Field Sexing Olrog's Gull (*Larus atlanticus*) Using Morphometry

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Abstract.—Olrog's Gull (*Larus atlanticus*) is a vulnerable species; however, no study has addressed the relationship between body measurements and sex. To provide an easy and reliable work tool to identify the sex of individuals, adult Olrog's Gulls ($n = 111$) were weighed, several measurements were taken, and the sexes were determined using DNA analyses. All measurements showed significant differences between sexes, with males being significantly larger (Range = 4.0-15.1%) in all measurements. Logistic regression models were selected using the Akaike information criterion and were validated using leave-one-out cross validation. The best set included three models. Model 1 performed best and included head-bill length, bill depth, wing length and body mass as independent variables, and was closely followed by model 2, which contained the same variables with the exception of wing length. Model 3 included head-bill length, body mass and wing length as independent variables. Model 1 was 1.3 and 2.1 times more likely to be the best model than models 2 and 3, respectively. Model 1 correctly assigned the sex in 94.6% of all birds and 93.4% of females. Model 2 performance was marginally better and correctly assigned the sex in 95.5% of all birds and 95.1% of females. The correct classification of males was 96.0% in both models. Results demonstrate the validity of these measurements, which can be easily taken in the field to reliably determine the sex of Olrog's Gulls. *Received 9 November 2017, accepted 28 July 2018.*

Key words.—*Larus atlanticus*, molecular sexing, Olrog's Gull, predictive modeling, sexual dimorphism.

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Sex identification of birds can be an important component of field studies examining the biology, ecology and demographics of seabirds (Weimerskirch and Jouventin 1987; La Sala *et al.* 2011b; Svagelj *et al.* 2012). However, determining the sex of live seabirds through visual assessment with a high degree of confidence can be extremely challenging, if not impossible, when no obvious differences in plumage or body size exist between sexes (Sheldon 1998).

A common characteristic of many gull species (family Laridae) is the absence of sexual dimorphism in plumage between adult males and females (Cramps *et al.* 1985). Several studies on Laridae species have shown that male body size is significantly larger than female body size (Bosch 1996; Palomares *et al.* 1997). However, relying arbitrarily on morphometry without any statistical validation of the optimal set of measurements can lead to errors, waste of time and resources, and prolonged handling of birds.

The Olrog's Gull (*Larus atlanticus*) is a vulnerable species (International Union for Conservation of Nature 2018) endemic to the Atlantic coast of Argentina, Uruguay and southern Brazil (Yorio *et al.* 2005). Few Olrog's Gull breeding sites have been identified, and all of them are located in Argentina (Yorio *et al.* 2005). Most of the breeding population concentrates in the Bahía Blanca Estuary, Buenos Aires province, with the largest colony reported to date being located in Isla del Puerto (Delhey *et al.* 2001).

Most studies of this species during the breeding period have concentrated on feeding and breeding ecology (La Sala *et al.* 2011a; Suárez *et al.* 2012) and health (La Sala and Martorelli 2007; La Sala *et al.* 2011b; La Sala *et al.* 2012). To date, no study has addressed the relationship between morphometry and sex, precluding sexing adult Olrog's Gull in the hand while conducting field research. The sex of birds can be determined by molecular methods (Ellegren 1996), and these methods have been employed to determine sex in a variety of avian species (Quintana *et al.* 2003; Li *et al.* 2015).

The goals of this study were to assess sexual dimorphism in Olrog's Gull by evaluating the use of external measurements for sex identification and comparing them with molecular sex. Having these data, the final goal was to generate and then validate predictive statistical models that could be applied during field work to readily and reliably determine the sex of Olrog's Gulls.

METHODS

Study Area

The study was conducted on the largest known breeding colony of Olrog's Gulls located in the Bahía Blanca Estuary (38° 49′ S, 62° 16′ W), Argentina. This colony comprises ~3,800 breeding pairs distributed in sub-colonies in a flat area by the intertidal zone.

Sampling Design and Data Collection

Fieldwork was conducted during the breeding season in 2003 (24 October through 10 December), 2004 (23 October through 26 October) and 2005 (2 November through 4 November). Due to logistic constrains related to the location of the studied colony and to minimize human disturbance to a vulnerable species during the breeding period, convenience sampling was conducted by placing modified Coulson traps (Weaver and Kadlec 1970) over active nests containing at least one egg, and which could be readily accessed with minimum disturbance to other breeding adults. After either adult was captured and sampled, the trap was changed to a different location. Only one adult Olrog's Gull per nest was captured. All birds were fitted with metal leg bands with unique identification codes to avoid recapture in the same or subsequent years. A hood was used to cover the birds' heads and reduce stress during manipulation. A total of 118 adult Olrog's Gulls were captured during the late incubation period.

Individuals were weighed with a hand-held spring scale (nearest 10 g), and four measurements were taken using a caliper: bill depth (at the proximal edge of the nostrils; nearest 0.01 mm), tarsus length (from joint between tarsus and toes to intertarsal joint; nearest 0.5 mm), head-bill length (from hindmost point of the head to the tip of the bill; nearest 0.01 mm), and wing length (from carpal joint to the tip of the wing in naturally folded wing; nearest mm) (Eck *et al.* 2011).

A blood sample was collected from each bird by venipuncture of the brachial vein using heparinized syringes with 23G×1-inch needles, and a few drops were placed on a small piece of commercial filter paper, air-dried, and stored at 4-8 °C until processed for sex identification (Quintana *et al.* 2008).

Molecular Sexing

Briefly, the size of an intron within the highly conserved chromo-helicase-DNA binding protein (CHD) gene was used to screen individuals for sex differentiation following Ellegren (1996). One pair of primers was used to amplify the CHD-W and CHD-Z genes located on the avian sex chromosomes: 2550F, 5' GTTACTGATTCGTCTGCGAGA 3' and 2718R, 5' ATTGAAATGATCCAGTGCTTG 3' (Fridolfsson and Ellegren 1999). The amplifications were performed (a) in 25 µl using either 1 µl of a 1:10 or 1:20 dilutions of each sample extraction, or (b) between 50-200 ng (1-5 µl) of purified genomic DNA, 2.5 U of Taq polymerase (Promega), 1 µl of stock solutions of each primer (10 µM), 0.5 µl of a dNTPs solution (10 µM), and a final concentration of 1.5 mM of MgCl2. An initial denaturing step of 5 min at 95 °C was followed by 35 cycles of 30 sec at 95 °C, 45 sec at 47 °C and 30 sec at 72 °C, followed by 5 min at 72 °C. In the case of negative controls, no DNA was added to the reaction, whereas purified DNA from a male was added for the positive control. Polymerase chain reaction products were resolved in 1.8% agarose gels following electrophoresis in TBE buffer and stained with ethidium bromide staining to reveal one band (male pattern) or two bands (female pattern).

Data Analysis

Sexual size dimorphism in each measurement was calculated by subtracting the mean values of females from the mean values of males and then dividing the absolute difference by the mean value of males. Results were expressed as percentages.

Because of bad weather conditions, not every measurement could be made on every individual, thus producing slightly varying sample sizes for two of the measurements (tarsus length and body mass). Model building using information criteria cannot be based on the comparison of models fitted on samples of different sizes For multivariate analysis, the original dataset was curated to retain only individuals with complete sets of measurements ($n = 111$), whereas the whole sample of individuals ($n = 118$) was used for analyses at the bivariate level.

Bivariate comparisons of measurements between sexes were made using Student's unpaired *t*-tests. Logistic regression was used to assess the association between body measurements and the sex of individuals.

The assumption of linearity of continuous independent variables and log odds of the dependent variable was checked (Hosmer and Lemeshow 2000).

All analyses were performed using the statistical program R (R Development Core Team 2017). When using the R function *glm* to fit the multivariate model, complete separation or quasi-complete separation of the data occurred, meaning that one or more predictors can perfectly predict the sex of an individual. To circumvent this issue, the logistic models were fit using the bias-reduction method (Firth 1993) implemented in the 'brglm' package of R (Kosmidos 2017). Next, functions in the R package 'MuMIn' were used to streamline model selection and generate subsets including all possible combinations of the global model (Barton 2016). An information-theoretic approach based on the Akaike information criterion (AIC; Akaike 1974) was used in model building. The AIC for small sample sizes (AIC_c) was used (Burnham and Anderson 2002). The model with the lowest AIC_c value was considered the best model, and models with AIC_c values within 2 units of distance from the best model were also considered to have substantial explanatory power. Akaike weights ($w_i AIC_c$) were calculated and interpreted as the probability that a model is the best one, given the data and the set of candidate models. Thus, the strength of evidence in favor of one model over the other was obtained by dividing their $w_i AIC_c$, and this ratio indicated how many times more likely the first, best-fitting, model was in comparison to an inferior model (Burnham and Anderson 2002).

Potential muiticollinearity problems associated with high correlation among predictor variables was evaluated in the final models by calculating the variance inflation factor (VIF) for each variable, where VIF values ≥ 5 indicate potential multicollinearity problems.

Model Validation

All models were validated using a leave-one-out cross-validation procedure (Dechaume-Moncharmont *et al.* 2011), where a single observation was used for the validation set, and the remaining observations made up the training set. The model was fit on the $n - 1$ training observations and a prediction was made for the excluded observation. This procedure allowed estimating the percentage of birds correctly classified as male or female by sequentially removing each individual's data and classifying the individual using the derived function (Gareth *et al.* 2013). Cross-validation allowed the estimation of the following model performance metrics: accuracy (the model's ability to correctly predict both sexes), sensitivity (proportion of correctly classified males), and specificity (proportion of correctly classified females). In all models, a decision probability boundary was set to 0.5 to classify an individual as male or female.

RESULTS

All captured birds were measured and sexed by polymerase chain reaction. Males were significantly larger than females in all measurements, with the magnitude of dimorphism ranging between 4.0% for wing length and 15.1% for body mass (Table 1).

A total of 32 logistic models were fitted with all possible combinations of variables, and three competing models with ΔAIC_c values < 2 provided a substantial level of support for the data (Table 2). These models are presented along with their performance metrics in Table 2, and fully described in Table 3.

Models 1 and 2 were almost equal in terms of data support ($\Delta AIC_c = 0.5$), but the first was more complex and it retained one extra variable, wing length, which was absent in model 2. Model 2 performed better than model 1 in terms of accuracy (95.5% and

Table 1. Body measurements in male and female Olrog's Gull (*Larus atlanticus*) from Bahía Blanca Estuary, Argentina. Differences are reported as significance *P* values and mean dimorphism percentages.

	Males		Females				
Measurement	Mean ± SD (*n*)	Range	Mean ± SD (*n*)	Range	*t*	*P*	Dimorphism (%)
Body mass (g)	916.2 ± 60.2 (50)	825-1,150	777.5 ± 52.2 (61)	675-880	-12.8	< 0.001	15.1
Head-bill length (mm)	115.9 ± 3.1 (54)	107.9-124.8	107.7 ± 3.1 (64)	100.7-117.8	-14.3	< 0.001	7.1
Tarsus length (mm)	61.5 ± 2.2 (51)	56.8-67.8	58.0 ± 2.1 (63)	51.5-63.0	-8.6	< 0.001	5.7
Bill depth (mm)	55.3 ± 1.9 (54)	51.7-59.0	50.9 ± 1.9 (64)	45.9-57.1	-12.8	< 0.001	8.0
Wing length (mm)	418.6 ± 9.8 (54)	394-439	402.0 ± 8.9 (64)	380-425	-9.6	< 0.001	4.0

Table 2. Logistic models with their accuracy, sensitivity and specificity reported as percentage and their 95% confidence interval in parentheses. AIC_c values, differences (ΔAIC_c) and AIC_c weights ($w_i AIC_c$) are presented.

Model	Accuracy	Sensitivity	Specificity	AIC_c	ΔAIC_c	$w_i AIC_c$
1. Sex ~ Bill depth + Head-bill length + Body mass + Wing length	94.6 (91.0-99.0)	96.0 (86.3-99.5)	93.4 (84.0- 98.2)	25.0	0.0	0.442
2. Sex ~ Bill depth + Head-bill length + Body mass	95.5 (89.8-98.5)	96.0 (86.3-99.5)	95.1 (86.3-99.0)	25.5	0.5	0.344
3. Sex ~ Head-bill length + Body mass + Wing length	93.7 (87.4-97.4)	92.0 (80.8-97.8)	95.1 (86.3-99.0)	26.4	1.44	0.215

94.6%, respectively) and specificity (95.1% and 93.4%, respectively), whereas sensitivity remained equal. This difference can be attributed to one more female being falsely classified as male when using model 1 compared with model 2, thus slightly reducing overall model accuracy and specificity. Model 3 had lower accuracy (93.7%) and sensitivity (92.0%), but its specificity remained the same as in model 2. Model 1 was 1.3 and 2.1 times more likely to be the best model than models 2 and 3, respectively, as evidenced by the ratio between the corresponding Akaike weights. In the selected models, the variables' VIF values were never above 1.4.

The equation below corresponds to model 1 and can be populated with the corresponding measurements to calculate the probability that an individual is male or female. If this probability is higher than 0.5, then $P(Y) = 1$ (male); otherwise $P(Y) = 0$ (female):

$$P(Y) = \frac{1}{1+e^{-(-182.6+Bill\ depth \times 0.47+Head\text{–}bill\ length \times 0.77+Body\ mass \times 0.039+Wing\ length \times 0.09)}}$$

Similar equations can be written and used for models 2 and 3 by changing the intercept and inserting new values for the corresponding measurements and their coefficients into the equation.

DISCUSSION

This work provides the first assessment of sex-related morphological measurements in Olrog's Gull and offers a reliable, yet inexpensive method for sexing adult individuals in the field. Based on genetic sexing our data show that, similar to other species from

Table 3. Parameters of the best three logistic regression models showing the association between body measurements and sex in Olrog's Gulls (n = 111). Response variable was the sex of individuals and explanatory variables were three different sets measurements. ΔAIC_c represents AIC_c value increment if the single variable is dropped from the model.

Variables	Coefficient	SE	ΔAIC_c
Model 1 = Sex ~ Bill depth + Head-bill length + Body mass + Wing length			
Intercept	-182.61	65.09	—
Bill depth (mm)	0.470	0.308	1.4
Head-bill length (mm)	0.770	0.328	7.8
Body mass (g)	0.039	0.015	18.7
Wing length (mm)	0.090	0.074	0.5
Model 2 = Sex ~ Bill depth + Head-bill length + Body mass + Wing length			
Intercept	-137.0	40.46	—
Bill depth (mm)	0.429	0.258	1.9
Head-bill length (mm)	0.703	0.272	7.9
Body mass (g)	0.041	0.015	20.6
Model 3 = Sex ~ Bill depth + Body mass + Wing length			
Intercept	-168.37	56.01	—
Head-bill length (mm)	0.828	0.295	21.6
Body mass (g)	0.043	0.015	24.2
Wing length (mm)	0.092	0.069	1.0

the family Laridae (Cramps *et al.* 1985; Burger and Gochfeld 1996), male Olrog's Gulls are larger than females.

Other research conducted on Herring Gulls (*Larus argentatus*), Lesser Black-backed Gulls (*L. fuscus*) and Kittiwakes (*Rissa tridactyla*) found that taking one measurement, head-bill length, provided high discriminatory power between sexes, and that the inclusion of additional measurements increased discrimination only slightly (Coulson *et al.* 1983). Contrary to these findings, our model that included head-bill length as the only variable ranked 27th among the 32 possible models.

Our modeling approach showed that there were three possible combinations of variables that yield high performing models. In terms of accuracy and specificity, model 2 was marginally better than model 1. Model 1 was more complex than model 2 and included one extra variable (wing length) which was absent in model 2. However, model 1 showed better trade off between model fit and complexity, as evidenced by the lowest AIC_c value and highest Akaike weight.

The four measurements included in the models were easy to take from adult Olrog's Gulls in the field. Therefore, our results suggest that field researchers could optimize data collection on each captured bird preferably by using one of the two best performing models presented, and preferably model 1, over more complex and expensive techniques such as molecular sexing.

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