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SEXING AND AGEING THE PURPLE SWAMPHEN *PORPHYRIO PORPHYRIO PORPHYRIO* BY PLUMAGE AND BIOMETRY

SEXADO Y EDAD DEL CALAMÓN COMÚN *PORPHYRIO PORPHYRIO PORPHYRIO* A TRAVÉS DEL PLUMAJE Y LA BIOMETRÍA

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SUMMARY.—Many bird families, including the Rallidae, are characterised by a lack of plumage sexual dimorphism and reduced sexual size dimorphism. In such cases, biometry may still allow the sex of captured birds to be determined. We investigated this possibility in the purple swamphen, a species for which biometric and moult data from southern Europe are scarce. We studied and measured a large sample of wild birds in order: 1) to assess the extent of sexual size dimorphism in adult and immature birds; 2) to determine the period during which plumage characteristics can be reliably used for ageing; and 3) to develop a discriminant function that allows purple swamphens to be sexed using a set of morphometric measurements. Ten biometric traits were measured for 421 wild birds that were also sexed molecularly. We used body and wing photographs from 425 and 232 birds, respectively, in order to classify bird age (adult versus immature, based on the evidence of immature plumage). For most measurements the overall size ranking was as follows: adult males > immature males > adult females > immature females. However, this ranking did not apply to culmen and shield width, because these were bigger in adult females than in immature males. Immature birds moulted gradually during the first winter. The best ageing criterion for immature birds was the presence of median underwing-coverts with whitish tips before the first complete summer moult (second-year birds). This extends the ageing period by another six months relative to prior knowledge. The discriminant analyses using six different biometric traits correctly assigned sex in 88% and 95% of immature and adult birds,

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respectively. Using only two variables (culmen and body mass) reduced correct sexing to 80%-92%. These equations are a simple and inexpensive way to sex purple swamphens of known or unknown age. Although some caution is necessary, these equations can be useful for sexing birds from other southern European populations.

Key words: discriminant function analysis, Ebro delta, moult, rallid, sexual size dimorphism, Spain.

RESUMEN.—Muchas familias de aves, como la Rallidae, se caracterizan por carecer de dimorfismo sexual en el plumaje y presentar un escaso dimorfismo en el tamaño. En estos casos, las aves capturadas pueden sexarse por medio de su biometría. Investigamos esta posibilidad en el caso del calamón común, una especie para la cual los datos de biometría y muda son escasos en el sur de Europa. Estudiamos una amplia muestra de aves capturadas con el fin de determinar (1) el grado de dimorfismo sexual en inmaduros y adultos, (2) el período en que el plumaje permite diferenciarlos y (3) desarrollar funciones discriminantes para sexarlos. En 421 individuos se tomaron 10 medidas morfométricas y se sexaron molecularmente. A partir de las fotos del cuerpo de 425 individuos y del ala de 232 se determinó la edad según la presencia de plumaje de inmaduro. Para la mayor parte de medidas las relaciones de tamaño fueron: machos adultos > machos inmaduros > hembras adultas > hembras inmaduras. Esta relación no se cumplió para el culmen y el ancho de la placa, ya que las hembras adultas presentaron tamaños superiores a los machos inmaduros. Los inmaduros mudaron gradualmente en el primer invierno las plumas del cuerpo. El mejor criterio para datar a los inmaduros fue la presencia de plumas con márgenes blancos en las infra-cobertoras medianas del ala, carácter que retuvieron hasta la primera muda total de verano (segundo año calendario). Esto permite alargar el período de determinación de la edad en seis meses más. Usando seis medidas en la función discriminante se obtuvieron entre un 88% y 95% de asignaciones de sexo correctas según la edad (inmaduros y adultos, respectivamente). Usando solo dos medidas (culmen y peso) el porcentaje de asignaciones correctas se redujo a 80%-92%. Estas ecuaciones son una forma sencilla y económica de determinar el sexo de individuos tanto de edad conocida como desconocida. Aunque se deben tomar precauciones, es posible que estas ecuaciones sirvan para determinar el sexo en otras poblaciones del sur de Europa.

Palabras clave: análisis discriminante, delta del Ebro, dimorfismo sexual en el tamaño, España, muda, rálido.

INTRODUCTION

Sexing and ageing birds is often important when studying their ecology, behaviour and genetics but also for carrying out conservation measures, for example reintroductions or breeding programmes. In particular, knowing the sexes and ages of birds allows detailed studying of several aspects of their ecology, such as demography (sex ratio, survival, age at maturity), movements and habitat selection (including during migration) and the use of trophic resources. Methods for sexing birds in monomorphic species are based on molecular techniques (reviewed in Dubiec and Zagalska-Neubauer, 2006), biometry (reviewed in Dechaume-Moncharmont, *et al*., 2011), behavioural observations, laparoscopy, cloacal examination or presence of incubation patch (the last two being reliable only during the breeding season; e.g. Baker, 1993). Moreover, ageing is often possible from plumage traits, when plumage characters change through moult until an individual reaches adult plumage i.e. its definitive adult appearance. Ageing by plumage may be possible over a few months or years, depending on the time taken to acquire full adult plumage.

Molecular techniques are among the most reliable methods for sex identification in birds (Dubiec and Zagalska-Neubauer, 2006).

However, these are not entirely free of error or misclassification (Robertson and Gemmell, 2006; Casey *et al*., 2009; Eilers *et al*., 2012) and require laboratory facilities and consumables that have associated costs. Morphometrics is an inexpensive method that allows sexing birds if they show some degree of sexual size dimorphism. However, a previous sample of known-sex birds is necessary to develop and validate a sexing method, such as a discriminant function analysis (Dechaume-Moncharmont *et al*., 2011).

Most species of the family Rallidae, including the five species comprising the genus *Porphyrio*, typically show no sexual dimorphism in plumage and reduced sexual dimorphism in size, with males being slightly larger than females (Taylor, 1996). In this genus, comprehensive works for assigning sex using morphometrics and molecular methods have been applied to two species inhabiting New Zealand, the takahe *P. mantelli* (Eason *et al*., 2001) and the pukeko *P. porphyrio melanotus* (Williams and Miers, 1958; Craig *et al*., 1980). Biometry has also been studied from museum specimens of the purple swamphen *P. p. porphyrio* from southern Spain (Hiraldo *et al*., 1974). All these studies report that males are slightly larger than females.

The purple swamphen *P. porphyrio* has a wide distribution that includes southern Europe, Africa, the Middle East, India, Southeast Asia (including several islands), Australia and New Zealand, with up to 13 different subspecies recognised (Taylor, 1996). The current distribution of *P. p. porphyrio* is restricted to countries bordering the western Mediterranean (Cramp and Simmons, 1980; Taylor, 1996), where there are scattered purple swamphen populations.

Here we report on the usefulness of plumage traits and biometry for ageing and sexing the purple swamphen *P. p. porphyrio*. We monitored a population at a coastal lagoon of the Ebro Delta during four years, allowing the capture and recapture of a large sample of wild birds. Our specific aims were: 1) to assess the extent of sexual and age-related size dimorphism in this population; 2) to determine plumage characteristics that allow ageing of birds and establish how long these remain reliable; and 3) to develop and validate a discriminant function that allows sexing purple swamphens using a set of morphometric measurements.

Material and methods

Study area and bird captures

In the first half of the 20th century the Spanish purple swamphen population was restricted to a few localities in southern Spain. In the Ebro Delta (NE Spain), it became extinct in the 1950s but recolonised this area in 1993, with breeding first confirmed in 1995 (Martí-Aledo, 2004). The origin of birds that started the recolonisation process is unknown. Nowadays the species is widespread and very abundant in this area, where groups of up to 150 individuals can be observed in some lagoons and rice fields.

This study was conducted during the "Delta Lagoon" Life+ project of the European Commission. Between January 2011 and May 2014, the project included intensive monitoring of purple swamphens in the marshes of the Alfacada lagoon in the Ebro delta (40° 40' 44'' N, 0° 50' 03'' E). The monitoring consisted of monthly capture sessions during two to three consecutive days, using seven large funnel traps located throughout the lagoon. The traps were baited with sorghum and inspected daily prior to and during capture sessions. All captured birds were marked with a metal ring and a plastic ring with an engraved two-digit individual code. Capture sessions were conducted monthly (except in August 2012 and

2013) but most morphological data were collected from winter to mid-spring (December to April) when most captures (90%) occurred.

Measurements

For each bird, we measured four bill dimensions: culmen plus shield length (culmen), nalospi measured from nostril to tip of bill (nalospi), depth at nostril (bill depth), maximum shield width (shield), head plus bill length (head), tarsus length (tarsus), tarsus plus mid-toe length without nail (tarsus-toe; after Baker 1993), maximum flattened wing chord (wing), length of the second outermost primary (P2) and body mass (mass) (fig. 1). Bill measurements were taken following Craig *et al*. (1980), except for bill depth. All measurements were made by the same person (AB) with a digital caliper (precision: ± 0.02 mm), except for wing, tarsus-toe and P2, which were measured with a ruler (precision: ± 1 mm) and mass, measured with a spring balance (precision: ± 10 g). In order to assess the repeatability of measurements (all biometrical traits except mass), a sample of 109 individuals was measured twice during the same capture event, removing the caliper or ruler between consecutive measurements. We estimated an intraclass coefficient of correlation [ICC] following Sokal and Rohlf (1995) and Lessels and Boag (1987). Repeatability values were high for all assessed

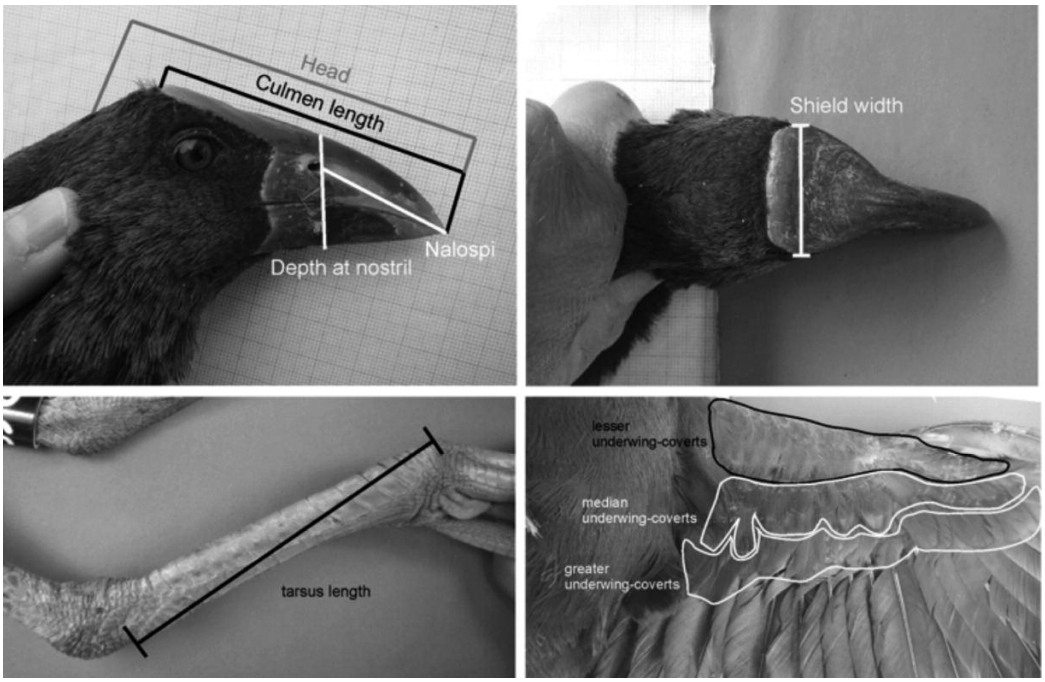

FIG. 1.—Biometrical measurements of study purple swamphens (morphometric analysis) and the area analysed for scoring lesser, median and greater underwing-coverts.
[Medidas biométricas del estudio del calamón común (análisis morfométrico) y del área analizada para marcajes de las coberteras inferiores pequeñas, medianas y mayores de las alas.]

variables and ranged between 0.905 (bill depth) to 0.997 (nalospi). All repeatability values were significant at $P < 0.0001$.

Age determination

During captures, we took a series of digital photographs in order to describe plumage characteristics (head, breast, underwing and upperwing, tail; figs. 2, S1). Juveniles have a partial post-juvenile moult, normally completed four months after hatching, and adults have a simultaneous complete moult in summer (Cramp and Simmons, 1980). The age at first breeding is unknown, but adult plumage is achieved after the first summer complete moult (see results). In southern Spain, reproduction was reported from February to September (Sánchez-Lafuente, 2004). However, at the Ebro Delta we only observed or captured chicks (< 2 months

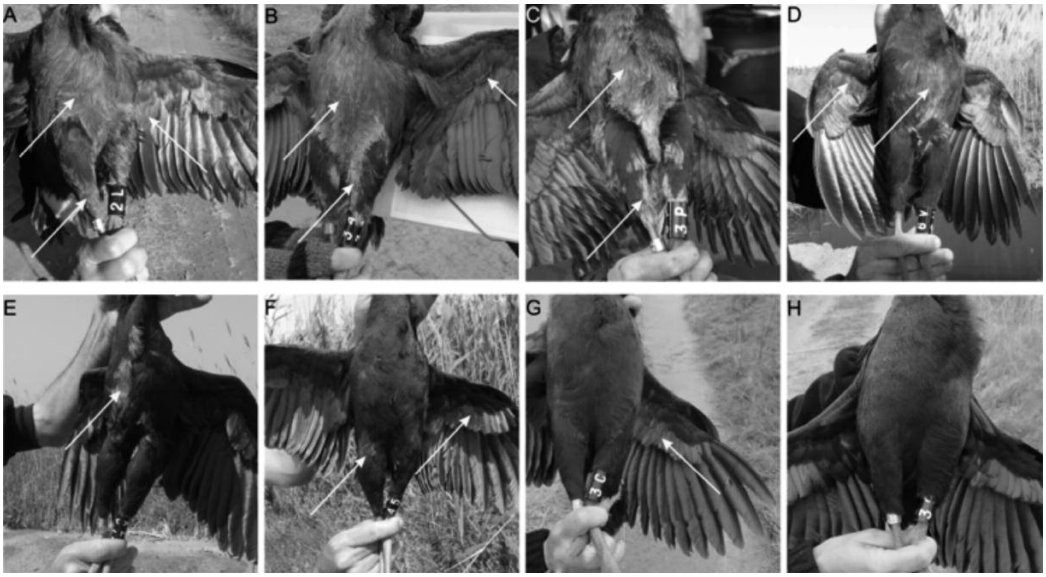

FIG. 2.—Examples of purple swamphen photographs used to score immature vs. adult plumages. A) and B) Immature plumage of breast, thighs and underwing-coverts (UWC), coded as 1-1-1 on 25 January 2011; C) immature plumage of breast and thighs (1-1-0 on 27 January 2011); D) immature plumage of breast and UWC (1-0-1 on 21 February 2012); E) immature plumage of breast only (1-0-0 on 27 March 2012); F) immature plumage of thighs and UWC (0-1-1 on 18 May 2011); G) immature plumage for UWC (0-0-1 on 26 January 2011); and H) adult plumage (0-0-0 on 26 January 2011). See methods for a more detailed explanation of plumage codes.

[Ejemplos de fotografías de calamón común usadas para puntuar el plumaje de los inmaduros y los adultos. A) y B) Plumaje de inmaduro en el pecho, los muslos y las infracoberteras (IC), codificado como 1-1-1 el 25 de enero de 2011; C) plumaje de inmaduro en el pecho y los muslos (1-1-0, 27 enero de 2011); D) plumaje de inmaduro en el pecho y las IC (1-0-1, 21 de febrero de 2012); E) plumaje de inmaduro solo en el pecho (1-0-0, 27 de marzo de 2012); F) plumaje de inmaduro en los muslos y las IC (0-1-1, 18 de mayo de 2011); G) plumaje de inmaduro únicamente en las IC (0-0-1, 26 de enero de 2011); y H) Plumaje adulto (0-0-0, 26 de enero de 2011). Para una mayor explicación de los códigos de plumaje ver el apartado de métodos.]

old) from July to September and juveniles from September onwards.

Bird handbooks state that juveniles and immatures (EURING age codes 3 and 5; EURING 2010) have duller plumage and bare parts than adults and can retain some grey body feathers, typical of the juvenile plumage, after their partial post-juvenile moult (e.g. Cramp and Simmons, 1980; Taylor, 1996). Throughout our fieldwork, we used these characters to age birds and we also recorded new plumage characteristics that could help ageing. For the sake of simplicity, we used the term *immature* to describe birds that have lost their chick plumage (dull black down) but do not yet display a typical adult plumage before first summer complete moult.

To assess the reliability of ageing criteria applied in the field, we examined the digital pictures of captured birds at the end of fieldwork (2014) and described plumage characteristics of three body areas: 1) breast and belly, frontal view (breast); 2) frontal view of both thighs (thighs); and, 3) underwing-coverts (UWC) (fig. 2). We only used data from birds for which we could clearly characterise these three body areas from good-quality pictures (425 captures in total). The same observer (SR) viewed all photographs and scored the presence (scored as 1) or absence (scored as 0) of grey or whitish feathers on the breast and thighs. In the same way, the presence (1) or absence (0) of underwing-coverts with whitish tips was recorded. Birds were classified as immature, independently of the age assigned during the fieldwork, if at least one of these three body parts (breast, thigh or UWC) had a score of 1. Although most birds were photographed on several occasions, we only used one picture for each individual, normally taken at first capture.

As field work progressed, we noticed that the underwing-covert characteristics could also be a reliable character for ageing (see results) so we took pictures of these systematically beginning in March 2013 (232 birds). The same observer (SR) separately scored the lesser, median and greater underwing-coverts as follows: (0) no whitish feather tips, (1) fewer than five feathers with a whitish tip, (2) up to 50% of feathers with whitish tips, and (3) over 50% of feathers with whitish tips (fig. 1).

To investigate the extent and chronology of immature plumage, we used a total score (hereafter body plumage score) that consisted of the sum of scores for each body area (breast, thigh, UWC; maximum score = 3) and a total score for underwing-coverts (hereafter UWC score) that consisted of the sum of scores for lesser, median and greater underwing-coverts (maximum score = 9).

Blood collection and DNA-based sexing

Blood samples (approx. 0.2 ml) were collected from the brachial vein or the intertarsal vein, kept in ethanol and stored at –20 ºC until analysis in the laboratory. After centrifugation and removing the ethanol supernatant from these samples, the blood cells were collected and DNA was extracted following the protocol of a commercial kit (QIAGEN Blood and Tissue kit). The quality of the DNA was assessed by spectrophotometry.

DNA samples were amplified using the P2 and P8 primers described for sex determination (Griffiths *et al.*, 1998). The PCR program included 35 cycles of three steps: 30s at 94 ºC for denaturing the DNA; 45s at 48 ºC for annealing primers; and 45s at 72 ºC for extension of the primers. This was preceded by five minutes at 94 ºC for complete separation of duplex template DNA and followed by ten minutes at 72 ºC for complete extension of amplicons. The amplicons were cut using the restriction enzyme Hae III. The digested products were run on

3% agarose gel and stained with ethidium bromide for visualisation. The CHD-Z and CHD-W can both be amplified using these primers. Digestion with Hae III generates distinctive banding patterns that differentiate the sexes.

Statistical analysis

We calculated multiple analyses of variance (MANOVA) to obtain multivariate descriptive statistics. We used Pillai's criterion to assess statistical differences between groups because sample size varied between groups (Hair *et al*., 2010). We tested for collinearity among study biometric traits using correlation analyses. We found pairs of redundant variables in the correlation matrix (wing – P2: r = 0.88; culmen – nalospi: r = 0.86; culmen – shield: r = 0.87). Hence, we dropped one variable from each pair of correlated variables (specifically, we dropped 'wing', 'nalospi' and 'shield') before performing the MANOVA analyses. We also excluded 'tarsus-toe' because this variable includes 'tarsus'. Multivariate homoscedasticity was tested using the Kullback test (Kullback test χ^2_{63} = 36.697, P = 0.997) (Legendre and Legendre, 1998). We carried out univariate comparisons (two-way ANOVA) to test for sexual and age differences in each biometric trait, including the variables 'sex', 'age' and the 'sex × age' interaction as explanatory variables. Body mass was cube-root transformed to achieve a normal distribution. We tested for normality and heteroscedasticity by examining the quartile-quartile plots and the plots of residual versus fitted values, respectively (Crawley, 2007).

We used linear discriminant function analyses (LDFA) to sex birds from biometrics. Discriminant analyses were conducted using all birds (irrespective of age in order to sex individuals of unknown age) or using each age class separately (adults and immatures). In a first LDFA, we used the same six variables as those used in the MANOVA analysis. In a second one, we included only two of these variables ('mass' and 'culmen'), which showed the stronger sexual dimorphism (see results). Finally, in a third LDFA, we used the two variables previously recommended by Craig *et al*. (1980) to sex *P. p. melanotus* ('nalospi' and 'bill depth'; note that our measure of bill depth was slightly different). Following Harrell (2001), we did not use a stepwise procedure to select the best model. Instead, we randomly divided the data into two sub-samples (Hair *et al*., 2010): the first to develop the discriminant function (70% of the sample of each sex); and the second to test the discriminant function (holdout sample, 30% of the sample of each sex). The use of the holdout method is considered to produce unbiased estimates of error rates (Chernick and LaBudde, 2011). For each model prior probabilities for each sex were set as equal.

In order to assess if ageing from plumage traits was reliable until the second complete summer moult, we analysed plumage score variation over time ('month', included as independent variable) using generalised linear models (GLMs) with a Poisson distribution and a log-link function.

We used the R 3.0.2 software (R Development Core Team, 2014) for all analyses (asbio, DiscriMiner, ICC, plyr and Rmisc packages).

Results

Age and plumage characteristics

Ageing from body plumage scores

All of the 425 birds whose age was determined from their body pictures were assigned the same age as during visual inspection in the field (298 immatures and

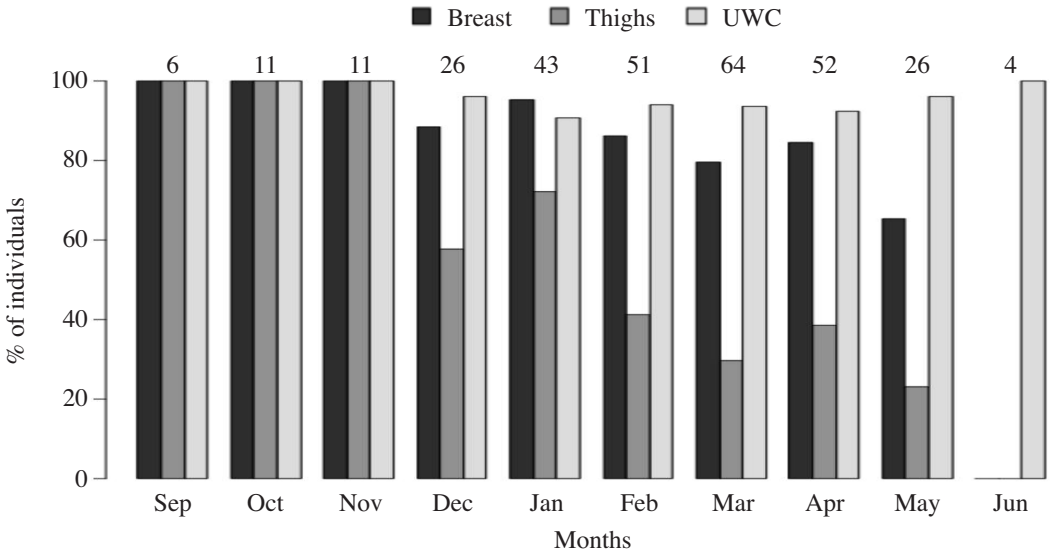

FIG. 3.—Monthly percentage of immatures showing typical immature-like plumage on breasts, thighs and underwing-coverts (UWC). Numbers above columns represent the total number of immatures sampled in each month.

[Porcentaje mensual de inmaduros mostrando el típico plumaje de inmaduro en su pecho, muslos y coberteras inferiores (IC). Los números bajo las columnas representan el número total de inmaduros muestreados en cada mes.]

127 adults overall). From September to November, all birds classified as immature showed breasts and thighs with grey or whitish feathers, and underwing-coverts with whitish tips (fig. 3). Thereafter, breast and thigh feathers were moulted gradually (partial winter moult) and total body plumage score decreased with date (score = 1.228 – 0.068 × month; GLM $z = -3.552$, $P < 0.001$; fig. 4a). In June, few immature birds were captured (n = 4); they showed adult like breast and thigh plumages, but had underwing-coverts with whitish tips (fig. 3). From September to June, we observed a high proportion of individuals with underwing-coverts with whitish tips (> 90% at each month; fig. 3).

Among the 298 immatures photgraphed, 21 were recaptured 13.4 ± 6.5 months later (range: 6-32). In all cases, they showed adult body plumage if at least one complete summer moult occurred between captures (table S1).

Ageing from underwing-covert scores

Of 232 birds with photographed underwing coverts, 82 were classified as adults and 149 as immatures, and the age assignment from photos coincided with the assignment made during visual inspection in the field in all cases. Only one adult bird, captured as an immature and recaptured two years later, showed a few grey median underwing-coverts with whitish tips in the left wing only (fig. S1). In all other cases, adult birds captured in September-July showed brown to grey greater underwing-coverts and blue underwing feathers without whitish tips.

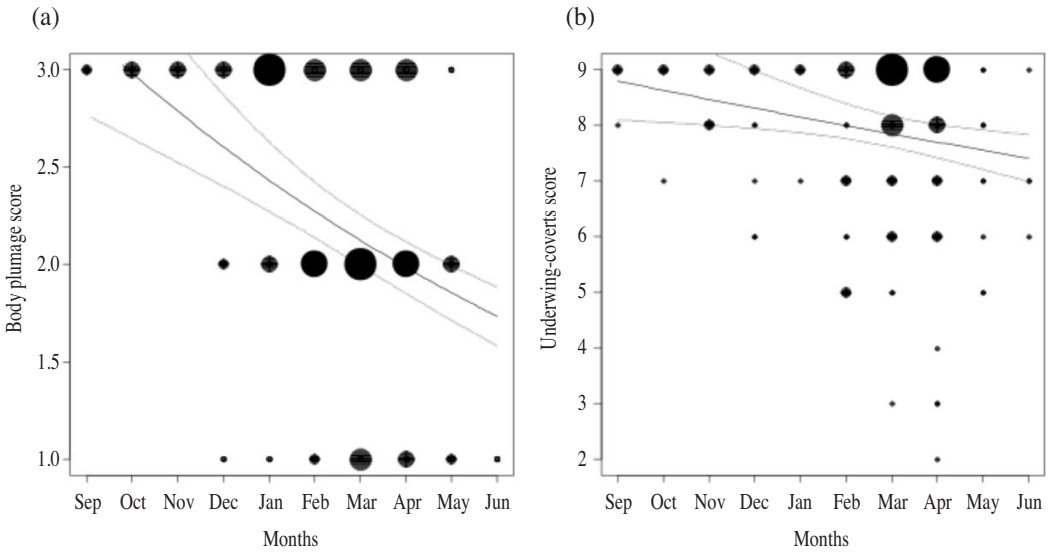

FIG. 4.—Temporal trends in (a) immature body plumage score (n = 294) and (b) underwing-covert score (n = 149). Lines show the fitted trend and 95% confidence interval. Dot sizes are proportional to the number of birds observed.

[Tendencia temporal en la puntuación (a) del plumaje del cuerpo (n = 294) y (b) de las infracoberteras (n = 149) en los inmaduros. La línea muestra la tendencia temporal obtenida en cada modelo y su intervalo de confianza al 95%. El tamaño de los puntos es proporcional al número de aves observadas.]

From September to June, all immature birds showed underwing-coverts with whitish tips (fig. 5). The median underwing-coverts showed a greater percentage of feathers with a whitish tip in all months (maximum score of 3; fig. 5). Hence, the median underwing-covert score seems to be the best for ageing purposes. The presence of underwing-coverts with whitish tips was a reliable character from September to June and the total underwing-covert score did not decrease throughout the year (GLM z = –1.391, P = 0.164; fig. 4b).

Biometry

Adults were significantly larger than immatures (Pillai's criterion = 0.635, MANOVA $F_{6, 394}$ = 113.99, P < 0.001) and males larger than females (Pillai's criterion = 0.494, MANOVA $F_{6, 394}$ = 64.08, P < 0.001). The interaction between age and sex was also significant (Pillai's criterion = 0.041, MANOVA $F_{6, 394}$ = 2.81, P = 0.011). Given the significance of the overall test, we further investigated differences trait by trait using univariate analyses (table 1). For all traits, males were bigger than females, and adults bigger than immatures. For most variables the size ranking was as follows: adult males > immature males > adult females > immature females. However, adult females showed a bigger culmen and shield than immature males. The degree of sexual dimorphism also differed between study traits: mass was the most dimorphic variable, while P2 and wing length were the least dimorphic (table 2).

For all morphometric characters, 95% confidence intervals did not overlap between

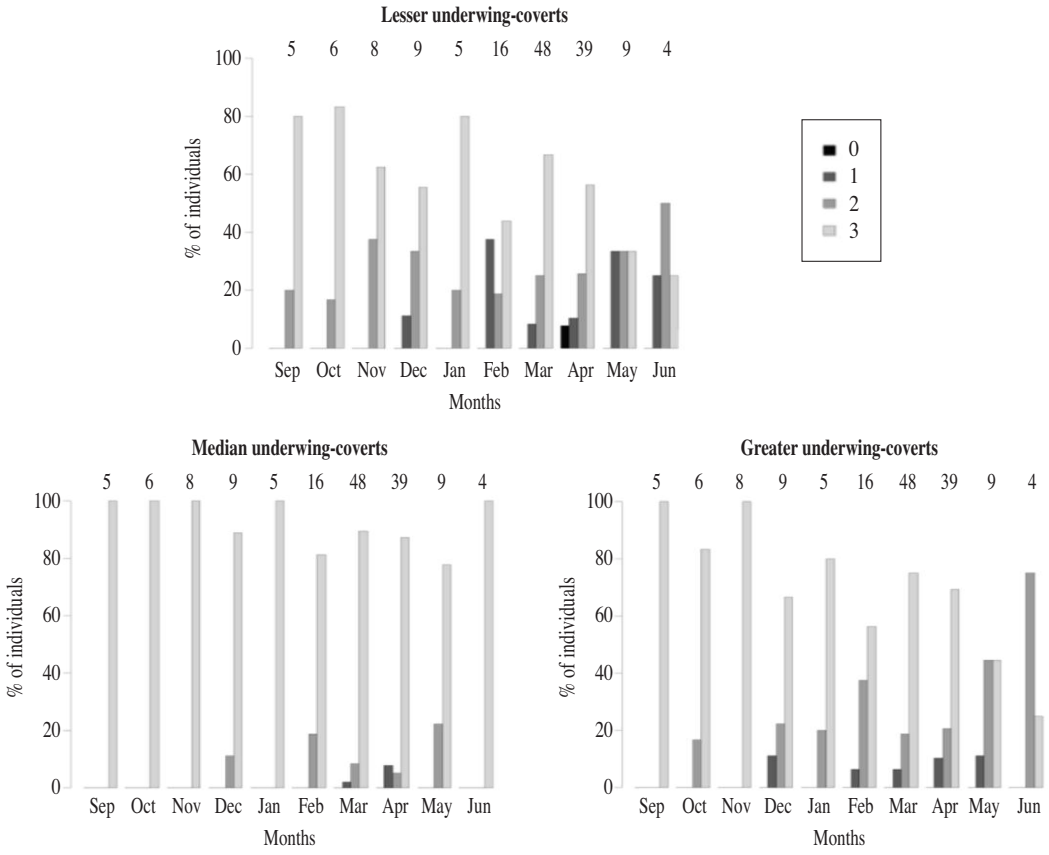

FIG. 5.—Monthly percentage of birds displaying lesser, median and greater underwing-coverts scores of 0–3. (0) no feather with whitish tips, (1) fewer than five feathers with whitish tips, (2) up to 50% of feathers with whitish tips, and (3) over 50% of feathers with whitish tips. Numbers above columns represent the total number of immatures sampled in each month.

[Porcentaje mensual de aves inmaduras que presentan infracoberteras pequeñas, medianas y grandes con puntuaciones entre 0-3. (0) todas las plumas sin bordes blanquecinos, (1) menos de cinco plumas con bordes blanquecinos, (2) hasta un 50% de las plumas con bordes blanquecinos, y (3) más de un 50% de las plumas con bordes blanquecinos. Los números encima las columnas indican el número total de inmaduros muestreados cada mes.]

immature females and adult males, but some overlap was found between adult females and immature males for some characters (table 1). However, since ranges overlapped and individuals could not be ascribed to each sex and age group with a high degree of confidence using a single measurement, we explored the usefulness of classification functions. The discriminant analyses (LDFA) using all biometric traits (except redundant ones) correctly assigned sex in 94.9% of adults and in 88.5% of immatures (table 3). When pooling adults and immature birds the percentage of correct sexing was slightly reduced to 87.6% (table 3). If the discriminant function did not include the

variable 'mass', which can show important short-term variations, the percentage of correct sexing was slightly reduced in all age categories (table 3). We obtained similar results using only the two most sexually dimorphic variables (same subset as for MANOVA analysis; table 2), which allowed predicting the sex of individuals of known age with an accuracy of 80-92% (table 3). Finally, the LDFA using the two variables recommended by Craig *et al*. (1980) to sex *P. p. melanotus* also gave similar results, with a prediction accuracy of 83-90% (table 3).

Discussion

Age determination

Body plumage differs between immatures and adults before the first partial winter moult is completed. After this first partial winter moult, immature and adult plumages differ only if some immature feathers are retained (Cramp and Simmons, 1980). Here, we found that body plumage characteristics were reliable for ageing all immatures until December (i.e. first-year birds; fig. 3). We also found that the presence of whitish tips on underwing-coverts was a reliable character to age all immature purple swamphens before the first summer complete moult (i.e. until June for second-year birds). This new criterion allows the ageing period to be extended by another six months. After their summer complete moult, second-year birds could not be distinguished from adults by plumage characteristics.

Sexual dimorphism

Few studies have reported morphometric data for the subspecies *P. p. porphyrio*. Information is available from a morphometric study carried out on museum specimens collected in Doñana (southern Spain; Hiraldo *et al*., 1974), and some descriptions published in bird handbooks, based on a very small sample sizes (e.g. Glutz von Blotzheim *et al*., 1973; Cramp and Simmons, 1980). As most of our morphometric measures were taken following Craig *et al*. (1980), they cannot be directly compared to those reported by Hiraldo *et al*. (1974). For the only two directly comparable measurements, wing length and shield width, we found similar values (t-test, all $P > 0.05$; pooling adults and immature birds, since age was not indicated by Hiraldo *et al*., 1974). We thus have no evidence that birds from the Ebro delta differ in biometry from those from southern Spain. This is consistent with the idea that the recent purple swamphen range expansion in Spain was due to a natural recolonisation process, probably helped by conservation actions (legal protection of both habitat and species and reintroduction projects) during the last 20 years of the 20th century (Sánchez-Lafuente *et al*., 1992, 2001).

We found significant sexual and age size dimorphism in the purple swamphen for all morphometric measurements taken in this study. Hiraldo *et al*. (1974) also found significant sexual size dimorphism for four of nine biometric traits studied. However, when we reanalysed their published data (table 2 in Hiraldo *et al*., 1974), we found evidence that the nine study characters differed significantly between sexes (t-test, all $P < 0.01$; table S2). In our study, we found that, for all characters, males were bigger than females and, for a given sex, adults were bigger than juveniles. However, this relationship was not met for culmen and shield width, which were bigger for adult females than immature males. These two characters are indicative of shield surface area, and the size of this ornament probably reflects reproductive and/or social status in *P. p. melanotus* (Craig *et al*., 1980; Dey *et al*., 2012, 2014). Shield size is testosterone-dependent in several species of

TABLE 1

Morphometric measurements of the purple swamphen from the Ebro Delta (NE Spain) according to age and sex (mean ± standard deviation, 95% confidence interval and range). Sample sizes = number of individuals with all traits measured. The results of two-way ANOVA indicate significant differences between sexes and age groups (df = 1,399 for all tests; mass was cube-root transformed for analysis). * Variables included in the MANOVA and the linear discriminant function analyses.

*[Medidas morfométricas del calamón común en el delta del Ebro (noreste de España) de acuerdo con la edad y el sexo (media ± desviación estándar, 95% de intervalo de confianza y rango). Tamaños muestrales = número de individuos con todas las características medidas. Los resultados de dos tipos de ANOVA muestran diferencias significativas entre sexos y edades grupales (df = 1.399 para todos los tests; la masa se transformó a raíz cúbica para los análisis). * Variables incluidas en el MANOVA y los análisis de función lineal discriminante.]*

Biometric characters	**Immature female (N = 147)**	**Adult female (N = 71)**	**Immature male (N = 124)**	**Adult male (N = 61)**	$\mathbf{F_{age}}$	**P**	$\mathbf{F_{sex}}$	**P**	$\mathbf{F_{age*sex}}$	**P**
Culmen*	58.19 ± 2.75	63.85 ± 2.42	61.47 ± 2.82	68.50 ± 2.65	482.93	< 0.0001	190.23	< 0.0001	5.66	0.0178
	57.74–58.64	63.28–64.42	60.97–61.97	67.82–69.18						
	[52.61–67.84]	[57.75–69.11]	[55.03–70.39]	[61.06–74.25]						
Nalospi	27.09 ± 1.15	28.48 ± 0.89	28.49 ± 1.07	30.30 ± 1.04	195.42	< 0.0001	208.27	< 0.0001	3.37	0.0672
	26.90–27.28	28.26–28.69	28.30–28.68	30.03–30.56						
	[24.13–30.81]	[26.34–30.51]	[25.94–30.93]	[27.43–32.50]						
Bill depth*	24.72 ± 1.18	25.39 ± 1.44	26.07 ± 1.42	26.60 ± 1.30	18.94	< 0.0001	97.28	< 0.0001	0.27	0.6052
	24.53–24.91	25.05–25.73	25.82–26.32	26.27–26.93						
	[22.64–27.82]	[23.09–28.84]	[22.57–29.76]	[22.94–30.08]						
Shield width	19.24 ± 1.62	22.73 ± 1.79	20.12 ± 1.83	24.87 ± 1.99	468.10	< 0.0001	53.26	< 0.0001	11.18	0.0009
	18.98–19.51	22.31–23.15	19.80–20.45	24.36–25.38						
	[15.45–28.05]	[19.42–26.82]	[17.18–28.69]	[19.89–29.93]						

TABLE 1 (cont.)

Biometric characters	Immature female (N = 147)	Adult female (N = 71)	Immature male (N = 124)	Adult male (N = 61)	F_{age}	P	F_{sex}	P	$F_{age*sex}$	P
Head*	76.57 ± 2.30 76.20–76.95 [70.05–85.06]	78.56 ± 1.82 78.13–78.99 [73.73–82.06]	80.02 ± 2.33 79.61–80.44 [74.67–85.73]	81.45 ± 2.31 80.86–82.04 [75.93–86.79]	54.38	< 0.0001	214.49	< 0.0001	1.42	0.2341
Wing	249.7 ± 6.1 248. 7–250.7 [237–266]	254.8 ± 4.5 253.7–255.8 [243–264]	257.4 ± 6.0 256.3–258.4 [243–271]	263.3 ± 5.6 261.8–264. 7 [246–279]	82.57	< 0.0001	194.63	< 0.0001	0.42	0.5156
P2*	175.5 ± 4.4 174.8–176.2 [166–190]	180.5 ± 4.0 179.5–181.4 [170–188]	180.8 ± 5.0 180.0–181.7 [170–195]	186.6 ± 4.7 185.4–187.8 [175–201]	119.80	< 0.0001	148.45	< 0.0001	0.66	0.4170
Tarsus*	87.63 ± 4.14 86.95–88.30 [77.08–97.65]	88.35 ± 3.45 87.54–89.17 [77.36–94.85]	91.37 ± 3.76 90.70–92.04 [79.81–99.91]	92.44 ± 3.48 91.55–93.33 [82.38–98.22]	5.00	0.0259	102.36	< 0.0001	0.18	0.6687
Tarsus-toe	195.4 ± 7.8 194.2–196.7 [176–217]	197.2 ± 6.0 195.8–198.6 [183–210]	203.5 ± 7.6 202.1–204.8 [176–218]	205.6 ± 5.8 204.2–207.1 [185–216]	6.82	0.0094	130.27	< 0.0001	0.07	0.7883
Mass*	626 ± 59 616–635 [500–880]	697 ± 63 682–712 [530–855]	722 ± 66 711–734 [585–875]	813 ± 63 797–829 [680–940]	143.73	< 0.0001	269.33	< 0.0001	0.76	0.3839

TABLE 2

Mean dimorphism index (MDI % = (mean female/mean male) × 100) of purple swamphen biometric traits. * Variables included in the MANOVA and the linear discriminant function analyses.
*[Índice de Media de Dimorfismo (MDI % = (media hembra/media macho) × 100) de las características biométricas del calamón común. * Variables incluidas en el MANOVA y los análisis de función lineal discriminante.]*

Biometric characters	MDI immature	MDI adult	MDI age combined
Culmen*	94.66	93.21	94.12
Nalospi	95.09	93.99	94.68
Depth bill*	94.82	95.45	95.02
Shield width	95.63	91.4	93.95
Head*	95.69	96.49	95.93
Wing	97.01	96.77	96.92
P2*	97.06	96.72	96.94
Tarsus*	95.91	95.58	95.79
Tarsus-toe	96.04	95.89	95.99
Mass*	86.7	85.73	86.29

the family Rallidae (Gullion, 1951; Eens *et al*., 2000) and may function as an indicator of individual quality, as found for many testosterone-dependent traits in birds (e.g. Mougeot *et al*., 2009). In the purple swamphen, shield size (determined by culmen length and shield width) is likely to be sexually determined and can be modified according to social status or season (Craig *et al*., 1980; Dey *et al*., 2014). The observed age differences in size suggest that the immatures can still grow slightly until they reach the adult size, as evidenced by some of the study characters in the sample of birds captured as immatures and recaptured as adults (see table S3).

The mean dimorphism index of each measurement was moderate within each age class, and body mass and culmen or shield width were the most dimorphic characters (table 2). Craig *et al*. (1980) found that these three measurements did not allow reliable determination of sex in *P. p. melanotus*, because of seasonal variations and an influence of social status. In contrast, we found here that, despite seasonal and social status variations, these variables could be used to predict sex accurately using linear discriminant functions. Discriminant functions including two variables (body mass and culmen) correctly assigned the sex of 80-95% of purple swamphens. The linear discriminant function proposed by Craig *et al*. (1980), using bill depth and nalospi, also correctly classified 74%-90% of our birds.

According to Dechaume-Moncharmont *et al*. (2011) the method that we used to test the accuracy of discriminant functions, splitting the data into training and test sets, produces similar results to the jackknife cross-validation, but with a much larger

TABLE 3

Results of the discriminant analyses applied for sexing purple swamphens of known age (adult and immature) and of unknown age (All birds = adults plus immatures). An individual is classified as male if constant + ∑(coefficient × variable) > 0 and female if the value is ≤ 0. * Discriminant functions with the morphological traits proposed by Craig *et al*. (1980).

[Resultados del análisis discriminante aplicado para el sexado de calamones de edad conocida (adulto e inmaduro) y de edad desconocida (todas las aves = adultos más inmaduros). Un individuo se clasifica como macho si la constante + ∑(coefficient × variable) > 0 y hembra si el valor es ≤ 0. * *Funciones discriminantes con las características morfológicas propuestas por Craig* et al. *(1980).]*

Age group	Constant	Culmen	Bill depth	Head	P2	Tarsus	Mass$^{1/3}$	Nalospi	Sample size		Holdout size (30%)		% of correct classification
									F	M	F	M	
Adults	−108.07	0.32	−0.27	0.22	0.25	0.03	3.00		50	43	21	18	94.9
Adults	−96.44	0.45	−0.22	0.20	0.27	0.07			50	43	21	18	87.2
Adults	−63.82	0.44					3.84		50	43	21	18	92.3
Adults*	−52.07		−0.06					1.81	50	43	21	18	89.7
Immatures	−79.07	−0.05	0.25	0.31	0.09	−0.03	4.36		108	94	47	40	88.5
Immatures	−65.25	0.18	0.26	0.35	0.14	−0.04			108	94	47	40	83.9
Immatures	−52.03	0.10					5.25		108	94	47	40	81.6
Immatures*	−36.19		0.47					0.87	108	94	47	40	82.8
All birds	−68.85	−0.21	0.17	0.35	0.08	0.02	3.88		153	129	65	56	87.6
All birds	−56.26	−0.03	0.19	0.36	0.12	0.04			153	129	65	56	80.2
All birds	−38.63	−0.04					4.64		153	129	65	56	80.2
All birds*	−29.96		0.42					0.68	153	129	65	56	73.6

variance. On the other hand, when sample size is small or moderate (< 200 birds), as in our dataset for adults, high discriminant success can be obtained by chance. However, our sample size for immatures and both ages combined was large enough to obtain reliable classification results (Dechaume-Moncharmont *et al*., 2011). The lack of apparent size differences, at least in wing length and shield width, between two of the most separate populations of the purple swamphen in the Iberian Peninsula (Ebro Delta and Doñana, about 750 km apart) suggests that it may be possible to apply these discriminant functions to sex birds elsewhere in southern Europe. However, the usefulness of these discriminant functions previously needs to be contrasted with a sample of known-sex birds if morphological differences between populations, or measurement differences between researchers, are detected.

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BIBLIOGRAPHY

BAKER, K. 1993. *Identification guide to European non-passerines*. BTO Guide 24. British Trust for Ornithology. Herts, UK.

CASEY, A. E., JONES, K. L., SANDERCOCK, B. K. and WISELY, S. M. 2009. Heteroduplex molecules cause sexing errors in a standard molecular protocol for avian sexing. *Molecular Ecology Resources*, 9: 61-65.

CHERNICK, M. R. and LABUDDE, R. A. 2011. *An introduction to bootstrap methods with applications to R*. John Wiley & Sons, New Jersey, USA.

CRAIG, J. L., MCARDLE, B. H. and WETTIN, P. D. 1980. Sex determination of the pukeko or purple swamphen. *Notornis*, 27: 287-291.

CRAMP, S. and SIMMONS, K. L. (Eds.). 1980. *The Birds of the Western Palaearctic*. *Vol. 2*. *Hawks to Bustards*. Oxford University Press. Oxford, UK.

CRAWLEY, M. J. 2007. *The R book*. John Wiley & Sons. West Sussex, UK.

DECHAUME-MONCHARMONT, F.-X., MONCEAU, K. and CEZILLY, F. 2011. Sexing birds using discriminant function analysis: a critical appraisal. *Auk*, 128: 78-86.

DEY, C. J., DALE, J. and QUINN, J. S. 2014. Manipulating the appearance of a badge of status causes changes in true badge expression. *Proceedings of the Royal Society B*, 281: 20132680. http://dx.doi.org/10.1098/rspb.2013.2680

DEY, C., JAMIESON, I. G. and QUINN, J. S. 2012. Reproductive skew and female trait elaboration in a cooperatively breeding rail. *Ibis*, 154: 452-460.

DUBIEC, A. and ZAGALSKA-NEUBAUER, M. 2006. Molecular techniques for sex identification in birds. *Biological Letters*, 43: 3-12.

EASON, D., MILLAR, C. D., CREE, A., HALVERSON, J. and LAMBERT, D. M. 2001. A comparison of five methods for assignment of sex in the takahe (Aves: *Porphyrio mantellii*). *Journal of Zoology London*, 253: 281-292.

EENS, M., DUYSE, E. V., BERGHMAN, L. and PINXTEN, R. 2000. Shield characteristics are testosterone-dependent in both male and female moorhens. *Hormones and Behavior*, 37: 126-134.

EILERS, A., SCHMITZ ORNÉS, A. and HAASE, M. 2012. Sex at second sight. Pitfalls of sexing water rails *Rallus aquaticus* and spotted crakes *Porzana porzana* using morphology and molecular techniques. *Acta Ornithologica*, 47: 1-9.

EURING – THE EUROPEAN UNION FOR BIRD RINGING. 2010. *The EURING Exchange Code 2000 Plus*. Thetford, UK. Available at: http://www.euring.org/data_and_codes/euring_code_list/index.html

GLUTZ VON BLOTZHEIM, U. N., BAUER, K. M. and BEZZEL, E. 1973. *Handbuch der Vögel Mitteleuropas. Band 5. Galliformes und Gruiformes*. Aula Verlag, Wiesbaden.

GRIFFITHS, R., DOUBLE, M. C., ORR, K. and DAWSON, R. J. G. 1998. A DNA test to sex most birds. *Molecular Ecology*, 7: 1071-1075.

GULLION, G. W. 1951. The frontal shield of the american coot. *Wilson Bulletin*, 63: 157-166.

HAIR, J. F. JR., BLACK, W. C., BABIN, B. J. and ANDERSON, R. E. 2010. *Multivariate Data Analysis*. (7th ed.). Pearson Prentice Hall. New Jersey, USA.

HARRELL, F. E. 2001. *Regression modeling strategies: With applications to linear models, logistic regression, and survival analysis*. Springer-Verlag. New York, USA.

HIRALDO, F., ÁLVAREZ, F. and VALVERDE, J. A. 1974. Biometría y dimorfismo sexual en el calamón (*Porphyrio porphyrio*). *Doñana Acta Vertebrata*, 1: 103-108.

LEGENDRE, P. and LEGENDRE, L. F. J. 1998. *Numerical Ecology*. Developments in Environmental Modelling. Elsevier Science. Amsterdam.

LESSELS, C. M. and BOAG, P. 1987. Unrepeatable repeatabilities: a common mistake. *Auk*, 104: 116-121.

MARTÍ-ALEDO, J. 2004. Polla blava *Porphyrio porphyrio*. In, J. Estrada, V. Pedrocchi, L. Brotons and S. Herrando (Eds.): *Atles dels ocells nidificants de Catalunya 1999-2002*, pp. 200-201. Institut Català d'Ornitologia (ICO)/Lynx Edicions. Barcelona.

R DEVELOPMENT CORE TEAM. 2014. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing. Vienna, Austria.

ROBERTSON, B. C. and GEMMELL, N. J. 2006. PCR-based sexing in conservation biology: wrong answers from an accurate methodology? *Conservation Genetics*, 7: 267-271.

SÁNCHEZ-LAFUENTE, A. M. 2004. Trade-off between clutch size and egg mass, and their effects on hatchability and chick mass in semi-precocial purple swamphen. *Ardeola*, 51: 319-330.

SÁNCHEZ-LAFUENTE, A. M., REY, P., VALERA, F. and MUÑOZ-COBO, J. 1992. Past and current distribution of the purple swamphen *Porphyrio porphyrio* L. in the Iberian Peninsula. *Biological Conservation*, 61: 23-30.

SÁNCHEZ-LAFUENTE, A. M., VALERA, F., GODINO, A. and MUELA, F. 2001. Natural and human-mediated factors in the recovery and subsequent expansion of the purple swamphen *Porphyrio porphyrio* L. (Rallidae) in the Iberian Peninsula. *Biodiversity and Conservation*, 10: 851-867.

SOKAL, R. R. and ROHLF, F. J. 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd ed. Freeman. New York, USA.

TAYLOR, P. B. 1996. Family Rallidae (rails, gallinules and coots). In, J. del Hoyo, A. Elliot and J. Sargatal (Eds.): *Handbook of the Birds of the World. Vol. 3. Hoatzin to Auks*, pp. 108-209. Lynx Editions. Barcelona.

WILLIAMS, G. R. and MIERS K. H. 1958. A field method of sexing the swamphen or pukeko. *Emu*, 58: 125-127.

SUPPLEMENTARY ELECTRONIC MATERIAL

Additonal supporting information may be found in the on-line version of this article. See volume 63(2) on www.ardeola.org.

Figure S1. Examples of pictures used to score under wing-coverts. Color pdf.

Table S1. Purple swamphens captured in immature plumage and recaptured as adults at the Ebro delta.

Table S2. Morphometric measurements (mean and standard deviation, SD) of purple swamphens from Doñana taken from table 2.

Table S3. Results of the one-tail paired t-tests comparing measurements of purple swamphens captured as immatures and recaptured as adults.

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