.004
Flipper length	113.2 ± 8.2 (111)	107.8 ± 11.3 (112)	4.03	< 0.001
Moulted chicks (55 days and after)				
Bill length	40.2 ± 1.6 (64)	37.0 ± 1.6 (57)	11.08	< 0.001
Bill depth	14.9 ± 0.7 (64)	13.8 ± 0.8 (57)	8.78	< 0.001
Flipper length	174.0 ± 4.5 (64)	169.0 ± 4.7 (57)	5.95	< 0.001

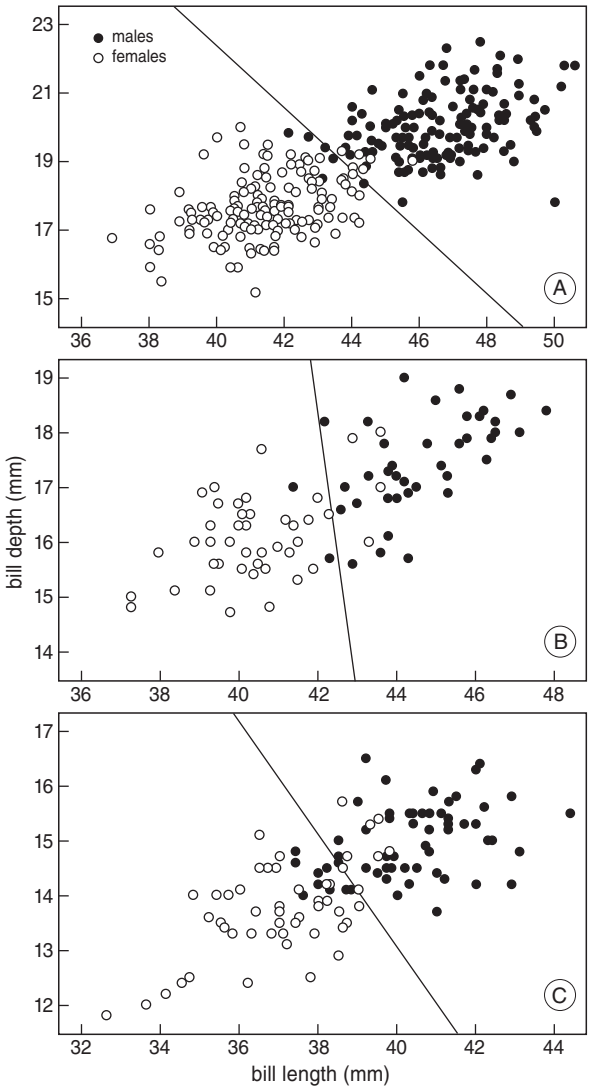


Figure 2. Relationship between bill length and bill depth for (A) adults (n = 144 males and 129 females), (B) immatures (n = 41 males and 44 females) and (C) moulted chicks (n = 60 males and 52 females) of Southern Rockhopper Penguins. The solid lines represent the statistical boundary between males and females, derived from the discriminant function (DF) given in the text.

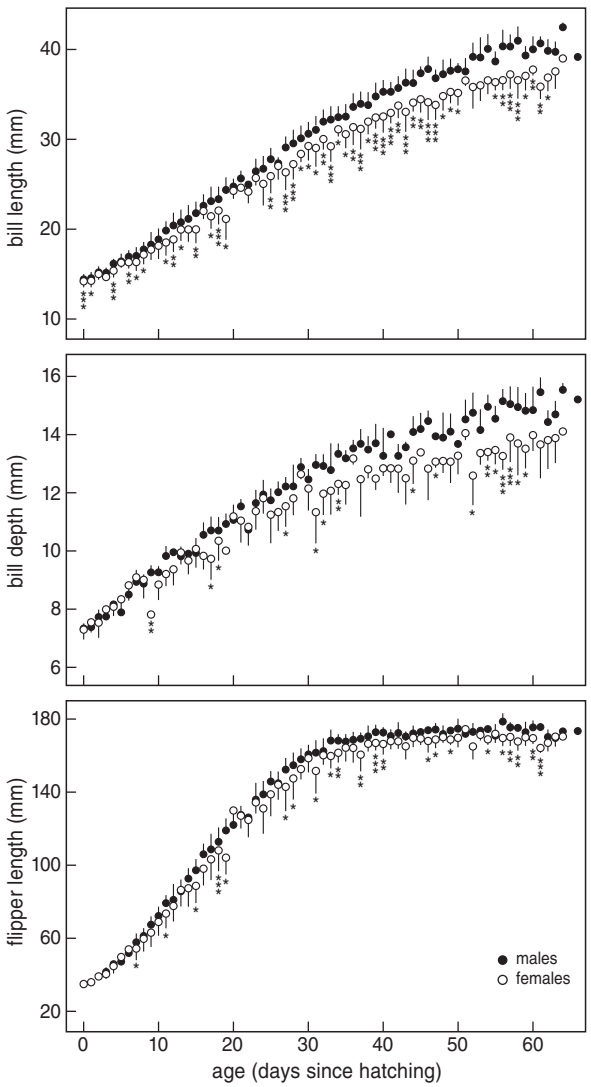


Figure 3. Change in bill length, bill depth and flipper length from hatching to fledging age for males (n = 832 for bill length and flipper length and n = 422 for bill depth) and females (n = 887 for bill length and flipper length and n = 339 for bill depth) for chicks of Southern Rockhopper Penguins. Means ± SD for ages with more than two measurements and only means for ages with one or two measurements. Significant differences between sexes tested with t-tests for each age are marked with stars under female dots: *: $P < 0.05$, **: $P < 0.01$ and ***, $P < 0.001$.

If we also removed bill depth from the DFA, we correctly sexed 90.6% (90.6% after cross-validation) of all birds with: DF = 0.635 Bill length – 26.877. We, therefore, obtained a cut-off point of 42.3 mm for bill length, above and below which birds are classified as males and females respectively.

Sex of chicks

Upon hatching (0 and 1 day), only bill length was different between sexes, males having a longer bill than females (Table 1, Fig. 3). However, when bill length was included in a DFA, we were able to sex only 63.5% of all birds (62.4% after cross-validation).

Just before the crèche age (18 and 19 days), all morphological measurements of chicks were significantly different between sexes, males being generally larger than females (Table 1, Fig. 3). However, data were not homoscedastic (Box's $M = 24.689$, $P = 0.001$) in the DFA with all the measured variables. Only after the removal of flipper length and bill depth from the DFA the data were homoscedastic (Box's $M = 0.217$, $P = 0.642$). However, we still correctly sexed only 68.2% (68.2% after cross-validation).

After their moult (55 days and after), males were significantly larger than females in all their morphological measurements (Table 1, Fig. 3). Flipper length had a very low discriminant coefficient (–0.023 compared to 0.776 and 0.360) in the analysis with all the measured variables. We then removed it and correctly sexed 84.3% (84.3% after cross-validation) of all birds with (see Fig. 2C): DF = 0.486 Bill length + 0.473 Bill depth – 25.605.

Including only bill length, we correctly sexed 82.6% (82.6% after cross-validation) of all birds with: DF = 0.632 Bill length – 24.478.

DISCUSSION

The comparison of the sexual dimorphism between studies shows that bill length is the most useful measurement to separate males from females. Indeed, this measurement both presents a higher dimorphism and varies less between individual scientists than bill depth. According only to this measurement, it seems that the sexual dimorphism of the adults does not vary among the three taxa of Rockhopper Penguins. Nevertheless, to confirm this observation, it would be useful to perform the same comparison with bill depth, the most dimorphic character. However, the same individual scientist should take the measurements using the same techniques for several populations to enable this comparison to be made. And, unfortunately, it is hardly ever the case for the studies found in the literature.

Similarly to the comparison of the sexual dimorphism between studies, the discriminant function analyses showed that, among the variables we measured, the most reliable single morphological measurement for assessing sex in Southern Rockhopper Penguins was bill length while flipper length was the least reliable measurement. Bill depth had a lower discriminant function than bill length. Removing this variable decreased the accuracy of the sexing method by only 1.2–2%. This observation was consistent among age categories. For the adults, it shows that, with an accuracy of 94.2%, using only bill length measurements was sufficient for accurate sexing, which is useful when time to manipulate birds is limited. Moreover, the cut-off points for bill length provide instant information in field studies. This rate is slightly higher than the 93.2% observed for adults of the sub-species *filholi* when subjected to the same procedure but incorporating both bill length and bill depth in the model (Hull 1996).

In any particular age class of crested penguin populations, males are significantly larger than females (Warham 1975). However, the smaller and growing bill size of chicks and immatures may preclude their use in sexing, thus necessitating separate discriminant functions from that of the adults. The use of bill length alone enabled us to sex 90.6% of the immatures. The addition of bill depth or flipper length did not significantly improve the model in our study whereas the use of tarsus length enabled to sex up to 95.7% of the Magellanic Penguin *Spheniscus magellanicus* yearlings (one year old bird, Scolaro 1987). Indeed, for this species where males are required to dig a burrow, feet may have evolved differently between sexes, leading to a sexual dimorphism between both (Scolaro 1987). The lower rates for immatures than for adults show that immatures are less sexually dimorphic than adults for the recorded measurements. The relatively high variation in morphological measurements in immatures compared with adults could be due to our inability to age them accurately as 'immatures' as they could be one or two years old and possibly still growing (Williams 1995). In practice, in an unbanded population, this information would not be available anyway. As such, the fact that the current sexing does not consider exact age actually makes it more robust.

Despite male and female bill length differing as soon as the chicks hatched, bill and flipper measurements did not enable us to sex young chicks with great precision. However, the discriminant measurements reach a plateau at the end of the pre-fledgling moult (see van Heezik 1990) and, after this moult, the accuracy of our discriminant function is greatly increased. It is, therefore, necessary to wait until the end of the moult to obtain an acceptable accuracy to correctly sex chicks. We also recommend performing measurements as synchronized as possible and when chicks are as old as possible. We accurately sexed 84.3% of the moulted chicks using both bill length and depth and 82.6% of them with only bill length. This result is better than the results obtained for Magellanic Penguins using the same two morphological measurements (78%, Bertellotti *et al.* 2002) but lower than results obtained for Yellow-eyed Penguins *Megadyptes antipodes* using

only foot length (88%, Setiawan *et al.* 2004), or for Magellanic Penguins using bill depth and length of middle toe (93%, Scolaro 1987). Maybe foot measurements are simply better across the board for sexing penguin fledglings (Scolaro 1987, Setiawan *et al.* 2004).

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SAMENVATTING

Dit onderzoek richtte zich op de vraag welke lichaamskenmerken het best gebruikt kunnen worden om onderscheid te maken tussen mannetje en vrouwtjes van de Zuidelijke Rotspinguïn *Eudyptes c. chrysocome*, een ondersoort die op de Falklandeilan-

den voorkomt. De snavel van volwassen en onvolwassen mannetjes bleek langer en hoger te zijn dan die van vrouwtjes uit dezelfde leeftijdsgroep. Ook waren de vleugels iets langer. Een rekenmodel dat de sekse vaststelt op grond van de lengte en hoogte van de snavel gaf een juiste voorspelling bij 92,6% van de adulte vogels en bij 91,8% van de onvolwassen vogels. Op het moment dat jongen uit het ei kruipen hebben mannetjes gemiddeld een langere snavel dan vrouwtjes maar de overlap is te groot om een betrouwbare voorspelling over de sekse te kunnen maken. Naarmate de vogels ouder worden, wordt het verschil tussen de seksen groter. Echter het percentage van de vogels waarvan de sekse juist geschat wordt, blijft beperkt (68,2% van de vogels wanneer ze 18–19 dagen oud zijn, en 84,3% wanneer ze ouder dan 55 dagen zijn). Voor alle leeftijdsgroepen is de snavellengte de lichaamsmaat die het sterkst tussen de seksen verschilde. (JS)

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