# Sexual Size Dimorphism and Sex Determination by Morphometric Measurements in Breeding Imperial Shags (*Phalacrocorax atriceps*)

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**Abstract.**—The Imperial Shag (*Phalacrocorax atriceps*) is monomorphic in plumage, but males are larger than females. We analyzed the sexual size dimorphism and variability of six morphometric characteristics (bill length, bill depth, head length, tarsus length, wing length, and body mass) measured on 291 breeding Imperial Shags at Punta León colony in coastal Patagonia, Argentina, during 2004 and 2005 breeding seasons. Discriminant analyses were performed on external measurements that we considered potentially useful in sexing Imperial Shags. All the birds were sexed by a distinctive behavior (vocalizations) and a sub sample of fifty were also sexed by DNA-based genetic techniques, showing 100% agreement between the two methods. All measured characteristics differed between the sexes, with males being larger than females. Body mass (17.8-18.0%) and bill depth (12.8-13.2%) showed the highest level of dimorphism whereas bill, head, tarsus, and wing length were less dimorphic (ranging 4.8-6.0%). Dimorphism in body mass for breeding shags was lower than previously reported during winter, prior to the breeding season. Although the wing length showed the lowest degree of dimorphism (5.3-5.4%), it presented the lowest coefficient of variation (1.9-2.0%) resulting in the most accurate single-measurement indicating sex. A cross validation process with a new sample, revealed that discriminant functions comprised by two characteristics were more accurate and reliable for sex determination than single-measurements. We obtained two functions correctly classifying 94-97% of shags. These functions were reliable (similar accuracy for discriminant analysis, Jackknifed validation, and cross-validation with a new sample) and seasonal unbiased, as body mass was not included in the analysis. *Received 15 April 2006, accepted 14 August 2006.*

**Key words.**—discriminant analysis, Imperial Shags, Phalacrocoracidae, *Phalacrocorax atriceps*, sexual size dimorphism.

Waterbirds 30(1): 97-102, 2007

Many bird species exhibit sexual dimorphism in terms of body size or plumage dichromatism. Shags and cormorants are sexually monomorphic in plumage, but they are dimorphic in size with males larger and heavier than females (Murphy 1936; Orta 1992; Johnsgard 1993). Such size dimorphism can be used to sex shags by discriminant analysis of the morphometric measurements (Potts 1969; Brothers 1985; Malacalza and Hall 1988; Glahn and McCoy 1995; Casaux and Baroni 2000; Quintana *et al.* 2003). Usefulness of body measurements as predictors of sex increases with increasing sexual size dimorphism and with decreasing in the variability within sexes (Weidinger and Franeker 1998; Fletcher and Hamer 2003).

The Imperial Shag (*Phalacrocorax atriceps*) inhabits southern South America on both the Atlantic and Pacific coasts (Johnsgard 1993). The only existing study of sexual determination of Imperial Shags was performed by Malacalza and Hall (1988) at Punta Lobería (44°35´S, 65°22´W) on the Patagonian coast during late winter, prior to the breeding season. In that study, birds were sexed by gonadal inspection and adult shags were not differentiated from juveniles and immature individuals, which could potentially affect the results. Although the authors provided two discriminant functions, one of these included body mass as variable, which could bias the resulting function as body mass present seasonal and annual variations (Brothers 1985; Croxall 1995; Casaux 1998). The other function includes total bird length as variable, which is not easy to take from live birds in the field. In the other hand, although DNA-based techniques have been recently used to determine the sex in birds (Ellegren 1996; Jodice *et al.* 2000; Quintana *et al.* 2003) these techniques require a time consuming process in the laboratory that impede the sex determination *in situ*, usually required during the fieldwork.

In this paper, we assess sexual size dimorphism and variability in six morphometric characteristics of breeding adult Imperial Shags (*Phalacrocorax atriceps*) measured during the breeding period. We also evaluate the reliability of sex determination by genetic DNA-based techniques compared to a behavior-based method. Finally, discriminant analyses were performed to obtain reliable and unbiased functions to determine the shag's sex based on external measurements easy to take in the field.

## METHODS

As part of a wider study on the behavioral ecology of the Imperial Shag, adult birds were captured and ringed during two consecutive breeding seasons (2004 and 2005) at Punta León colony (43°05'S, 64°30'W) in coastal Patagonia, Chubut, Argentina. A total of 291 shags (188 and 103 for 2004 and 2005 breeding seasons, respectively) were handled to obtain external measurements between early November and late December. All sampled shags belonged to the *albiventer* morph (Murphy 1936; Orta 1992).

*Behavioral and genetic sex determination.* As many "blue-eyed shag" species, male and female of the Imperial Shag can be distinguished by their vocalizations displayed during the courtship and nest defense behavior (Bernstein and Maxson 1982; Brothers 1985; Malacalza and Hall 1988; Casaux and Baroni 2000). Thus, all sampled shags were sexed based on this behavioral method. A sub-sample of 50 shags (25 of each sex) was also sexed by genetic DNA-based techniques to test its applicability to this species and to verify the accuracy compared to the behavioral method. The different size of an intron within the highly conserved chromo-helicase-DNA binding protein (CHD) gene was used to differentiate sex in birds, following Ellegren (1996). For a detailed description of these procedures, see Quintana *et al.* (2003).

*Morphometric measurements.* Six body measurements were taken: bill length (exposed culmen), bill depth (minimum depth, which is about at the mid length of the bill), head length (from the tip of the bill to the posterior ridge formed by the parietalsupraoccipital junction), tarsus length (from the middle of the midtarsal joint to the distal end of the tars-metatarsus), and wing length (as the length of flattened and extended closed wing, from carpal joint to tip of longest primary). Tarsus measurements were taken on the right side of the body. For bill, head and tarsus measures, we used a digital vernier caliper (nearest 0.01 mm). Wing length was measured with a ruler (nearest 1 mm). We also recorded the body mass using spring scales (nearest 10 g). Body mass measures represent shag's weight without food loads. All measurements were taken after birds completed their clutches and by the same person (WSS) to avoid any bias between observers.

*Data analyses.* One-way ANOVA's were used to determine whether the overall external morphology varied with sex (Sokal and Rohlf 1995). All measured variables were normally distributed (Lilliefors tests; n.s.). For all variables we calculated the sexual size dimorphism index as: $SSD = \{(\bar{x}_m - \bar{x}_f)/x_f\} \times 100$ (from Weidinger and Franeker 1998); where $\bar{x}_m$ and $\bar{x}_f$ are the mean values of males and females, respectively. For all variables, coefficient of variation ($CV = (SD/\bar{x}) \times 100$) were calculated for each sex and averaged between them (Fletcher and Hamer 2003) to indicate the degree of variability of each measurement (Sokal and Rohlf 1995).

Discriminant analyses were applied to 188 shags (93 males and 95 females) sampled during 2004 breeding season to predict the sex of unknown shags by their morphometric characteristics. Given that body mass may not be a reliable measure to include in discriminant functions since it may vary throughout the year and also during the day (Brothers 1985; Croxall 1995; Casaux 1998; Svagelj and Quintana, unpubl. data), we excluded this variable from the analysis to avoid any bias in our discriminant functions.

The performance of each single-variable as discriminant (univariate discriminant analysis) was evaluated. Forward discriminant analyses were applied to obtain combinations of characteristics (discriminant functions) that best distinguished the sexes (Tabachnick and Fidell 1996; Phillips and Furness 1997). For each discriminant analysis the associated cut-off value was calculated (see Phillips and Furness 1997). Shags with a discriminant score (measurements values for univariate analysis) higher than the cut-off value were classified as males, and those with a lower score as females. Following the criteria proposed by Phillips and Furness (1997), cut-off values were those discriminant score values corresponding to a posterior probability value of 0.5 for each group (i.e. the probability of being male and female). Cut-off values were obtained by fitting data (each discriminant score and the associated posterior probability of being a male) to logistic curves (Phillips and Furness 1997).

The effectiveness of the discriminant analyses was assessed, first in terms of the proportion of birds of known sex that were classified correctly, second by a Jackknifed validation, and finally by a cross-validation process through the classification of an additional new sample (Sokal and Rohlf 1995; Tabachnick and Fidell 1996; Phillips and Furness 1997). The Jackknifed classification is a validation process in which each individual case is classified using a function obtained from the total sample, excluding the individual case to be classified (Tabachnick and Fidell 1996). As correct classification rates tended to be overestimated when discriminant analyses are validated with the same sample used to generate them (Tabachnick and Fidell 1996), we performed a cross-validated process to classify a new sample of 103 shags (46 males and 57 females) from the 2005 breeding season.

We provided cutt-off values and discriminant functions obtained. We also report the F-value with degrees of freedom, significance level (P-value), Wilk's Lambda, and the percentage correctly classified for each sex and for all birds pooled.

## RESULTS

All DNA samples showed one of the typical band patterns that differentiate males (one band) from females (two bands). The fifty shags sexed by both behavioral and genetic techniques were classified the same by both

methods. Although the ranges overlapped, males were significantly larger than females for all measured characteristics in both years ($P < 0.0001$ for all tests; Table 1). Characteristics that showed the highest sexual size dimorphisms were body mass and bill depth whereas bill, head, tarsus, and wing length were less dimorphic (Table 1). Body mass showed the highest within-sex variation whilst wing length had the lowest (Table 1).

All single measured characteristics significantly predicted the sex of shags ($P < 0.0001$ for all analyses; Table 2). Wing length was the most accurate single variable indicator of sex correctly classifying 94% of shags (Table 2). Shags with wing length values larger than 291mm (cutt-off value) were classified as males while those with lower values were classified as females. Bill depth (cutt-off value = 11.76mm) and tarsus length (cutt-off value = 67.5mm) were also accurate predictors of sex correctly classifying 89% and 86% of shags, respectively (Table 2). Head and bill length had less discriminatory power (Table 2). Jackknifed validation process provided the same classifications than those produced by discriminant analyses for all single measurements except for the bill length, where only one shag differed between classifications. For all single measurements, cross-validation provided slightly different classifications respect to discriminant analysis, decreasing the accuracy for wing and bill length and increasing for bill depth, head and tarsus length (Table 2).

Forward discriminant analysis provided two significant functions ($P < 0.0001$). The best classification of sex produced by discriminant functions was obtained including wing length and bill depth as discriminatory variables. The resulting function ($D_1$, with an associated cut-off value of 0.024) was:

**Table 1. Male and female body measurements (mean ± SD, and range), coefficients of variation (CV) and sexual size dimorphism (SSD) of adult Imperial Shags from Punta León, Argentina, sampled during 2004 and 2005 breeding seasons. All measurements are given in mm, except body mass in g. All measured characteristics differed between the sexes (see text; one-way ANOVA; P < 0.0001 for all tests).**

| Body measurement | Males | Females | One-way ANOVA | CV (%) | SSD (%) |
|---|---|---|---|---|---|
| 2004 | N = 93 | N = 95 | $F_{1,186}$ | | |
| Bill length | 58.7 ± 2.2<br>53.8 - 64.0 | 55.3 ± 2.3<br>47.1 - 60.8 | 103.1 | 4.0 | 6.0 |
| Bill depth | 12.5 ± 0.6<br>11.0 - 14.4 | 11.1 ± 0.5<br>10.0 - 12.7 | 285.7 | 4.9 | 12.8 |
| Head length | 139 ± 4<br>132 - 154 | 132 ± 3<br>122 - 140 | 192.1 | 2.7 | 5.8 |
| Tarsus length | 69.4 ± 1.6<br>65.4 - 73.0 | 65.7 ± 1.7<br>61.3 -70.0 | 237.8 | 2.5 | 5.7 |
| Wing length | 298 ± 6<br>287 - 317 | 283 ± 6<br>269 - 298 | 336.2 | 2.0 | 5.4 |
| Body mass | 2323 ± 168<br>1920 - 2720 | 1972 ± 123<br>1720 - 2300 | 268.1 | 6.7 | 17.8 |
| 2005 | N = 46 | N = 57 | $F_{1,101}$ | | |
| Bill length | 58.9 ± 2.2<br>52.7 - 64.9 | 56.2 ± 2.2<br>51.3 - 62.0 | 37.7 | 3.9 | 4.8 |
| Bill depth | 12.7 ± 0.7<br>11.2 - 14.2 | 11.2 ± 0.5<br>10.3 - 12.7 | 167.7 | 4.8 | 13.2 |
| Head length | 137 ± 3<br>132 - 146 | 131 ± 3<br>122 - 138 | 115.4 | 2.4 | 5.2 |
| Tarsus length | 69.0 ± 1.2<br>66.2 - 72.7 | 65.1 ± 1.6<br>61.6 - 68.2 | 192.0 | 2.1 | 6.0 |
| Wing length | 298 ± 6<br>287 - 315 | 283 ± 5<br>270 - 292 | 196.6 | 1.9 | 5.3 |
| Body mass | 2306 ± 118<br>2040 - 2560 | 1955 ± 115<br>1590 - 2160 | 232.8 | 5.5 | 18.0 |

**Table 2. Accuracy of sexing Imperial Shags (as percentages correctly classified) using single measurements and discriminant functions ($D_1$ and $D_2$) for 2004 (original sample included in discriminant analyses) and 2005 (the new sample to cross-validation (see text). All discriminant analyses are significant (P < 0.0001).**

| | | | 2004 (N = 188) | | | 2005 (N = 103) | | |
|---|---|---|---|---|---|---|---|---|
| | Wilk's Lambda | F - value | Males | Females | Total | Males | Females | Total |
| Bill length | 0.643 | $F_{1,186} = 103.1$ | 80 | 78 | 79 | 83 | 61 | 71 |
| Bill depth | 0.394 | $F_{1,186} = 285.7$ | 86 | 92 | 89 | 96 | 91 | 93 |
| Head length | 0.492 | $F_{1,186} = 192.1$ | 77 | 89 | 84 | 74 | 96 | 86 |
| Tarsus length | 0.439 | $F_{1,186} = 237.8$ | 87 | 84 | 86 | 89 | 95 | 92 |
| Wing length | 0.356 | $F_{1,186} = 336.2$ | 94 | 94 | 94 | 91 | 88 | 89 |
| $D_1$ | 0.251 | $F_{2,185} = 276.3$ | 97 | 97 | 97 | 98 | 95 | 96 |
| $D_2$ | 0.311 | $F_{2,185} = 204.8$ | 92 | 96 | 94 | 98 | 98 | 98 |

$$D_1 = (\text{Bill Depth} \times 1.100) + (\text{Wing Length} \times 0.123) - 48.662$$

This function correctly sexed 97% of the breeding shags (Table 2), misclassifying only three males and three females (Figure 1A). We also obtained an additional discriminant function including bill depth and tarsus length, correctly classifying 94% of shags (Table 2 and Figure 1B). This function ($D_2$, with an associated cut-off value of 0.023) was:

$$D_2 = (\text{Bill Depth} \times 1.174) + (\text{Tarsus Length} \times 0.345) - 37.133$$

The jackknifed validation provided the same classifications than those produced by the discriminant functions. Cross-validation process provided similar classifications than those produced by discriminant functions (Table 2), with 96% and 98% of shags sampled during 2005 correctly classified by $D_1$ and $D_2$, respectively.

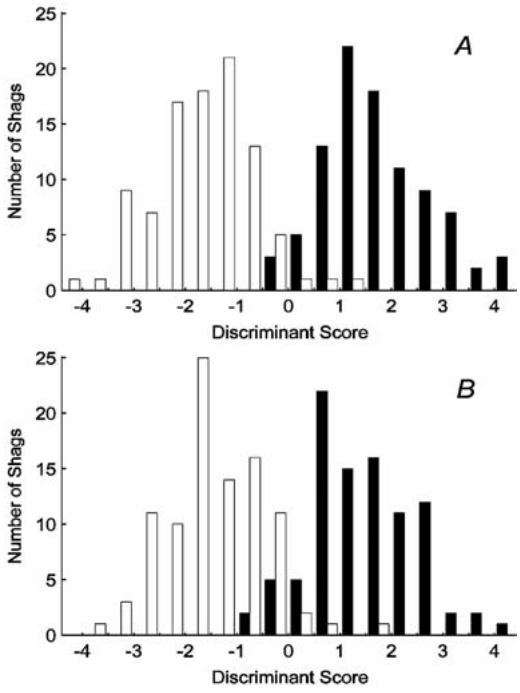


**Figure 1. Classification of male (black bars) and female (white bars) Imperial Shags sampled during 2004 breeding season, applying $D_1$ (A) and $D_2$ (B) discriminant functions.**

## DISCUSSION

We found that all body measurements differed between sexes, with males being larger and heavier than females. Our findings support the idea of a consistent sexual size dimorphism in the Phalacrocoracidae family (Johnsgard 1993). For all body measurements, sexual size dimorphism and coefficients of variation were consistent between years. Body mass (17.8-18.0%) and bill depth (12.8-13.2%) showed the highest sexual size dimorphism. However, wing length was the most accurate single variable to distinguish between males and females. Although the wing length showed a small degree of sexual size dimorphism (5.3-5.4%), it presented the lowest coefficient of variation (1.9-2.0%) resulting in the most accurate single predictor of sex. Our findings were in accordance with previous studies that showed that the wing length (Glahn and McCoy 1995; Casaux and Baroni 2000), bill depth (Potts 1969; Malacalza and Hall 1988; Glahn and McCoy 1995) and tarsus length (Glahn and McCoy 1995; Casaux and Baroni 2000)

are key characteristics to differentiate sexes in cormorants and shags.

Except for body mass, most of the body dimensions registered at this study were similar to those measured by Malacalza and Hall (1988) on the same morph during late winter. Those authors reported a sexual dimorphism in mass during winter (prior to the breeding season) of 27.7%, which is notably higher than that we found during the breeding season (17.8-18.0%). Beside the seasonally variation of body mass (which is scarcely knowledge in shags; see Brothers 1985; Casaux 1998), body mass may substantially vary since the last food intake (Grémillet *et al.* 1996; Casaux 1998; Svagelj and Quintana, unpubl. data). Therefore, differences in dimorphism in body mass between studies may be a consequence of differences in the time elapsed between food intake and the capture to weight the birds and/or seasonal variations.

As show by the total agreement between behavioral and genetic techniques found in this study, adult Imperial Shags can be almost unequivocally sexed by their vocalizations during breeding season. Sex determination by behavior is a non-invasive method that does not require manipulating the bird. However, it can only be applied for breeding adult shags. Chicks, fledglings, and juveniles of the Imperial Shag cannot be sexed by behavioral methods, and the DNA-based techniques seems particularly useful in such circumstances.

The two discriminant functions found at this study were more accurate and reliable predictors of sex than every single-measurements, as revealed by two validation process. Our results also emphasized the importance of the different kind of validations to discriminant analyses. While Jackknifed validation reveals influential observations biasing analysis, cross-validation with a new sample is a more realistic process corresponding to a new sample of different individuals potentially measured on different times, locations, conditions and by different observers (Tabachnick and Fidell 1996).

Our discriminant functions included combinations of only two morphometric variables (with the bill depth included in both functions) and correctly classified 94-97% of shags. These functions were not seasonally biased as body mass was not included in the analyses. Some authors (Fox *et al.* 1981; Jodice *et al.* 2000), but not others (Glahn and McCoy 1995; Phillips and Furness 1997; Casaux and Baroni 2000), avoid the inclusion of wing length in discriminant functions because it may be affected by wing-tip wear and consequently by time elapsed between moult and measurement. Consequently, function $D_2$ (including bill depth and tarsus length) seems to be more reliable than function $D_1$ which include wing length as variable. All measurements considered are relatively easy to take in the field and should be reproducible, allowing a quick sex determination for both breeding and non-breeding adult Imperial Shags.

## ACKNOWLEDGMENTS

Research was supported by Wildlife Conservation Society and CONICET (PIP N° 5387). V. E. Borrell, L. A. Silva, and M. C. Sueiro provide invaluable help in the field. We thank A. J. Gatto, B. Urrutia, and J. Rua for their collaboration in the field. Genetic sex determination was performed by G. Somoza from Instituto de Investigaciones Biotecnológicas, (INTECH, CONICET–UNSM). S. Copello and P. Edelaar made valuable comments on early versions of the manuscript. We appreciate the improvements in English usage made by S. Small through the Association of Field Ornithologists' program of editorial assistance and P. Edelaar. We also thank Organismo Provincial de Turismo for permits to work in the area. WSS was supported by a doctoral fellowship from CONICET, and FQ is a Research Fellow of the CONICET.

## LITERATURE CITED

Bernstein, N. P. and S. J. Maxson. 1982. Behaviour of the Antarctic Blue-eyed Shag *Phalacrocococorax atriceps bransfieldensis*. Notornis 29: 197-207.

Brothers, N. P. 1985. Breeding biology, diet and morphometrics of the King Shag, *Phalacrocorax albiventer purpurascens*, at Macquarie Island. Australian Wildlife Research 12: 81-94.

Casaux, R. 1998. Biología reproductiva y ecología alimentaria del Cormorán Antártico *Phalacrococorax bransfieldensis* (Aves: Phalacrocoracidae) en las Islas Shetland del Sur, Antártida. Unpublished Ph.D. thesis, University of La Plata, La Plata, Argentina.

Casaux, R. and A. Baroni. 2000. Sexual size dimorphism in the Antarctic Shag. Waterbirds 23: 489-493.

Croxall, J. P. 1995. Sexual size dimorphism in seabirds. Oikos 73: 399-403.

Ellegren, H. 1996. First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. Proceedings of the Royal Society of London B 263: 1635-1641.

Fletcher, K. L. and K. C. Hamer. 2003. Sexing terns using biometrics: the advantage of within-pair comparisons. Bird Study 50: 78-83.

Fox, G. A., C. R. Cooper and J. P. Ryder. 1981. Predicting the sex of Herring Gulls by using external measurements. Journal of Field Ornithology 52: 1-9.

Glahn, J. F. and R. B. McCoy. 1995. Measurements of wintering Double-crested Cormorants and discriminant models of sex. Journal of Field Ornithology 66: 299-304.

Grémillet, D., R. Dey, S. Wanless, M. P. Harris and J. Regel. 1996. Determining food intake by Great Cormorants and European Shags with electronic balances. Journal of Field Ornithology 67: 637-648.

Jodice, P. G. R., R. B. Lanctot, V. A. Gill, D. D. Roby and S. A. Hatch. 2000. Sexing adult Black-legged Kittiwakes by DNA, behavior, and morphology. Waterbirds 23: 405-415.

Johnsgard, P. A. 1993. Cormorants, darters, and pelicans of the world. Smithsonian Institution Press, Washington, D.C.

Malacalza, V. E. and M. A. Hall. 1988. Sexing adult King Cormorants (*Phalacrocorax albiventer*) by discriminant analysis. Colonial Waterbirds 11: 32-37.

Murphy, R. 1936. Oceanic birds of South America. MacMillan, New York, NY.

Orta, J. 1992. Family Phalacrocoracidae (Cormorants). Pages 326-353 *in* Handbook of the birds of the world, Vol. 1 (J. del Hoyo, A. Elliot and J. Sargatal, Eds.), Lynx Editions, Barcelona.

Phillips, R. A. and R. W. Furness. 1997. Predicting the sex of parasitic Jaegers by discriminant analysis. Colonial Waterbirds 20: 14-23.

Potts, G. R. 1969. The influence of eruptive movements, age, population size and other factors on the survival of the shag (*Phalacrocorax aristotelis* (L.)). Journal of Animal Ecology 38: 53-102.

Quintana, F., G. Somoza, M. Uhart, C. Cassará, P. Gandini and E. Frere. 2003. Sex determination of adult Rock Shags by molecular sexing and morphometric parameters. Journal of Field Ornithology 74: 370-375.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry. 3rd edition. W. H. Freeman and Company, New York.

Tabachnick, B. G. and L. S. Fidell. 1996. Using Multivariate statistics. 3rd edition. Harper Collins Publishers, New York.

Weidinger, K. and J. A. van Franeker. 1998. Applicability of external measurements for sexing of the Cape Petrel *Daption capense* at within-pair, within-population and between-population scales. Journal of Zoology of London 245: 473-482.