Morphometric sex determination of young Ospreys *Pandion haliaetus* using discriminant analysis

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Capsule Discriminant functions based on morphometric variables provide a reliable method for sex identification of free-living and hacked young Ospreys.

Aims To describe an easy, accurate and low-cost method for sex determination of fully grown nestling and fledgling Ospreys *Pandion haliaetus* based on morphometric measurements.

Methods Four different measurements were taken in 114 birds (40–73 days old) and a DNA analysis, using PCR amplification, was carried out for sex identification. A forward stepwise discriminant analysis was performed to build the best explanatory discriminant models, which were subsequently validated using statistics and external samples.

Results Our best discriminant function retained forearm and tarsus as the best predictor variables and classified 95.1% of the sample correctly, supported also by external cross-validations with both hacked and free-living birds. Moreover, a discriminant function with only forearm as predictor showed a similar high correct classification power (93.4%).

Conclusions These discriminant functions can be used as a reliable and immediate method for sex determination of young Ospreys since they showed high discriminant accuracy, close to that of molecular procedures, and were supported by external cross-validations, both for free-living and hacked birds. Thus, these morphometric measurements should be considered as standard tools for future scientific studies and management of Osprey populations

ACCURATE AND EASY METHODS TO DETERMINE THE SEX OF INDIVIDUALS ARE VALUABLE FOR THE STUDY OF DIFFERENT ASPECTS OF AVIAN BIOLOGY SUCH AS EVOLUTIONARY ECOLOGY AND GENETICS (CLUTTON-BROCK 1986, BENNETT & OWENS 2002), POPULATION DYNAMICS (NEWTON 1998), BEHAVIOUR, DISPERSION/MIGRATION AND CONSERVATION GENETICS (GRIFFITH & TIWARI 1995, SUTHERLAND *ET AL.* 2004), AND ALSO FOR THE ACTIVE MANAGEMENT OF SPECIES AND POPULATIONS (SARRAZIN & BARBAULT 1996, BIRD & BILDSTEIN 2007). DISCRIMINANT FUNCTION ANALYSIS HAS BEEN LARGELY USED AS A TOOL FOR SEXING BIRDS WHICH DO NOT SHOW SEXUAL DIMORPHISM IN PLUMAGE, BUT WHICH DO SHOW SEXUAL SIZE DIMORPHISM (BRENNAN *ET AL.* 1991). EXTERNAL MORPHOMETRIC MEASUREMENTS ARE TAKEN FROM BIRDS OF KNOWN SEX TO DEVELOP AND TEST DISCRIMINANT FUNCTIONS USING SKINS FROM MUSEUMS AND COLLECTIONS, FRESHLY DEAD ANIMALS AND LIVE ANIMALS, BOTH CAPTIVE AND WILD (BORTOLOTTI 1984, COUNSILMAN *ET AL.* 1994, DONOHUE & DUFTY 2006). METHODOLOGICAL STUDIES ASSESSING THE POWER AND LIMITATIONS OF THESE DISCRIMINANT TECHNIQUES HAVE ALSO BEEN PUBLISHED (WILLIAMS 1983, BRENNAN *ET AL.* 1991).

THE BIOLOGY AND ECOLOGY OF OSPREYS *PANDION HALIAETUS* HAVE BEEN WIDELY STUDIED FROM DIFFERENT POINTS OF VIEW (POOLE 1989, SAUROLA 1997, KJELLÉN *ET AL.* 2001), AND SEVERAL CONSERVATION PROGRAMMES INVOLVING THE REINTRODUCTION OF NESTLING INDIVIDUALS HAVE BEEN CARRIED OUT IN NORTH AMERICA AND EUROPE (RYMON 1989, MARTELL 1995, DENNIS & DIXON 2001, CASADO & FERRER 2005). THE SEXING OF YOUNG OSPREYS, WHICH ARE OFTEN RINGED,

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MEASURED OR FITTED WITH RADIO TRANSMITTERS AS NESTLINGS AS PART OF SUCH MONITORING AND CONSERVATION STUDIES, IS CRUCIAL. ALTHOUGH SEX DETERMINATION USING DISCRIMINANT ANALYSIS HAS BEEN EXTENSIVELY APPLIED FOR BIRDS OF PREY, ONLY A FEW DESCRIPTIVE STUDIES HAVE BEEN PERFORMED IN ADULT OSPREYS (MACNAMARA 1977, POOLE 1989, SCHMIDT *ET AL.* 2000). IN THOSE STUDIES SEX IDENTIFICATION WAS ACCOMPLISHED BY MEANS OF BODY WEIGHT, BIOMETRIC MEASUREMENTS, PLUMAGE CHARACTERISTICS, NESTING BEHAVIOUR OR A MULTIVARIATE COMBINATION. HOWEVER, NO RELIABLE CRITERIA HAVE BEEN DESCRIBED FOR SEX IDENTIFICATION OF YOUNG OSPREYS. ONLY SCHAADT & BIRD (1993) PERFORMED A SEX-SPECIFIC STUDY ON GROWTH IN NORTH-AMERICAN OSPREY NESTLINGS IN WHICH SEX WAS DETERMINED ON THE BASIS OF DISTINCT MASS CLASSES THAT WERE SUBSEQUENTLY CONFIRMED USING KARYOTYPE ANALYSIS AND BY A NON-SPECIFIED DISCRIMINANT ON A SMALL SUBSAMPLE OF 17 INDIVIDUALS. GIVEN THIS LACK OF PUBLISHED METHODOLOGY, AN EASY AND ACCURATE SEXING TECHNIQUE FOR NESTLINGS AND FLEDGLINGS OF THIS SPECIES WOULD PROVIDE A USEFUL TOOL FOR SCIENTISTS AND FOR PEOPLE INVOLVED IN THE MANAGEMENT OF OSPREYS.

THE AIM OF THIS STUDY WAS TO FIND A VERSATILE AND RELIABLE MORPHOMETRIC CRITERION FOR SEX DETERMINATION IN NESTLING AND PRE-FLEDGLING EURASIAN OSPREYS USING DISCRIMINANT MODELS.

METHODS

Sampled birds

ONE HUNDRED AND FOURTEEN YOUNG OSPREYS WERE STUDIED. OF THESE, 91 WERE INVOLVED IN REINTRODUCTION PROGRAMMES IN THE PROVINCES OF CÁDIZ (36°24′N, 05°44′W) AND HUELVA (37°15′N, 06°55′W), SOUTHWEST SPAIN. THESE PROGRAMMES STARTED IN 2003 AND 2004 RESPECTIVELY, AND BY 2009, 129 BIRDS HAD BEEN RELEASED IN THE TWO AREAS BY MEANS OF HACKING TECHNIQUES. ALL OF THEM WERE WILD-HATCHED BIRDS OF THE PALAEARCTIC SUBSPECIES (SSP. *HALIAETUS*) AND TRANSLOCATED AS NESTLINGS FROM GERMANY, SCOTLAND AND FINLAND WHEN 30–45 DAYS OLD. THEY WERE KEPT IN HACKING CAGES FOR AN AVERAGE PERIOD OF 23.5 DAYS UNTIL RELEASE. THE REMAINING 23 BIRDS WERE MEASURED IN WILD NESTS IN THE LAND OF BRANDENBURG, GERMANY, DURING A STUDY OF THE POST-FLEDGING PERIOD IN 2006.

A SUB-SAMPLE OF 61 HACKED BIRDS WAS MEASURED FOR ALL THE VARIABLES CONSIDERED IN THE ANALYSIS AND USED FOR THE DISCRIMINANT ANALYSIS. THE 53 REMAINING BIRDS, 30 HACKED AND 23 FREE-LIVING BIRDS, SHOWED MISSING VALUES FOR SOME MORPHOMETRIC VARIABLES AND WERE USED AS TWO EXTERNAL SUB-SAMPLES FOR SUBSEQUENT CROSS-VALIDATIONS OF THE DERIVED MODELS.

Morphometric measurements

FOUR MORPHOMETRIC MEASUREMENTS WERE TAKEN FROM THE SAMPLE OF PRE-FLEDGLING OSPREYS. WE MEASURED BODY MASS (MASS) WITH 2500 G PESOLA SCALES TO THE NEAREST 10 G (SCALE OF 20 G DIVISIONS, BUT READ TO THE NEAREST 10 G WHEN THE MEASUREMENT WAS EQUIDISTANT BETWEEN DIVISIONS); THE LENGTH OF FLATTENED WING CHORD (WING) USING A METAL RULER TO THE NEAREST 1 MM; TARSUS LENGTH (TARSUS) WITH A DIGITAL CALIPER TO THE NEAREST 0.1 MM, FROM THE FRONT OF THE TARSOMETATARSAL BONE AT THE TOE JOINT TO THE END OF THE BONE BELOW THE ANKLE JOINT (FIG. 1); AND THE FOREARM LENGTH (FOREARM), OR THE LENGTH FROM THE FRONT OF THE FOLDED WRIST TO THE PROXIMAL EXTREMITY OF THE ULNA USING A DIGITAL CALIPER (FERRER & DE LE COURT 1992) (FIG. 2). WE ALSO CONSIDERED THE AGE OF NESTLINGS AND FLEDGLINGS (AGE) IN DAYS AS A COVARIATE IN THE ANALYSIS. AGE WAS ESTIMATED ACCORDING TO PLUMAGE DEVELOPMENT AND WING CHORD LENGTH, IF HATCHING DATE WAS NOT EXACTLY KNOWN (SCHAADT & BIRD 1993).

MEASUREMENTS WERE TAKEN A FEW DAYS BEFORE REINTRODUCED BIRDS WERE RELEASED AND BEFORE WILD-REARED BIRDS LEFT THE NEST, WHEN YOUNG WERE 40–73 DAYS OLD. BIRDS YOUNGER THAN 40 DAYS OF AGE WERE NOT INCLUDED SINCE WE CONSIDERED THIS AGE AS AN ADEQUATE CUT-OFF AT WHICH THE SIZE OF MOST SKELETAL STRUCTURES TENDS TO REACH AN ASYMPTOTE, BUT FEATHERS ARE STILL GROWING (SCHAADT & BIRD 1993). THE AGE RANGE CONSIDERED IS CLOSE TO THE AVERAGE FLEDGING AGE FOR OSPREYS; 53 DAYS (GREEN 1976, BUSTAMANTE 1995).

Sex identification

APPROXIMATELY 2 ML OF BLOOD WERE EXTRACTED FROM THE BRACHIAL VEIN OF EACH BIRD AND STORED AT LEAST 50 µL IN

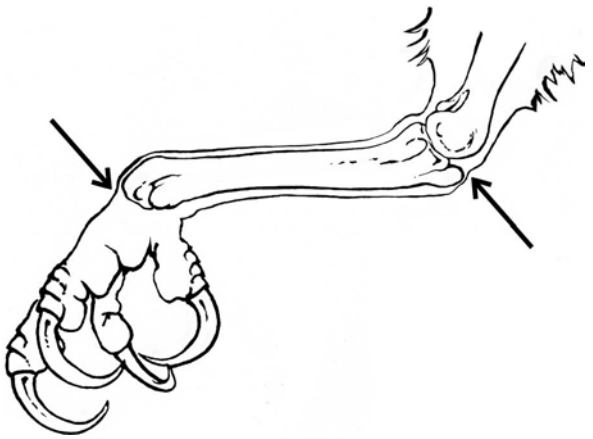

Figure 1. Measurement of tarsus length in Ospreys.

Figure 2. Measurement of forearm length in Ospreys.

tubes with 96% ethanol that were kept refrigerated until analysis in the laboratory. The cellular fraction was used for sex identification by means of PCR amplification of sections from CHD1-Z and CHD1-W genes that are located on the avian sex chromosomes. The CHD1-W gene is found on the W-chromosome, and therefore is unique to females, while the CHD1-Z gene occurs both in males and females. We followed Griffiths *et al.*'s (1998) amplification protocol, that employs the P8 (5′-CTCCCAAGGATGAGRAAYTG-3′) and P2 (5′TCTGCATCGCTAAATCCTTT-3′) primers, which provide optimum amplification and fragment separation with Osprey samples. In order to avoid possible experimental errors, all the results were subjected to reliability tests by repetition of all or at least 25% of the analyses randomly. If any discrepancy was detected, the whole process was then repeated. Using this technique, we identified 30 females and 31 males from the 61 birds used in the discriminant analysis, and 23 females and 30 males from the 53 extra birds used for the external cross-validations. The analyses were carried out in the laboratory of Molecular Ecology of the Doñana Biological Station (CSIC), Seville, Spain.

Statistical analysis

Mean, standard deviation and range for all measurements were calculated for each sex. All the variables were normally distributed and met homogeneity of variance. In order to check for overall differences in size between sexes we performed a MANOVA. We also conducted Student's *t*-tests for each morphometric measurement and age to check for sexual differences. Sexes were discriminated using forward stepwise discriminant analysis procedures that build the best explanatory discriminant model with the minimum possible number of morphometric variables. Body mass was first cube-root transformed to place it in the same linear scale as the rest of the biometric measurements. Then, each variable was moved into the model in successive steps, with an F to enter set to 3.84 (0.95 probability) and an F to remove set to 2.71 (0.90 probability). Wilk's lambda statistic was derived to quantify the discriminant power of each model. Cohen's kappa statistic was also calculated and a significance test was performed for each of the resulting discriminant functions in order to reinforce the model interpretation. This statistic estimates the correct classification rate adjusted by chance (Titus *et al.* 1984). Finally, we used two different posterior cross-validations approaches to assess the predictive accuracy of the resulting functions. First we used a jackknife procedure that classified each individual using a model derived from the total sample minus the individual being classified (Manly 1986). For the second cross-validation we used two external subsamples not involved in the discriminant model estimation, one constituted by 30 hacked birds from the same reintroduction project and the second one by 23 free-living birds. Hence, the derived models could be validated not only for Ospreys under temporary captivity (hacking) but also for free-living birds, so their application could be extended to free-ranging young Ospreys.

All the statistical analyses were performed using Statistica 8.0 (StatSoft Inc., Tulsa) and the jackknife validations were conducted using SPSS 14.0 (SPSS Inc., Chicago).

RESULTS

Young Ospreys differed significantly in overall size between males and females (MANOVA: $F_{4,56} = 32.26$, $P < 0.001$). The *t*-tests showed males to be significantly smaller than females for all the morphometric measurements, with forearm and tarsus the most dimorphic characteristics (Table 1). No significant differences in mean age were found between males and females, thus none of the differences between sexes in mean body measurements could be attributed to variation in age.

Table 1. Morphometric measurements of young Ospreys and statistical results for gender differences.

	Females				Males					
	Mean	sd	Range	*n*	Mean	sd	Range	*n*	*t*	*P*
Forearm (mm)	198.5	5.0	185.0–210.1	30	185.6	5.1	170.0–196.0	31	−10.0026	< 0.0001
Tarsus (mm)	57.9	2.5	51.5–63.4	30	53.6	3.1	46.4–58.0	31	−6.0201	< 0.0001
Wing (mm)	447.4	29.5	369–483	30	426.6	33.6	280–468	31	−2.5761	0.0125
Mass (g)	1538.5	169.9	1200–1830	30	1356.8	130.7	1010–1600	31	−4.6920	< 0.0001

THE STEPWISE DISCRIMINANT ANALYSIS RETAINED ONLY FOREARM AND TARSUS AS THE BEST PREDICTOR VARIABLES IN THE DISCRIMINANT MODEL AND EXCLUDED WING CHORD, CUBE-ROOT OF BODY MASS AND ESTIMATED AGE. THIS MODEL CLASSIFIED CORRECTLY 93.3% OF THE FEMALES AND 96.8% OF THE MALES (OVERALL SUCCESS 95.1%, TWO FEMALES AND ONE MALE MISCLASSIFIED) WITH A LOW VALUE FOR WILK'S LAMBDA AND A HIGH VALUE FOR COHEN'S KAPPA (TABLE 2). THE JACKKNIFE PROCEDURE ALSO CLASSIFIED CORRECTLY 95.1% OF CASES AND THE EXTERNAL CROSS-VALIDATIONS A SLIGHTLY LOWER OVERALL VALUE OF 84.6% OF THE WHOLE SAMPLE (HACKED BIRDS: 83.3%; FREE-LIVING BIRDS: 86.4%) (TABLE 3).

THE DISCRIMINANT FUNCTION OBTAINED WAS:

$$D_1 = 0.466\,(\text{FOREARM}) + 0.389\,(\text{TARSUS}) - 111.288.$$

VALUES OF $D > 0$ IDENTIFIED FEMALES AND VALUES OF $D < 0$ IDENTIFIED MALES. THE RESULTS OF THIS MODEL ARE SHOWN IN A SCATTERED-PLOT GRAPH WHERE THE CLASSIFIED OSPREYS ARE REPRESENTED ACCORDING TO THE FOREARM AND TARSUS MEASUREMENTS (FIG. 3). THE DISCRIMINANT THRESHOLD BETWEEN MALES AND FEMALES WAS ALSO INCLUDED AS THE LINE THAT REPRESENTS 50% PROBABILITY OF GROUP MEMBERSHIP ($D = 0$).

IN ORDER TO ACCOUNT FOR EVENTUAL VARIATION IN BODY SIZE OWING TO GROWTH, THE ESTIMATED AGE WAS FORCED INTO THE MODEL TOGETHER WITH FOREARM AND TARSUS. THE RESULTING CORRECT ASSIGNMENT OF SEX DID NOT IMPROVE AND ONLY WILK'S LAMBDA VALUE WAS SLIGHTLY LOWER (TABLE 2).

A DISCRIMINANT FUNCTION WITH ONLY ONE MEASUREMENT MAY BE MORE USEFUL AS A PRELIMINARY REFERENCE TO SEX IDENTIFICATION, BEFORE MORE ACCURATE DETERMINATIONS. A DISCRIMINANT FUNCTION WITH ONLY FOREARM AS PREDICTOR VARIABLE CLASSIFIED 93.4% OF CASES CORRECTLY, WITH A VALUE OF WILK'S LAMBDA AND A VALUE OF COHEN'S KAPPA CLOSE TO THE BEST MODEL WITH FOREARM AND TARSUS TOGETHER. BOTH VALIDATIONS, JACKKNIFE AND EXTERNAL SUB-SAMPLES, SHOWED ALSO SIMILAR PERCENTAGES OF CORRECT CLASSIFICATIONS THAN THOSE FROM THE VALIDATION OF THE BIVARIATE MODEL (TABLE 3). NEVERTHELESS, THE PERCENTAGE OF CORRECT CLASSIFICATION WAS SLIGHTLY HIGHER FOR THE SUB-SAMPLE OF HACKED BIRDS THAN FOR THE FREE-LIVING ONES. THIS DISCRIMINANT FUNCTION WAS:

$$D_2 = 0.508\,(\text{FOREARM}) - 97.592.$$

BY SOLVING THE FUNCTION FOR $D_2 = 0$ WE OBTAINED A THRESHOLD FOREARM LENGTH OF 192.1 MM THAT SEPARATES FEMALES (> 192.1) AND MALES (< 192.1).

BY CONTRAST, THE MODEL CONSTRUCTED ONLY WITH TARSUS AS PREDICTOR VARIABLE SHOWED A NOTICEABLY HIGHER VALUE OF WILK'S LAMBDA AND A LOWER VALUE OF COHEN'S KAPPA THAN THE MODEL WITH ONLY FOREARM. IN FACT, THE PERCENTAGES OF CORRECT CLASSIFICATION DERIVED WITH THIS MODEL AND WITH THE JACKKNIFE AND THE EXTERNAL CROSS-VALIDATIONS, ESPECIALLY FOR THE SUB-SAMPLE OF HACKED OSPREYS, WERE LOWER THAN THE PERCENTAGE IN THE MODEL

Table 2. Accuracy[a] of sexing young Ospreys obtained from discriminant analysis using single measurements or combinations of morphometric variables.

		Cases correctly separated								
		Females		Males		Overall				
Variables	Wilk's Lambda	%	*n*	%	*n*	%	*n*	Cohen's Kappa	*Z*	*P*
Forearm + Tarsus	0.3344	93.3	30	96.8	31	95.1	61	0.9016	7.0357	< 0.001
Forearm	0.3709	93.3	30	93.5	31	93.4	61	0.8687	6.7797	< 0.001
Tarsus	0.6195	86.7	30	71.0	31	78.7	61	0.5734	4.4750	< 0.001
Forearm + Tarsus + Age[b]	0.3315	93.3	30	96.8	31	95.1	61	0.9016	7.0357	< 0.001

[a]Accuracy is assessed by the percentage of cases correctly classified, Wilk's Lambda and Cohen's Kappa statistics; [b]discriminant accuracy of best predictor model (Forearm + Tarsus) when estimated age of Ospreys was forced into the analysis.

Table 3. Accuracy of discriminant models assessed by both Jackknife procedure and external cross-validations by means of two external sub-samples of hacked and free-living birds.

	Cases correctly separated																					
							External sample validation															
	Jackknife cross-validation						Hacking						Free-living						Total sample			
	Females		Males		Overall		Females		Males		Overall		Females		Males		Overall		Females		Males		Overall	
Variables	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
Forearm + Tarsus	93.3	30	96.8	31	95.1	61	85.7	14	81.5	16	83.3	30	75.0	8	92.9	14	86.4	22	81.8	22	86.7	30	84.6	52
Forearm	93.3	30	90.3	31	91.8	61	85.7	14	93.7	16	90.0	30	88.9	9	78.6	14	82.6	23	87.0	23	86.7	30	86.8	53
Tarsus	86.7	30	67.7	31	77.0	61	64.3	14	75.0	16	70.0	30	75.0	8	92.9	14	86.4	22	68.2	22	83.3	30	76.9	52

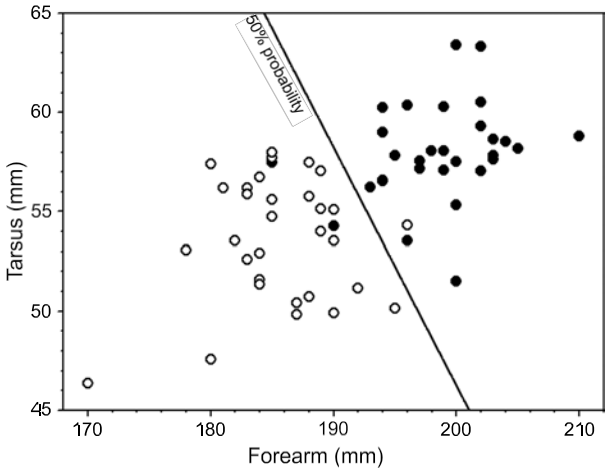

Figure 3. Distribution of the 61 young Ospreys according to forearm length and tarsus length. ♀, males; •, females; straight line, discriminant threshold between males and females, with a 50% probability of classification; two females were misclassified as males and one male as a female.

WITH FOREARM AS PREDICTOR, WHICH SHOWS THE GENERAL LOWER PREDICTIVE POWER OF THE TARSUS MEASUREMENT.

DISCUSSION

OUR RESULTS INDICATE SIGNIFICANT SEXUAL SIZE DIMORPHISM IN YOUNG OSPREYS, WITH MALES SMALLER THAN FEMALES. THEREFORE, SEXES MAY BE SEPARATED BY MEANS OF EXTERNAL MORPHOMETRIC MEASUREMENTS IN AN EASY AND RELIABLE WAY. HOWEVER, THERE WAS A CONSIDERABLE OVERLAP IN THE RANGES OF THESE MEASUREMENTS, THUS DISCRIMINANT FUNCTIONS USING THESE MEASUREMENTS AS PREDICTOR VARIABLES COULD BE USEFUL AND MORE ACCURATE TOOLS FOR SEX IDENTIFICATION.

IN MOST STUDIES DEALING WITH GENDER DETERMINATION IN BIRDS, DISCRIMINANT ANALYSIS HAS SHOWN SOME VARIATION IN ACCURACY WHICH IS DIRECTLY RELATED TO THE DEGREE OF SIZE DIMORPHISM OF THE STUDIED SPECIES. MOST BIRDS OF PREY SHOW A DEGREE OF REVERSED SEXUAL SIZE DIMORPHISM (FEMALES LARGER THAN MALES) THAT VARIES FROM VULTURES, WITH A GREAT OVERLAP OF MORPHOMETRICS BETWEEN SEXES, TO SOME FALCON AND HAWK SPECIES WITH EXTREME DIMORPHISM, WITH OSPREYS IN AN INTERMEDIATE POSITION (NEWTON 1979, FERGUSON-LEES & CHRISTIE 2001). THE RESULTS PROVIDED BY A DISCRIMINANT ANALYSIS WILL BE MORE RELIABLE AS THE SIZE DIMORPHISM INCREASES, AND MAY EVEN ACHIEVE ACCURACY COMPARABLE TO THAT OF MOLECULAR PROCEDURES WHEN THE DIMORPHISM IS EXTREME. NONETHELESS, MOLECULAR TECHNIQUES USING DNA ANALYSIS REPRESENT THE MOST RELIABLE NON-INVASIVE METHOD FOR SEX DETERMINATION OF ANIMALS, ESPECIALLY IN SPECIES WITHOUT PLUMAGE DIFFERENCES AND APPARENT SIZE DIMORPHISM, FOR WHICH DISCRIMINANT MODELS MAY BE INACCURATE. FURTHERMORE, DNA AMPLIFICATION ON SEX CHROMOSOMES SHOWS CLEAR ADVANTAGES SUCH AS AGE AND MORPHOLOGICAL INDEPENDENCE OR RELIABLE RESULTS ON VERY SMALL TISSUE SAMPLES AS BLOOD OR FEATHERS, AND IT IS NOT SUBJECT TO OTHER FACTORS THAT COULD LEAD TO BIASED RESULTS WITH BIOMETRIC VARIABLES: DIFFERENCES AMONG OBSERVERS IN MEASUREMENTS AND SIZE VARIATIONS BETWEEN POPULATIONS DUE TO LOCAL ADAPTATIONS OR ECO-GEOGRAPHIC PATTERNS (E.G. BERGMANN'S RULE OR ALLEN'S RULE). FOR THOSE REASONS, THE DNA ANALYSIS IS WIDELY USED AS REFERENCE METHOD FOR SEX IDENTIFICATION WHEN TESTING THE ACCURACY OF DISCRIMINANT FUNCTIONS ON MORPHOMETRICS.

THE BEST MODEL OBTAINED IN OUR DISCRIMINANT ANALYSIS SHOWED A HIGH LEVEL OF OVERALL CORRECT CLASSIFICATION OF GENDERS (95.1%), SUPPORTED BY SEVERAL STATISTICS AND CROSS-VALIDATIONS, BOTH FOR HACKED AND FREE-LIVING SUBSAMPLES. IN THIS ANALYSIS ONLY TARSUS AND, IN PARTICULAR, FOREARM WERE RETAINED AS THE BEST PREDICTIVE VARIABLES IN THE FINAL MODEL, WHEREAS CUBE-ROOT OF BODY MASS, WING CHORD LENGTH AND ESTIMATED AGE WERE REMOVED. MEASURES LIKE BODY MASS, WING LENGTH, BILL LENGTH, TAIL LENGTH, LENGTH OF WING FEATHERS, LENGTH OF CLAWS OR FOOT PAD LENGTH ARE WIDELY USED IN GENDER DETERMINATION OF RAPTORS, THOUGH THESE KIND OF CHARACTERISTICS ARE MORE VARIABLE AND DEPENDENT ON OTHER FACTORS. IN PARTICULAR, BODY MASS SHOWED HIGH VARIATION, EVEN WITHIN THE SAME DAY, AND IS VERY DEPENDENT ON FOOD SUPPLY, GROWING STAGE, ENVIRONMENTAL CONDITIONS OR POPULATION OF ORIGIN (POOLE 1989, SCHMIDT *ET AL.* 2000). IN THE SAME WAY, CAPTIVITY DURING THE HACKING PERIOD MAY ALSO LEAD TO WEIGHT VARIATIONS OWING TO DIFFERENCES IN FOOD SUPPLY, PHYSICAL ACTIVITY OR HANDLING STRESS. HENCE, A SUBSAMPLE OF FREE-RANGING BIRDS IS NECESSARY FOR EXTERNAL VALIDATION TO GENERALIZE THE DISCRIMINANTS AND APPLY THEM ON FREE-LIVING POPULATIONS. ON THE OTHER HAND, WING FEATHERS KEEP GROWING AFTER THE FIRST FLIGHT OF YOUNG BIRDS AND ARE SUBJECTED TO FEATHER LOSSES AND MOULT. HOWEVER, IN ADULT OSPREYS STRUCTURAL MEASUREMENTS SUCH AS BILL, TAIL OR WING LENGTH, BUT MAINLY BODY WEIGHT, HAVE PROVIDED GOOD RESULTS FOR SEX DIFFERENTIATION SINCE THEY PRESENT STABLE RANGES WITH LITTLE OR NO OVERLAP BETWEEN THEM (MACNAMARA 1977, POOLE 1989, SCHMIDT *ET AL.* 2000). THUS, MORPHOMETRIC VARIABLES DERIVED FROM HARD BODY STRUCTURES, LIKE FOREARM OR TARSUS, WOULD BE GENERALLY PREFERABLE AS MORE RELIABLE PREDICTORS (COUNSILMAN *ET AL.* 1994). SINCE THEY TEND TO REACH ASYMPTOTIC GROWTH BEFORE FLEDGING, THEY ARE NO LONGER AGE-DEPENDENT OR CONDITIONED BY OTHER EXTERNAL FACTORS. IN THIS REGARD,

ESTIMATED AGE WAS EXCLUDED FROM THE DISCRIMINANT MODEL, WHICH IS CONGRUENT WITH THE LACK OF SIGNIFICANT DIFFERENCES IN MEAN AGE BETWEEN SEXES. EVEN WHEN FORCING AGE INTO THE MODEL, THE DISCRIMINANT POWER DID NOT INCREASE NOTICEABLY. THEREFORE, THE MORPHOMETRIC VARIABLES RETAINED IN THE MODEL WERE ROBUST ENOUGH FOR SEX DETERMINATION, WITH NO RELEVANT INFLUENCE OF ESTIMATED AGE. MOREOVER, SKELETAL MATERIAL, SPECIMENS FROM MUSEUMS OR DEPREDATED BIRDS COULD ONLY BE SEXED FROM SUCH BONE MEASUREMENTS.

ACCORDING TO OUR RESULTS, FOREARM WAS THE MOST POWERFUL VARIABLE FOR SEX IDENTIFICATION OF NESTLING AND FLEDGLING OSPREYS. ALTHOUGH IT HAS NOT BEEN USED TRADITIONALLY IN SEX DETERMINATION STUDIES IN BIRDS, THE FOREARM HAS BECOME MORE FREQUENTLY USED (DELGADO & PENTERIANI 2004, SARASOLA & NEGRO 2004). PROPOSED ORIGINALLY BY FERRER & DE LE COURT (1992), THE FOREARM HAS BEEN SHOWN TO BE AN EASIER AND RELIABLE MEASUREMENT WITH LOWER VARIANCE AMONG DIFFERENT OBSERVERS AND SOURCES OF SAMPLE (LIVE OR DEAD ANIMALS) THAN OTHER HARD BODY STRUCTURES LIKE THE TARSUS. THE MODEL CONSTRUCTED ONLY WITH THIS MEASUREMENT PROVIDES A FOREARM LENGTH THRESHOLD OF 192.1 MM BETWEEN FEMALES AND MALES, AND THE PREDICTIVE POWER WAS SIMILAR TO THE BEST MODEL WITH FOREARM AND TARSUS TOGETHER.

TAKING INTO ACCOUNT ALL THE ADVANTAGES, THE TARSUS LENGTH, BUT MAINLY THE FOREARM LENGTH, COULD BE USED AS EASY, IMMEDIATE, LOW-COST AND ACCURATE EXPLANATORY REFERENCES FOR GENDER DETERMINATION. THEY MAY BE USED ON HACKED/CAPTIVE BIRDS OR IN THE WILD AS SHOWN BY THE EXTERNAL CROSS-VALIDATIONS, THOUGH CARE MUST BE TAKEN WHEN APPLYING TO OTHER POPULATIONS THAN THOSE FROM CENTRAL OR NORTHERN EUROPE, FROM WHICH THE SAMPLES WERE COLLECTED. MOST OF THE SCIENTIFIC STUDIES AND POPULATION MONITORING WORKS ON BIRDS OF PREY ARE PERFORMED ON THE YOUNG FRACTION OF THE POPULATIONS. THUS, THESE MORPHOMETRIC MEASUREMENTS SHOULD BE CONSIDERED STANDARD WORK TOOLS FOR FUTURE SCIENTIFIC STUDIES AND THE MANAGEMENT OF OSPREY POPULATIONS, BOTH FOR ADULT AND YOUNG BIRDS, AT LEAST FOR >40-DAY-OLD NESTLINGS WHEN THEY HAVE ALMOST COMPLETED THEIR STRUCTURAL GROWTH.

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