

## **Gender Determination in the Western Marsh Harrier (*Circus aeruginosus*) Using Morphometrics and Discriminant Analysis**

Authors: Bavoux, Christian, Burneleau, Guy, and Bretagnolle, Vincent

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## GENDER DETERMINATION IN THE WESTERN MARSH HARRIER (*CIRCUS AERUGINOSUS*) USING MORPHOMETRICS AND DISCRIMINANT ANALYSIS

CHRISTIAN BAVOUX

*Le Marais aux Oiseaux, Les Grissotières, 17550 Dolus-d'Oléron, France*

GUY BURNELEAU

*2, rue du Port de Chiffeu, Mauzac, 17320 Saint-Just-Luzac, France*

VINCENT BRETAGNOLLE<sup>1</sup>

*Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique,  
79360 Beauvoir-sur-Niort, France*

**ABSTRACT.**—When adult, the gender of Western Marsh Harriers (*Circus aeruginosus*) is determined on the basis of the presence of gray feathers on the wing and on the tail in the males. However, males of this species in southwestern France have female-like plumages, lacking gray feathers, and thus, gender determination is impossible by using coloration alone. We investigated sex determination using biometric parameters in a study site located in southwestern France. From one to six measurements (body mass, bill length, wing chord length, tarsus length and width, and tail length) were taken from 243 marsh harriers of known gender (156 males and 87 females), marked in the study site, and subsequently re-sighted. Additionally, 30 marsh harrier specimens were measured by two observers to establish within and between observer repeatability values, as well as within bird repeatability for the six biometric parameters. Using quadratic discriminant analysis, we showed that it was possible to sex individuals of this species with >99% accuracy on the basis of only two parameters: bill length and body mass. Actually, using bill length alone provides very good indication of gender (accuracy >96%). These two parameters are also those for which observer and within-bird repeatability values were highest. When we applied the discriminant equation to 648 fledged birds that have been captured so far, the adult sex ratio (percent of males) was 49.1, which did not depart from parity.

**KEY WORDS:** Western Marsh Harrier; *Circus aeruginosus*; sexual dimorphism; biometrics; discriminant analyses; gender determination; repeatability; sex ratio.

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### DETERMINACIÓN DEL SEXO EN *CIRCUS AERUGINOSUS* USANDO MORFOMETRÍA Y ANÁLISIS DISCRIMINANTE

**RESUMEN.**—El sexo de los individuos adultos de *Circus aeruginosus* puede determinarse con base en la presencia de plumas grises en las alas y la cola de los machos. Sin embargo, los machos de esta especie que habitan en el suroeste de Francia tienen plumajes parecidos a los de las hembras (carecen de plumas grises), por lo que determinar el sexo sólo con base en la coloración es imposible. Investigamos la determinación del sexo empleando parámetros biométricos en un sitio de estudio ubicado en el suroeste de Francia. Se tomaron entre una y seis medidas (peso corporal, longitud del pico, longitud de la cuerda alar, longitud del tarso, ancho del tarso y longitud de la cola) en 243 individuos de sexo conocido (156 machos y 87 hembras) que fueron marcados en el sitio de estudio y posteriormente observados nuevamente. Además, 30 especímenes de esta especie fueron medidos por dos observadores para establecer la repetibilidad de las mediciones de un observador y entre observadores, además de la repetibilidad de las mediciones de los seis parámetros biométricos en aves individuales. Usando un análisis discriminante cuadrático, demostramos que es posible determinar el sexo de los individuos en *C. aeruginosus* con más del 99% de exactitud con base en sólo dos parámetros: la longitud del pico y el peso corporal. De hecho, el uso de sólo la longitud del pico provee una muy buena indicación del sexo (exactitud

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<sup>1</sup> Email address: breta@cebc.cnrs.fr

mayor que el 96%). Además, para estos dos parámetros la repetibilidad de las mediciones, tanto para los observadores como para las aves, es máxima. Cuando aplicamos la ecuación discriminante a las 648 aves que han sido capturadas hasta ahora, el cociente de sexos de los adultos (porcentaje de machos) fue de 49.1, un valor que no difiere de la igualdad.

The sexing of individuals in monomorphic bird species has always been problematic, although of utmost importance for many behavioral and ecological studies. Discriminant analyses performed on morphological measurements are now widely used for sexing birds in such cases (e.g., Gruys and Hannon 1993, Counsilman et al. 1994, Genevois and Bretagnolle 1995, Sweeney and Tatner 1996, Calvo and Bolton 1997). Raptors are generally not as problematic, because most species are measurably dimorphic (Newton 1979) and sometimes dichromatic (Ferguson-Lees and Christie 2001).

Among raptors, juvenile harriers (*Circus* spp.) are not noticeably dimorphic (Simmons 2000), although most species are dichromatic when adult. The Western Marsh Harrier (*Circus aeruginosus*) is no exception: sexual dimorphism is rather weak (males are 14% smaller than females, based on cube root wing chord length, following Ferguson-Lees and Christie 2001). However, males are strikingly different in plumage compared to females, with gray secondaries and tail feathers (e.g., Porter et al. 1981, González 1991, del Hoyo et al. 1994, Clarke 1995, Génsbøl 1999). This plumage coloration varies with sex and age. More importantly, while this species is reported to be clearly dichromatic over its distribution range (Clarke 1995 and Génsbøl 1999), in some populations, especially in western France, males have female-like plumages (Bavoux et al. 1988, 1993). In such cases, gender determination based on coloration can be difficult or confusing, and sometimes impossible. Moreover, fledglings of both sexes have similar female-like plumage, and, in contrast with Montagu's Harriers (*C. pygargus*; Leroux and Bretagnolle 1996) and Northern Harriers (*C. cyaneus*; Balfour 1970), eye color does not vary between the sexes (Bavoux et al. 1991, 1993).

Therefore, for the Western Marsh Harrier, analysis of morphometrics represents an important method for gender determination in fledglings (see Zijlstra et al. 1992 for a methodology based on tarsus). For fledged or adult birds (in their first year or older), a discriminant analysis has been used successfully to assign gender (Bavoux et al. 1988). Here we: (1) reanalyze such data using a much larger data set, (2)

establish repeatability estimates (within and between observers and within birds) to determine the least-biased biometric measurements, and (3) use our analyses to classify the gender of all birds captured so far in this population.

#### METHODS

**Study Species and Locality.** Since 1982, the Western Marsh Harrier was intensively studied by two of the authors (C. Bavoux and G. Burneleau), in the marshes of Charente-Maritime (45°57'N, 01°09'W; western France). The species is relatively abundant in this area (between 200–250 nesting pairs in 120 km<sup>2</sup>, 2000–02), with locally high breeding densities (e.g., 35 laying pairs in 15.6 km<sup>2</sup>; Bavoux and Burneleau 2004). Chicks have been color ringed since 1983 and fledged and adult birds since 1984. Harriers have been ringed and wing-tagged since 1987. To date, a total of 2240 birds have been ringed or wing-tagged in the study area.

**Capture and Measurements.** Our data set is composed of 652 different fledged birds trapped between 1984–2003. Most of them ( $N = 632$ ) were trapped in the wild throughout the year. The other birds ( $N = 20$ ) were found wounded or sick, brought to a recovery center, and subsequently released in the wild in the study area.

These 652 individuals produced, over the study period, 824 captures (including 31 “captures” reported by the recovery center). We caught 510 birds only once, 116 individuals twice, 24 individuals three times, and two individuals four and six times, respectively. Several different techniques resulted in 793 captures in the wild: 456 were made at night roosts, principally with mist nets, and 337 in daylight (252 by clap-nets [Bub 1991] baited generally with mammalian lung tissues, 57 with bal-chatri traps with a European Starling [*Sturnus vulgaris*] as a lure, and 28 by other techniques). The birds were released 1–2 hr after capture during daylight or at dawn the next morning when captured in the evening and when weather conditions were too adverse for a safe release. Capture effort was not constant through years or seasons: most catches (85.2%) were made from October–March. From April–October, the majority of captures was in daylight (84.7%), while captures at roosts were more numerous (66.3%) from November–March.

Once captured, the birds could not normally be sexed with certainty, except in the presence of light or dark gray coloring on the feathers of the wings or tail that verified the bird was male. However, once wing-tagged, some birds could be sexed later by their breeding behavior: females could be classified by their breeding behavior (incubation behavior because only females incubate, copulation position, or the presence of a brood patch). We verified the gender of 245 individual birds: 156 males and 89 females.

At least two biometric parameters were measured for 644 of the 652 birds captured: (1) body mass—measured within 10 g with a spring-balance (1983–89) and then within 5 g with an electronic-balance and (2) bill length—from the edge of the cere to the tip of the bill ( $\pm 0.1$  mm). Additionally, the wing chord length ( $\pm 1$  mm), tarsus length and width ( $\pm 0.1$  mm following Spencer 1984), and the tail length ( $\pm 1$  mm) were measured for different subsamples of birds. Therefore, sample size varied according to the variable under analysis.

Of the 652 measured birds, 243 were considered local (resident) birds, defined as those either hatched in the study area or present during the breeding period. Three birds were reared elsewhere (one in the United Kingdom and two in the Netherlands, based on rings) and were trapped during migration (April and October). The origin of all other birds was unknown.

**Statistical Analyses.** Univariate comparisons (analysis of variance [ANOVA]) were first performed for each biometric character. Then, we used multiple analysis of variance (MANOVA) to obtain multivariate statistics (Wilks's lambda statistics) and carried out quadratic discriminant function analysis (DFA) on the biometric parameters. We used quadratic DFA rather than linear DFA as the criterion of homoscedasticity was not met (except in one case). DFA is a multivariate technique which combines variables in order to maximize separation between groups (Pimentel 1979, James and MacCulloch 1990). It provides a quantitative measure on the discriminatory power of the functions in allocating single observations into the *a priori* group with the closest centroid in the multivariate space (Footitt and MacKauer 1980). Thus, the percentage of correctly classified observations into their *a priori* groups gives an indication of the extent of the separation. Following each analysis, four estimates of classification power were calculated. The first one, derived from re-substitution of all individuals, provides an apparent error rate (the error count), but has an optimistic bias. The second was obtained by a cross-validation technique (a jackknife procedure) and is probably more realistic. However, both measures have a high variance, due to the allocation rule which is either 0 or 1 depending on whether the observation is incorrectly or correctly classified. Therefore, we also applied to both re-substitution and cross validation techniques, a smoothing procedure, which uses a continuum of values between 0 and 1 (i.e., the probability of allocation of the observation in each of the possible groups). This latter technique assumes that the population follows a normal distribution (SAS 1999). The Stepwise Discriminant Function was also used to detect the biometric parameters that were the most informative in discriminating gender, taking into account the inter-correlation among variables. Finally, canonical Discriminant Analysis was used to derive scores for each individual to provide a graphic representation of gender separation.

To assess that the biometric characters that had the most discrimination power were also highly repeatable (i.e., provided similar values in successive measurements), we established among- and within-observer, as well as within-bird, repeatability values for each parameter. To this end, we used a sample of marsh harrier specimens ( $N = 30$ ). All birds were measured by two of the authors (C. Bavoux and

G. Burneleau) twice. The birds were selected at random, and the observers never knew which birds they were measuring. Additionally, we used birds captured twice in the wild (for birds captured more than twice, we used only the first two measurements) and applied the same repeatability analysis. To calculate repeatability, we used ANOVA, between and within observers or between and within birds, for each biometric character. We used the methodology described in Lessels and Boag (1987) to calculate repeatability, although much simplified, as the sample size was always identical (two repeated measurements for each observer or bird).

We checked for normality of variables, before performing parametric tests. All statistical analyses were performed on SAS 8.0 software (SAS 1999).

## RESULTS

We found that females were significantly larger than males for all six characters measured (Table 1). Using mean values for birds that were captured more than once did not change the mean values (at least at the first decimal place). The assumption of normality held for most biometric variables (Table 1), although in some cases it differed according to gender. The magnitude of sexual dimorphism varied with the biometric measurement employed.

Repeatability values were high in general (Table 2), with all *F*-values providing significance probabilities below 0.001. Repeatability within and between observers was particularly high for the body mass and the wing chord length, and to a lesser extent, for bill and tail lengths. Conversely, the tarsus measurements had a relatively low repeatability. Repeatability within birds was highest for bill length, and least for tarsus width, tail length, and body mass. In contrast with observer repeatability, bird repeatability values were obtained over longer time periods, typically spanning one or more years. Overall, by combining the highest values for the observer and the bird, the biometric characters that showed the highest repeatability were the body mass and the bill length (Table 2).

The birds were captured over a 20-yr period, in a variety of contexts; in some cases, different types of baits were used, and many birds were caught on roosts, but others were not. We tested the potential effects of these additional factors on biometry using a two-way ANOVA, with gender being one of the explanatory factors. Significant effects on the body mass were detected for year ( $F = 2.85$ ,  $P = 0.0001$ ), hour ( $F = 3.49$ ,  $P < 0.0001$ ), and the bait used for capture ( $F = 21.5$ ,  $P < 0.0001$ ), and on tail measurement also for the presence of bait ( $F =$

Table 1. Summary statistics for the six morphometric parameters of Western Marsh Harriers from western France (1983–2004), according to gender (only birds definitively sexed are considered: 156 males and 87 females). An ANOVA was performed to test for a significant effect of gender. Normality was tested using Shapiro-Wilk statistic. Sample size refers to the number of measurements available; not all measurements were collected on all birds.

CAPTURES OF BIRDS OF KNOWN SEX		STATISTIC						
		BODY MASS (g)	BILL (mm)	WING CHORD (mm)	TARSUS LENGTH (mm)	TARSUS WIDTH (mm)	TAIL (mm)	
Males	Mean	609.0	22.3	389.9	85.6	7.9	213.5	
	SD	55.1	0.8	10.2	2.6	0.5	10.7	
	Range	460–750	20.4–24.6	353–416	79.6–96.8	7.0–9.4	165.0–259.0	
	N	213	213	130	107	106	104	
Females	Mean	813.8	25.2	411.1	90.5	8.8	226.5	
	SD	77.6	0.8	10.8	3.0	0.5	10.4	
	Range	640–1030	22.5–27.3	382–430	85.8–97.4	7.5–9.9	205.0–250.0	
	N	121	120	74	55	51	47	
Test for normality Males	W	0.99	0.99	0.95	0.97	0.98	0.89	
	(Pvalue)	0.68	0.08	0.001	0.02	0.18	0.001	
Test for normality Females	W	0.98	0.98	0.97	0.95	0.98	0.98	
	(Pvalue)	0.10	0.12	0.06	0.02	0.71	0.53	
Between sex comparison	F-statistic (Pvalue)	805.2 0.0001	1059.2 0.0001	194.3 0.0001	117.9 0.0001	119.0 0.0001	47.5 0.0001	

6.09,  $P = 0.03$ ). However, in all cases, the significance of such other factors was much lower than the gender effect, and in none of the cases were the interactions significant.

The data set with birds of known sex, whether captured once or more, and with complete measurements (i.e., the six morphometric variables), contained 144 captures: 100 for males and 44 for females. Three dependant variables (bill length, body mass, and tarsus width, in order of importance) entered a stepwise discriminant function with gender as the categorical variable. Using a stratified data set in which each bird was only present once (using mean biometric values when a bird was caught more than once), which contained 113 different birds (80 males and 33

Table 2. Repeatability values for each morphometric parameter measured on a sample of 30 Western Marsh Harrier specimens that were measured twice (first and second measure) by two observers (first and second), and a subset of live marsh harrier that were measured twice in the field. Within-bird repeatability (in this subset, only birds captured twice were used) is given with  $F$ -value. All repeatability values were significant at least at  $P = 0.001$ .

BIOMETRIC CHARACTERS	MEASUREMENT REPEATABILITY (N = 30 BIRDS) <sup>a</sup>							
	MEASURE		OBSERVER		OVERALL	BIRD REPEATABILITY		
	FIRST	SECOND	FIRST	SECOND		N	F-VALUE	REPEATABILITY
Body mass	1.000	1.000	1.000	1.000	1.000	109	7.89	0.775
Bill length	0.859	0.952	0.946	0.973	0.961	106	19.86	0.904
Wing chord	0.997	0.997	0.997	0.996	0.998	53	12.74	0.854
Tarsus length	0.716	0.668	0.919	0.866	0.862	47	11.21	0.836
Tarsus width	0.728	0.690	0.662	0.692	0.821	48	3.50	0.556
Tail	0.916	0.917	0.969	0.976	0.966	45	5.37	0.686

<sup>a</sup> “First measure probability” (left column) was calculated using the first measurement made by the two observers; similarly, “first observer repeatability” was calculated using the two measurements made by the first observer.

Table 3. Results of the discriminant function analyses on gender classification according to the number of parameters used. Due to heterogeneity between variances in groups, a within-group covariance matrix and quadratic discriminant functions were used except for the second analysis where a linear discriminant analysis was performed. Discriminant power is evaluated using re-substitution and cross validation (see Methods). The percentage of correctly classified birds, and posterior probabilities of error rate in classification are reported (posterior probabilities are smoothed error rates).

PARAMETERS	N <sup>a</sup>		PERCENT CORRECT CLASSIFICATION				POSTERIOR PROBABILITY ERROR RATE	
			RE-SUBSTITUTION		CROSS VALIDATION		RE-SUBSTITUTION	CROSS VALIDATION
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES		
Mass, bill, wing, tarsus length and width, tail	100	44	100	97.7	100	97.7	0.009	0.012
Mass, bill, tarsus width	106	49	100	98.0	100	98.0	0.008	0.009
Mass, bill	213	119	99.1	97.5	99.1	97.5	0.012	0.012
	(205) <sup>a</sup>	(113)	(99.0)	(98.2)	(99.0)	(98.2)		
Bill	213	120	97.7	96.7	97.7	96.7	0.027	0.027
	(205)	(114)	(98.0)	(97.4)	(98.0)	(97.4)		

<sup>a</sup> Values in parentheses refer to analyses that excluded birds from the recovery center.

females), did not change the results of the stepwise discriminant analysis.

When using at least two biometric parameters (body mass and bill length) or more, error count estimates were always low; only two males were misclassified in one of the analyses using re-substitution (no males were misclassified in the other analyses), and between one and three females were misclassified (Table 3). Using the cross-validation technique did not reduce the statistical power of the discrimination, which remained more or less identical. Posterior smoothed error probabilities were less, or about 0.01, in all analyses with at least two parameters. Therefore, the gender of a bird could be determined using these discriminant functions with a confidence of 99%, even on the basis of only two measurements (Fig. 1), using the following formula:

Gender Index = 0.0000769 × (body mass)

+ 0.5645 × (bill length) − 13.2205

Gender Index for males have negative values and for females, positive values.

Actually, using a single measurement, the bill length, provided reliable gender determination results, with a threshold value of 23.6 mm for this marsh harrier population (Table 3). Only 3 or 4 of the 120 females and 2–5 of the 213 males were misclassified on the sole basis of bill length (error

rates below 4% in all cases and posteriori error rates probabilities less than 0.03). Birds from the recovery center might have altered slightly the discriminant functions, as these birds were more likely in bad physical condition. However, excluding such birds from the sample did not change the results (Table 3).

We used the discriminant function (based on body mass and bill length) to assign gender to the remaining 403 birds of unknown sex for which these two measurements were available. They were distributed between 162 males and 241 females. Overall, of 648 birds for which we could classify, 318 were males and 330 females. The adult sex ratio (percent of males) of 49.1 did not differ from parity (two-sided binomial test,  $P = 0.67$ ). Using the larger data set, we used a MANOVA to test for a population variation in sexual dimorphism among local versus others (including birds of unknown origin and the three birds from outside the study area). The effect was not significant (population: Wilks's lambda = 0.97,  $F = 1.48$ ,  $P = 0.18$ ; interaction: Wilks's lambda = 0.97,  $F = 1.57$ ,  $P = 0.15$ ).

DISCUSSION

**Gender Discrimination in the Western Marsh Harrier.** Female marsh harriers were larger than males for all measurements, particularly so for bill length and, to a lesser extent, body mass (Table 1). Within our study population, the Reversed Size



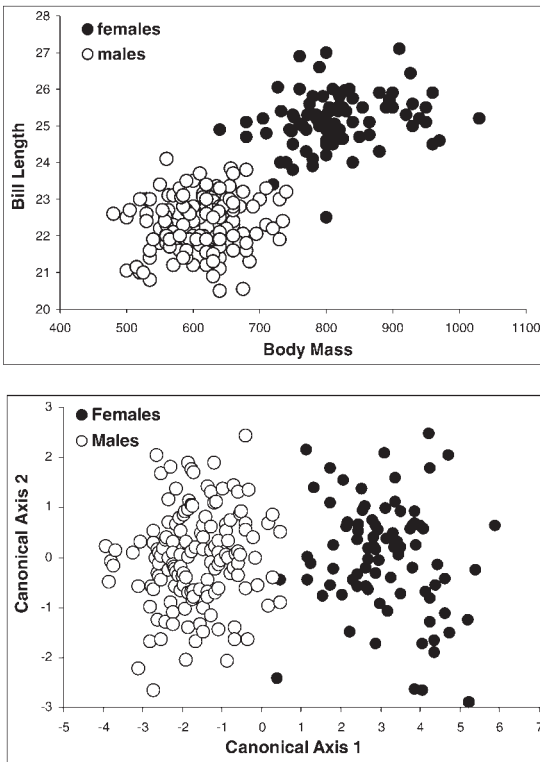


Figure 1. Comparison between bivariate plots of male and female measurements of Western Marsh Harriers from western France (1983–2004), based either on body mass and bill length (upper figure) or on canonical scores (lower figure). The Discriminant Analysis was performed on birds of known gender (156 males and 85 females).

Dimorphism index (RSD, difference in mass between the gender following Simmons 2000) was 29, indicating that the marsh harrier is one of the least dimorphic species in the genus *Circus*. The other measured characters explained very little residual variance, probably because of their tight correlation with body mass and bill length. A discriminant function including body mass and bill length correctly sexed more than 99% of the individuals (Table 3), a result that cannot be improved much further, even using genetic markers. These two biometric parameters were furthermore shown to have high levels of repeatability, both between and within observer's measurements, but also within birds (Table 2). This is surprising, given that: (1) body mass is highly variable in this species and (2) consecutive measurements of the same individual spanned periods of up to 10 yr.

There are few published data on sexual dimorphism in the Western Marsh Harrier, and most of them are based on small samples of museum specimens (e.g., Cramp and Simmons 1980, Glutz von Blotzheim et al. 1989, González 1991, Clarke 1995). The first study with a large sample size of live birds was Bavoux et al. (1988). Error rates found in our study are actually very close to those found by Bavoux et al. (1988), though the sample size is now far greater (243 birds of known sex analyzed against the previous 108 in Bavoux et al. [1988]). We found that bill length alone has a high classification reliability. Moreover, it is highly repeatable within birds, thus providing an effective technique to classify correctly >96% of birds (compared to Bavoux et al. [1988] correct classification of 65%). Presumably, bill length is a better predictor than body mass because the latter can vary considerably within individuals according to physiological status.

Our gender determination formula is presumably valid only for our local population, but could be applied to other populations by using the method developed by Biernacki et al. (2002). The absence of differences in biometry between the local birds and those of unknown origin may suggest that there is relatively little geographic variation in body size in this species or that birds of unknown origin are actually breeding close to our study site. The biometric data already collected elsewhere on the Western Marsh Harriers show rather high geographical variation in body size (Cramp and Simmons 1980, Bavoux et al. 1988, Glutz von Blotzheim et al. 1989, Simmons 2000). Therefore, we suggest that the birds of unknown origin in this study are probably birds from nearby areas. This is not surprising because the Western Marsh Harrier is largely sedentary in our region, irrespective of age and sex (Bavoux et al. 1992, 1994, 1995, 1997). Our study site is an important wintering area; in the *Marais de Brouage* (about 120 km<sup>2</sup>), ca. 400 birds were counted at roosts in mid-January 2003. Although the possibility remains that birds of more northern populations winter in this area, up to now, there are no ringing data to support this suggestion.

**Western Marsh Harrier Sex Ratio.** Avian sex ratio does not generally depart from parity (Clutton-Brock 1986). However, in harriers, secondary sex ratios (at the fledgling stage) were found to differ from parity in the three European species (Northern Harrier—Picozzi 1984; Montagu's Harrier—Leroux and Bretagnolle 1996, Arroyo 2002, Millon

and Bretagnolle 2005; Marsh Harrier—Zijlstra et al. 1992). In our population, we found an unbiased adult sex ratio. This was unexpected because polygyny is relatively common in our study population (Bavoux et al. 1995, 1998), and polygyny has been suggested, among other hypotheses, to result from a biased sex ratio; specifically, excess adult females may promote polygamy (Simmons 2000).

All other published results on Western Marsh Harrier sex ratio suggested biased sex ratios. In the Netherlands, Zijlstra et al. (1992) found a significant excess of males (54.8%) for 2260 nestlings ( $N = 735$  broods). Adult sex ratio data on Western Marsh Harrier populations are not numerous and were collected in wintering populations. In Spain, there was a slight bias toward females (González 1991, Martínez and Ortega 1995, Estrada et al. 1995). In the Ebro Delta for instance, 1982 marsh harriers were observed in the winter of 1993–94 (Estrada et al. 1995): 65% of the birds were juveniles (males and females), 13% males (5% immature and 8% adults), and 22% females (immature and adults). Similarly, Clarke (1995) found that females (and juveniles) tend to predominate at winter sites in the Netherlands. In contrast with the Dutch and Spanish studies, in our study population the sex ratio was unbiased, at least for fledged birds and adults. However, sex ratio data from wintering populations are based on the proportion of gray birds, specifically, males (e.g., Schipper et al. 1975, Zijlstra 1987, Clarke et al. 1993, Arroyo and King 1995). Unfortunately, at least in some populations, adult males are female-like. In our study site, a study based solely on sightings would have also provided a biased sex ratio toward females: among the 3966 Western Marsh Harriers observed in roosts from October to March, only 12.4% were gray males (Bavoux et al. 1997), a similar percentage to that obtained by Estrada et al. (1995). In conclusion, we advocate extreme caution when using data on population structure with regard to sex-ratio in the Western Marsh Harrier when based only on sightings, because of the existence of large male plumage variability. Our study highlights that sex ratio data should come from a large number of captured individuals in this species.

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