Ardeola 56(2), 2009, 281-286

TESTING EFFECTIVENESS OF DISCRIMINANT FUNCTIONS TO SEX DIFFERENT POPULATIONS OF MEDITERRANEAN YELLOW-LEGGED GULLS *LARUS MICHAHELLIS MICHAHELLIS*

EFECTIVIDAD DE LAS FUNCIONES DISCRIMINANTES PARA SEXAR DIFERENTES POBLACIONES MEDITERRÁNEAS DE GAVIOTAS PATIAMARILLAS *LARUS MICHAHELLIS MICHAHELLIS*

José I. AGUIRRE* [1], Pilar ARANA** and María Teresa ANTONIO***

SUMMARY.—In this study a series of morphological measurements were taken and individuals of *Larus michahellis* from the Chafarinas Islands were sexed genetically. New discriminants have been developed which, in spite of correctly sexing 84 % of the individuals, may be applicable at any time of year as only purely skeletal measurements were used, such as the tarsus and the nalospi and also the maximum chord of the folded wing. This study shows that it is necessary to develop specific sexual discrimants for different populations of the same species and to include, where possible, skeletal variables which are easily taken in the field.

RESUMEN.—En este estudio se han tomado una serie de medidas morfométricas y se ha sexado genéticamente individuos de *Larus michahellis* en las islas Chafarinas. Se han desarrollado nuevos discriminantes, que a pesar de determinar el sexo del 84 % de los individuos, puede ser aplicable en cualquier momento del año ya que usa medidas puramente esqueléticas, como el tarso y el nalospi, así como la cuerda máxima del ala plegada. El estudio demuestra que es necesario desarrollar discriminantes sexuales específicos para distintas poblaciones de la misma especie y que hay que incluir, en la medida de lo posible, variables esqueléticas fáciles de tomar en el campo.

Knowledge of the sex of individuals is an important requirement in many field studies but sometimes sex-discrimination in the field is difficult, especially in species monomorphic in plumage and size such as many seabirds (e.g. Sphenisciformes, Gaviiformes, Procellariiformes). A way for accurately sexing birds are DNA-based methods (reviewed in Ellegren and

* Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad Complutense de Madrid, Madrid, E-28040, Spain.

** Departamento de Genética, Facultad de biología, Universidad Complutense de Madrid, Madrid, E-28040, Spain.

*** Departamento de Fisiología Animal II (Fisiología Animal), Facultad de Biología, Universidad Complutense de Madrid, Madrid, E-28040, Spain.

1 Corresponding author: jaguirre@bio.ucm.es

Sheldon, 1997). This procedure is affected by the level of technical expertise, demands relative cost, and its applicability to large number of samples. Another technique to determine the sex of adult individuals in gull species (Arizaga, *et al.* 2008; Galarza, *et al.*, 2008; Bosch, 1996) is to apply a discriminant analysis function which is used to determine which variables discriminate between two naturally occurring groups. Such analysis decreases the costs and allows using external measures easy to record in the field.

However, it may be inappropriate to apply a discriminant analysis function obtained in one population to other populations of the same subspecies in different geographic locations (Evans, *et al.*, 1993). To the present date, not very many papers have addressed such concern. Most of the variables commonly used to establish discriminant functions are based on skeleton measurements with low covariation over the year (Bertellotti, *et al.*, 2002; Bluso, *et al.*, 2006; Genovart, *et al.*, 2003) but high sex-specific variation (Copello, *et al.*, 2006). Several studies have described sexual dimorphism in body mass for gull species so this measurement should be use with caution due to the high seasonal variation (Croxal, 1995).

In this paper we studied the effectiveness of the discriminant function proposed by Bosch (1996) for the population of *Larus michahellis* from the Medes Islands in a different population of the same species but breeding at the Chafarinas Islands. We also explored how the inclusion of variables with high intraseasonal variation (e.g. body mass) may affect the results of the effectiveness of the discriminant functions.

Adult yellow-legged gulls were captured with a shooting net during the winter (February) (8 males and 19 females) and breeding season (April-May) (15 males and 24 females) of 2007 in a colony on the protected area Chafarinas Islands (Melilla, Spain: 35° 11' N 46' 35'' E) (see Ruiz *et* al., 1995 for further details of the sampling area). At present the islands hold *c.* 5602 breeding pairs (RNC Chafarinas' own data). Eight body measurements were taken by the same researcher: (i) tarsus length (TL) from the depression in the angle of the intertarsal joint to the base of the last complete scale before de toes diverge; (ii) wing length (WL) from the carpal joint to the tip of the longest primary; (iii) body-mass (BM); (iv) head plus bill length (HB) from the tip of the bill to the posterior ridge formed by the parietal-supraoccipital junction; (v) long bill length (LB) distance from the tip of the upper mandibule to the corner of the mouth; (vi) bill width (BW); (vii) nalospi (NA) distance from the tip to the bill to the nostril; (viii) bill depth (BD) minimum depth of the bill posterior to the gonys. Measurements of both wing and tarsus were taken on the right side of the body. Wing length was measured using a topper ruler (± 1 mm), while the rest of the measurements were measured using digital Vernier callipers (± 0.01 mm). All birds were weighed to the nearest 10 g. using Pesola® spring balances. Subsequent to being measured, all birds were sexed by molecular procedures (Fridolfsson and Ellegren, 1999) with a drop of blood (0.5 ml) obtained by venipuncture from the brachial vein of the gulls.

The measurements of 66 sexed gulls (27 males and 39 females), during the winter (n = 23) and breeding season (n = 43) were used to evaluate sexual size dimorphism and to obtain several discriminant functions. Multivariate analyses of variance (MANOVA) was used to determine whether the overall external morphology varied with sex and time of the season. We used the statistical package STATISTICA 6.0 (StatSoft. Inc. 2001) to apply the discriminant analysis to the biometric data of birds on known sex. The performance of each variable was evaluated using Wilks' Lambda, which decreases as discriminatory power increases and with the allocation rate of the model (the percentage of individuals correctly identified for each sex). To avoid discriminant functions to be tested against the same sample form which it was derived and

TABLE 1

Body measurements (mm. and gr.) of male and female yellow-legged gulls from the Chafarinas Islands.
[Medidas corporales (mm. y gr.) de machos y hembras de gaviota patiamarilla en las islas Chafarinas.]

Variable	**Males (N = 18) Mean ± SD**	**Females (N = 19) Mean ± SD**	**MANOVA**	
			F	**P**
Tarsus length (TL)	69.04 ± 4.94	66.26 ± 3.52	4.16	0.049
Wing-length (WL)	458.33 ± 11.17	439.47 ± 13.94	20.21	< 0.001
Body-mass (BM)	1091.22 ± 111.43	914.94 ± 107.17	20.95	< 0.001
Head-bill length (HB)	123.28 ± 17.46	118.89 ± 14.18	0.06	0.804
Bill-depth (BD)	28.13 ± 17.46	28.33 ± 18.83	0.09	0.766
Long Bill length (LB)	80.23 ± 8.11	77.55 ± 4.27	1.12	0.297
Bill width (BW)	13.28 ± 3.59	12.24 ± 3.96	0.44	0.509
Nalospi (NA)	33.16 ± 4.71	29.55 ± 4.89	6.29	0.017

not from an independent sample, resulting in an exaggerated effectiveness, we applied a jackknife procedure (Amat, *et al.,* 1993), in which each individual in the sample was classified using a discriminant function derived from the total sample, excluding the individual being classified (Chardine and Morris, 1989; Amat, *et al*., 1993). This algorithm chooses the function that has the lowest percentage of misclassification. Student's t tests were performed in order to compare Medes and Chafarinas Islands biometric measurements. Values reported are means ± SD.

We also applied Bosch function for yellow-legged gulls at the Medes Islands (Bosch, 1996: D1 = 1.403* HB + 5.135* BD + 0.114* WL + 0.262*TL – 366.988) to the data. Comparisons of the yellow-legged data presented in this study and those in Medes Island study (Bosch, 1996) indicate significant differences in all body measurements ($p < 0.0283$) except for head plus bill length in females ($p = 0.9480$) and wing-length in males ($p = 0.9271$).

Males were significantly heavier, with longer wings and tarsus and bigger nalospi measure than females, none of the other head and bill measurements showed significant differences between sexes (table 1).

Of the measurements that showed significant differences between the two sexes, only nalospi showed significant differences between males and females when introducing the seasonal period (MANOVA, males $F_{1,25} = 111.72$, $p < 0.0001$; females $F_{1,37} = 19.87$, $p < 0.0001$). Body mass showed significant differences between the winter and breeding season only in females (MANOVA $F_{1,37} = 5.1826$, $p = 0.02869$), (see table 2 and figure 1)

After the discriminant analysis, body mass emerged as the single most accurate indicator of sex, correctly identifying 84 % of the individuals (table 3). A function requiring only three measurements and excluding tarsus length was determined (D_1, table 3) as the best way to discriminate between sexes since it increases detectability up to 92 % of the individuals.

$D_1 = 0.0363\ WL + 0.0055\ BM + 0.1005\ NA – 24.9182$

Mass was included in one of the logistic regressions in despite of some papers (Rodriguez, *et al.*, 1996) which affirm that adults lose mass seasonally, both males and females (Pugesek and Diem, 1990). According to our data, the variation in body mass occurs only in females but not in males suggesting a higher energetic demand during reproduction despite of the presence of a rubbish dump nearby the

TABLE 2

Body measurements (mm. and gr.) of male and female yellow-legged gulls during winter and breeding seasons from the Chafarinas Islands.
[Medidas corporales (mm. y gr.) de machos y hembras de gaviota patiamarilla durante el invierno y la temporada de cría en las islas Chafarinas.]

Variable	**Males Mean ± SD**		**Females Mean ± SD**	
	Winter (8)	**Breeding (19)**	**Winter (15)**	**Breeding (24)**
Tarsus length (TL)	70.92 ± 2.64	69.61 ± 4.88	67.64 ± 4.57	64.52 ± 3.36
Wing-length (WL)	462.75 ± 15.01	459.84 ± 11.81	444.66 ± 17.00	380.08 ± 140.72
Body-mass (BM)	1053.25 ± 89.98	1112.10 ± 116.12	963.06 ± 124.84	864.16 ± 136.15
Head-bill length (HB)	129.12 ± 4.87	125.53 ± 21.99	122.11 ± 6.18	116.88 ± 17.26
Bill-depth (BD)		28.13 ± 17.46		28.33 ± 18.83
Long-Bill length (LB)	82.91 ± 3.29	81.01 ± 8.22	79.11 ± 5.04	73.00 ± 9.84
Bill width (BW)	19.29 ± 0.74	11.41 ± 0.73	18.79 ± 1.66	9.78 ± 0.88
Nalospi (NA)	25.40 ± 2.35	35.32 ± 2.17	23.21 ± 2.41	29.82 ± 5.39

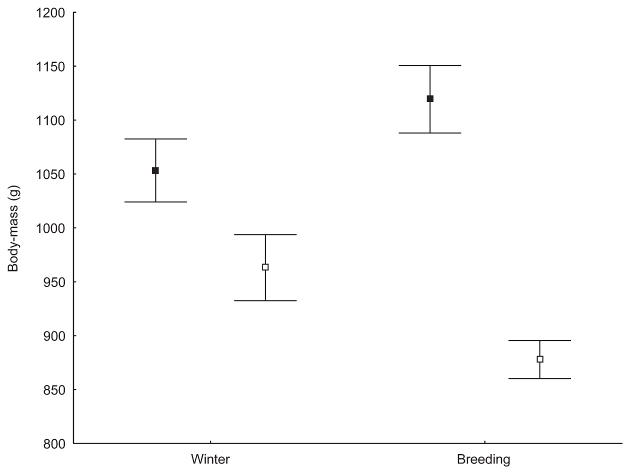

FIG. 1.—Body-mass of male (black) and female (white) yellow-legged gulls during winter and breeding season for the Chafarinas Islands.
[Peso de machos (negro) y hembras (blanco) de gaviota patiamarilla durante el invierno y la temporada de cría en las islas Chafarinas.]

colony (pers. obs.) which provides a constant and reliable source of food. However, the function excluding body mass and including tarsus, wing length and nalospi, (D_2 table 3) correctly sexed 86% of the individuals, without including any measurement that might change seasonally and therefore improving the accuracy of our function throughout the year.

TABLE 3

Accuracy of sexing yellow-legged gulls obtained by discriminant analysis using single measurements or combined functions.
[Precisión en el sexado de gaviotas patiamarillas obtenida mediante análisis discriminante usando medidas sencillas o funciones combinadas.]

Variable	F	Wilks' λ	Males (%)	Females (%)
Tarsus length (TS)	3.854	0.898	66.6 % (12 / 18)	78.9 % (15 / 19)
Wing-length (WL)	20.465	0.631	77.8 % (14 / 18)	78.9 % (15 / 19)
Body-mass (BW)	24.059	0.593	83.3 % (15 / 18)	84.2 % (16 / 19)
Nalospi (NA)	5.148	0.872	77.8 % (14 / 18)	47.4 % (9 / 19)
Combined function				
Bosch (1996)		0.114	27.7 % (5 / 18)	68.4 % (13 / 19)
Present study D1	11.78	0.483	88.9 % (16 / 18)	94.7 % (18 / 19)
Present study D2	9.542	0.527	83.3 % (15 / 18)	89.5 % (17 / 19)

$D_2 = 0.0617\ TL + 0.0658\ WL + 0.1075\ NA - 37.0547$

To apply a discriminant analysis function derived from one population to populations of the same subspecies in different geographic locations has been proven ineffective in the case of the yellow-legged gull. Bosch (1996) pointed out that measurements of gulls from the Medes Islands do not significantly differ from those taken from gulls at colonies as far away as the Chafarinas Islands, nevertheless, we were only able to correctly sex 52 % of the individuals using that function.

The three variables needed for our discriminant function are easy to obtain and allow birds to be released unharmed. However the usefulness of the discriminant function on the same population in other times of the season should be confirmed. Bosch's (1996) study was carried out at a different colony than this study, and whereas one cannot discount the possibility that there are morphological differences between the various colonies along the Mediterranean, one likely cause may be the differences in measurements taken by different researchers. Therefore, comparative studies with data derived by different colonies of the same species should be conducted with caution.

ACKNOWLEDGEMENTS.—This research was funded by the Organismo Autónomo de Parques Nacionales. We thank the Reserva Nacional de Caza de las Islas Chafarinas and its director. P. Laiolo provided advice on statistical analysis; E. Banda reviewed the early versions and two anonymous referees and F. de Lope substantially improved the manuscript.

BIBLIOGRAPHY

AMAT, J. A., VIÑUELA, J. and FERRER, M. 1993. Sexing Chinstrap Penguins (*Pygoscelis antarctica*) by morphological measurements. Colon. *Waterbirds*, 16: 213-215.

ARIZAGA, J., ALDALUR, A., HERRERO, A. and GALICIA, D. 2008. Sex differentiation of Yellow-legged Gull (*Larus michahellis lusitanius*): the use of biometrics, bill morphometrics and wing tip coloration. *Waterbirds*, 31(2): 211-219.

BERTELLOTTI, M., TELLA, J. L., GODOY, J. A., BLANCO, G., FORERO, M., DONÁZAR, J. A. and CEBALLOS, O. 2002. Determining sex of Magellanic Penguins using mollecular procedures and discriminant functions. *Waterbirds*, 25: 479-484.

BLUSO, J. D., ACKERMAN, J. T., TAKEKAWA, J. Y. and YEE, J. L. 2006. Sexing Forster's Terns using morphometric measurements. *Waterbirds*, 29: 512-517.

BOSCH, M. 1996. Sexual size dimorphism and determination of sex in Yellow-legged Gulls. *Journal of Field Ornithology*, 67: 534-541.

CHARDINE, J. W. and MORRIS, R. D. 1989. Sexual size dimorphism and assortative mating in the Brown, Noddy. *Condor,* 91: 868-874.

COPELLO, S., QUINTANA, F. and SOMOZA, G., 2006. Sex determination and sexual size-dimorphism in Southern Giant-Petrels (*Macronectes giganteus*) from Patagonia, Argentina. *Emu,* 106: 141-146.

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

ELLEGREN, H. and SHELDON, B. C. 1997. New tools for sex identification and the study of sex allocation in birds. *Trends in Ecology and Evolution,* 12: 255-259.

EVANS, D. R., HOOPES, E. M. and GRIFFIN, C. R. 1993. Discriminating the sex of Laughing Gulls by linear measurements. *Journal of Field Ornithology,* 64: 472-476.

FRIDOLFSSON, A. K. and ELLEGREN, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology,* 30: 116-121.

GALARZA, A., HIDALGO, J., OCIO, G. and RODRÍGUEZ, P. 2008. Sexual size dimorphism and determination of sex in Atlantic yellow-legged gulls *Larus michahellis lusitanius* from Northern Spain. *Ardeola*, 55(1): 41-47.

GENOVART, M., MCMINN, M. and BOWLER, D. 2003. A Discriminant function for predicting sex in the Balearic Shearwater. *Waterbirds,* 26: 72-76.

PUGESEK, B. H. and DIEM, K. L. 1990. The relationship between reproduction and survival in Known-Aged California Gulls. *Ecology,* 71: 811-817.

RODRÍGUEZ, E. F., PUGESEK, B. H. and DIEM, K L. 1996. A sexing technique for California Gulls breeding at Bamforth lake, Wyoming. *Journal of Field Ornithology*, 67: 519-524.

RUIZ, X., ORO, D. and GONZÁLEZ-SOLÍS, J. 1995. Incidence of a Haemoproteus lari parasitemia in a threatened Gull: *Larus audouinii*. *Ornis Fennica*, 72: 159-164.

[Recibido: 17-03-09]
[Aceptado: 05-10-09]