Age and Sex Differences in Fuel Load and Biometrics of Aquatic Warblers *Acrocephalus paludicola* at an Autumn Stopover Site in the Loire Estuary (NW France)

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AGE AND SEX DIFFERENCES IN FUEL LOAD AND BIOMETRICS OF AQUATIC WARBLERS *ACROCEPHALUS PALUDICOLA* AT AN AUTUMN STOPOVER SITE IN THE LOIRE ESTUARY (NW FRANCE)

DIFERENCIAS DE EDAD Y SEXO EN LAS RESERVAS CORPORALES Y BIOMETRÍA DEL CARRICERÍN CEJUDO *ACROCEPHALUS PALUDICOLA* EN UN SITIO DE PARADA MIGRATORIA OTOÑAL EN EL ESTUARIO DEL LOIRA (NOROESTE DE FRANCIA)

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SUMMARY.—The aquatic warbler *Acrocephalus paludicola* is the only globally threatened passerine breeding in Europe. We studied morphometric traits of 176 individuals captured at one of the key European autumn stopover sites, the Loire estuary on the Atlantic coast of France, in relation to age (first-year and older) and sex (based on molecular determination). We found significant sex differences in wing length, third primary feather length and body mass, with males being longer-winged and heavier than females; adult females also had deeper bills. However, high overlap in all measurements between the sexes meant the best discriminant functions based on wing length and bill depth (adults) and wing length (inmatures) correctly classified only 87% and 75% of individuals, respectively. The mean potential non-stop flight range of autumn staging birds at the Loire estuary with high fuel load was estimated at 1,178 km for adults and 926 km for immatures. We conclude that in autumn migrant aquatic warblers probably do not fly directly to wintering grounds in west Africa. Instead they gain significant body mass for onward migration at a few key stopover sites in western Europe and the southern Mediterranean region. Conservation of a series of important refuelling stopover sites, especially wet grassland habitats, along the migration route is therefore essential for effective protection of the aquatic warbler.

Key words: biometry, discriminant function, flight range, migration strategy, sex identification.

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RESUMEN.—El carricerín cejudo es el único paseriforme nidificante en Europa que está globalmente amenazado. Estudiamos rasgos morfométricos de 176 individuos capturados en uno de los principales sitios de parada durante la migración otoñal –el estuario del Loira en la costa atlántica de Francia– en relación a la edad (primer año y más viejos) y sexo (determinado por procedimientos moleculares). Encontramos diferencias sexuales significativas en longitudes de ala y tercera primaria, y en masa corporal, siendo los machos mayores y más pesados que las hembras; los picos de estas últimas presentaron mayor altura. No obstante, hubo mucho solapamiento entre sexos en todas las medidas, lo que influyó en que las mejores funciones discriminantes basadas en la longitud de ala y la altura del pico (adultos) y la longitud del ala (inmaduros) clasificasen correctamente solo al 87% y 75% de los individuos, respectivamente. El rango de vuelo potencial sin paradas de las aves que se detienen en otoño en el estuario del Loira con más reservas corporales se estimó en 1.178 km para los adultos y 926 km para los inmaduros. Concluimos que los carricerines cejudos que migran en otoño probablemente no vuelen directamente a los sitios de invernada en África occidental, sino que tengan que ganar peso en unos pocos sitios clave de parada migratoria en Europa occidental y la región mediterránea meridional para poder progresar con la migración. La conservación de unos pocos sitios de paradas migratorias donde las aves ganan masa corporal (especialmente hábitats de pastizales encharcados) a lo largo de la ruta migratoria es, por consiguiente, crucial para una protección eficiente del carricerín cejudo.

Palabras clave: biometría, estrategia migratoria, función discriminante, identificación de sexos, rango de vuelo.

INTRODUCTION

The aquatic warbler *Acrocephalus paludicola* is one of the most specialised of reedbed warblers and the only globally threatened passerine that breeds in mainland Europe (BirdLife International, 2004). It prefers rich, flooded, fen mires that are now globally threatened by agricultural intensification and drainage. As a result of habitat destruction, the breeding range of the aquatic warbler continues to shrink. It has completely disappeared from former breeding grounds in France, Belgium, the Netherlands and Austria (Bargain, 1999) and the core of the current breeding range is now in Belarus, Ukraine and Poland (Aquatic Warbler Conservation Team, 1999). In autumn, aquatic warblers migrate to tropical sub-Saharan Africa (Buchanan *et al.*, 2011; Flade *et al.*, 2011) through western Europe, many of them staging at known stopover sites in marshes in the Netherlands, Belgium, France and Spain (de By, 1990; Cramp, 1998; Aquatic Warbler Conservation Team, 1999). France is believed to host the largest number of individuals during autumn migration (Julliard *et al.*, 2006; Jiguet *et al.*, 2011). However, important and rapid losses in marshland extent have occurred along the aquatic warbler migratory route: 50% of marsh areas in France were lost during 1970-1990 (Bernard, 1994) and 40% of freshwater wetlands were destroyed or degraded in the Netherlands in just 10 years (Holland *et al.*, 1995).

The aquatic warbler would be expected to be particularly affected by the degradation or loss of important refuelling stopover sites along the migratory route (Kerbiriou *et al.*, 2011). The migrants spend a quarter of the year in transit between wintering and breeding grounds (Newton, 2008) and their survival outside the breeding season has an obvious impact on the number of breeders (Newton, 2006). Thus, the quality of stopover sites is crucial for sustaining efficient fuel deposition rates, which affect both the potential flight range and the probability of reaching the next stopover site successfully. Migratory passerines spend far more time and energy at stopover sites than they do flying between the breeding and wintering grounds (Hedenström and Alerstam, 1997; Wikelski *et al.*, 2003). Thus, detailed knowledge of migration strategy and stopover ecology is of importance for the development and implementation of conservation strategies that cover the entire annual cycle of this globally threatened species.

Several studies have examined stopover ecology during the autumn migration of the aquatic warbler (de By, 1990; Atienza *et al.*, 2001; Julliard *et al.*, 2006; Miguélez *et al.*, 2009; Neto *et al.*, 2010; Le Névé *et al.*, 2011), but the sample sizes involved in such studies were usually small and the sex of birds was not considered. The only recent study involving sexed individuals revealed sex differences in the timing of autumn migration (Wojczulanis-Jakubas *et al.*, 2013), indicating the importance of including sex when investigating the stopover ecology of this species. In this study, we analysed body mass, relative fuel load and potential non-stop flight range of molecularly sexed aquatic warblers captured during their autumn migration through one of the most important stopover sites: Donges in the Loire estuary, France. Based on molecularly sexed adult and immature (we use this general term for non-adult birds due to controversy over partial post-juvenile prior to autumn migration; Svensson, 1992) individuals, we assessed the accuracy of using external measurements as means of gender identification. Significant differences in wing length between adult males and females have been reported (Dyrcz, 1993; Cramp, 1998), suggesting that body size measurements may be useful for sex identification. Considering the current limited breeding range of the global population: almost 80% is concentrated at only four sites in Belarus, Poland and Ukraine (Flade and Lachmann, 2008), and its low genetic diversity (Gießing, 2002), measurements taken during migration may be representative of the entire remaining population.

MATERIALS AND METHODS

Study area and field work

We carried out this study at the Donges Ringing Station on the Loire estuary (47° 18' N, 2° 2' W) in the Department of Loire-Atlantique, NW France, which has been operative since 2002. In terms of numbers caught, it is one of the most important sites in France for *Acrocephalus* warblers (Le Névé *et al.*, 2011). In 2011, 1317 aquatic warblers were caught in Loire-Atlantique and of those, 179 individuals were in Donges (i.e. 47% and 14% of all aquatic warblers captured in France, respectively; Foucher *et al.*, 2011). The habitat at the netting site comprises reedbeds of common reed *Phragmites australis* mixed with hedge bindweed *Calystegia sepium* and saltmarshes with sea clubrush *Scirpus maritimus*, sea aster *Aster tripolium*, samphire *Salicornia* sp., hastate orache *Atriplex prostrata*, long-stalked orache *Atriplex longipes* and common saltmarsh-grass *Puccinellia maritima* (Foucher, 2010). Fieldwork was carried out from 18 July to 15 September 2011, spanning the entire autumn migration period of adult aquatic warblers and almost the whole period for immatures in this area (Chenaval *et al.*, 2011; Le Névé *et al.*, 2011; Foucher *et al.*, 2012). We captured the birds in 25 mist-nets, opened just before local sunrise and left open for about six hours (weather permitting). The nets were distributed throughout habitat much dominated by reedbeds, attractive for migrating aquatic warblers. Birds stay in the area for an average of 7 days (Chenaval *et al.*, 2011). We used tape-lures throughout the catching period, which were played from about 1 hour before local sunrise until the nets were closed. We ringed and aged the captured birds on external characters and took the following measurements: wing length (maximum flattened chord), head-bill length (distance from the back of the head to the tip of the bill), bill length (exposed culmen), bill depth (depth at widest point), hindclaw length (all measurements after Svensson, 1992) and the length of the third primary. We used a wing-rule for wing length (with 0.5 mm accuracy) and dial calipers for all other measurements (with 0.1 mm accuracy). We weighed the birds with an electronic scale (My Weigh PointScale 5.0, accurate to 0.1 g). To estimate fat reserves, we determined the fat score according to the four-score scale (1-4) proposed by the French ringing centre (CRBPO-Museum Paris).

Laboratory work

We took a few (1-5) body feathers for molecular sexing. We extracted DNA from the proximal tip of a feather using the Sherlock AX kit for biological tracks (A&A Biotechnology, Gdynia, Poland). We performed the molecular sexing with the primer pair P2 and P8 according to the protocol described by Griffiths *et al*., (1998), using a 50 °C annealing temperature for the PCR. These primers amplify a 390-bp fragment on the W chromosome (in females only) and a 370-bp fragment on the Z chromosome (in both sexes) (Griffiths, *et al*., 1998). This size difference is clearly visible when separating the fragments on 3% agarose gel. We sampled 176 of 179 (98%) birds captured during the whole study period, sexing all of them successfully. Of all birds captured, only seven were retrapped (two captured twice, one re-caught five times), but we did not include retrap records in the analyses. In total, we collected data from 42 adults (29 males and 13 females) and 134 immatures (99 males and 35 females).

Statistical analyses

To estimate body reserves of captured birds we calculated the relative fuel load using the equation $f = (m - m_0) / m_0$, where m is the actual body mass and m_0 is the size-specific lean body mass. Due to the small sample size, it was not possible to calculate m_0. Hence, we used the lowest body mass in the studied data set (9.5 g) following the method proposed by Neto *et al*. (2010). This value corresponds well with that recorded in Portugal (9.3 g; Neto *et al*., 2010).

To analyse the effects of categorical factors on studied variables, we performed analysis of variance (ANOVA) with age and sex as independent factors and with interaction term sex × age. During the analysis of the effects of categorical factors on body mass and relative fuel load, we used analysis of covariance (ANCOVA) including two covariates: trapping date (Julian date was significantly correlated to body mass: Pearson correlation coefficient, $r_{175} = 0.16$, $P = 0.03$, and relative fuel load: $r_{174} = 0.16$, $P = 0.04$), and head-bill length (to control for structural size differences; García-Berthou, 2001). We chose head-bill length to represent the adult individual body size due to a significant correlation between this variable and body mass in individuals with the lowest fat score (fat = 1, $N = 66$; both ages combined; Pearson correlation coefficient, males: $r_{44} = 0.36$, $P = 0.01$; females: $r_{18} = 0.53$, $P = 0.02$). We also used ANCOVA in the case of one morphometric variable that was significantly correlated with the trapping date, i.e. the claw length ($r_{166} = 0.23$, $P = 0.003$). Other morphometric variables were not related to the trapping date ($P > 0.05$). Before performing ANCOVA, we tested the assumption of homogeneity of regression slopes by checking significance of interaction between independent factors and covariates.

We estimated the potential non-stop flight range (Y in km) using the equation proposed by Delingat *et al*. (2008): $Y = 100 * U * \ln(1 + f)$, where U is the air speed [km/h] and f is the relative fuel load. For the flight range calculation, we assumed an air speed of

10 m/s (i.e. 36 km/h), as expected for small passerines (Alerstam *et al.*, 2007). To visualise ranges of possible non-stop flights, we represented the distances that could be travelled non-stop with different fuel load levels as concentric circles around Donges: low fuel load (0-25% of distribution), moderate (26-50%), high (51-75%) and very high (76-100%). Due to different distributions of fuel load, levels were different for adults and immatures.

We used a discriminant function (DFA) based on structural biometrics to determine the sex of the aquatic warbler, separately for adults and immatures. To find the best measurements to sex identification, we did not use the stepwise method since several authors strongly recommend avoiding this procedure (reviewed in Dechaume-Moncharmont *et al.*, 2011). Instead, we selected the DFA with highest effectiveness among various functions with manually selected variables. We assessed the effectiveness of the DFA firstly by examining the proportion of molecularly sexed individuals that were classified correctly using all individuals in the analysis (self-test), and secondly by cross-validation (each case is classified by the functions derived from all cases other than that case). Due to unequal sample size for males and females, we used chance-corrected procedure (Cohen's kappa statistic) to determine if the classification was better than random or chance alone (Titus *et al.*, 1984). We em-

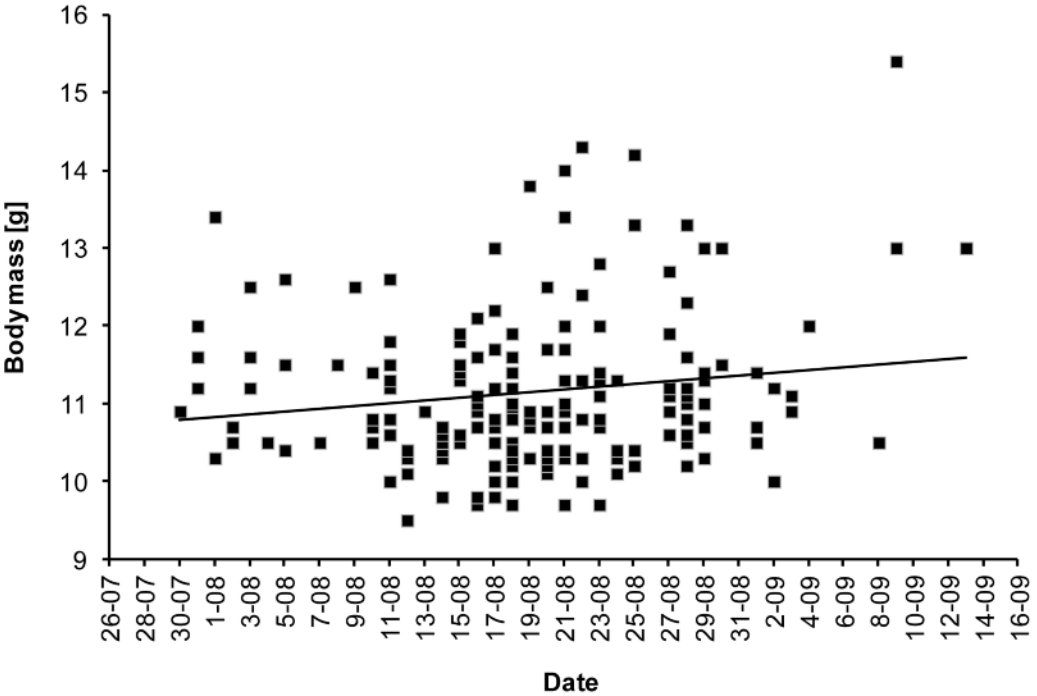

FIG. 1.—The relationship between body mass and trapping date in aquatic warblers (all sex and age categories combined).

[Relación entre la masa corporal y la fecha de captura en carricerines cejudos (todas las categorías de sexo y edad combinadas.]

ployed only data that met the assumption of homogeneity of variance, normality and lack of multicollinearity in the function.

We performed all statistical calculations using STATISTICA 8.0 (StatSoft, Inc., Tulsa, Oklahoma) and IBM SPSS 21.0 (IBM SPSS, Chicago, Illinois). To represent potential non-stop flights, we used a map created in Ocean Data View software (Schlitzer, 2011).

RESULTS

Body mass, fuel loads and potential non-stop flight ranges

Body mass was significantly related to trapping date (ANCOVA, $F_{1, 168} = 28.54$, $P < 0.001$), sex ($F_{1, 168} = 8.85$, $P = 0.003$) and age ($F_{1, 168} = 26.54$, $P < 0.001$). No other factors, i.e. sex × age interaction ($F_{1, 168} = 2.54$, $P = 0.11$) and head-bill length ($F_{1, 168} = 2.29$, $P = 0.13$), were significantly associated with body mass. Body mass increased with the trapping date (fig. 1). Adults were heavier than immatures (table 2) and males heavier than females (table 2).

Relative fuel load was significantly related to trapping date (ANCOVA, $F_{1, 167} = 28.0$, $P < 0.001$), age ($F_{1, 167} = 27.6$, $P < 0.001$) and sex ($F_{1, 167} = 8.82$, $P = 0.003$). Relative fuel loads increased with the trapping date. Adults had higher relative fuel loads than immatures (table 2) and males larger fuel loads than females (table 2). Neither the interaction term age × sex ($F_{1, 167} = 2.56$, $P = 0.11$) nor head-bill length ($F_{1, 167} = 2.30$, $P = 0.13$) were significantly associated with relative fuel load.

Considering the potential non-stop flight range, birds with very high fuel load would be able to reach sites situated an average (± SD) of 926 ± 282 km (immatures) and 1,178 ± 149 km (adults) from Donges (fig. 2). Individuals with high fuel load (51-75% of the distribution) would reach sites situated 551 ± 48 and 822 ± 114 km from Donges (immatures and adults, respectively). The corresponding figures for birds with a moderate fuel load were 397 ± 49 and 607 ± 36 km from Donges respectively. Individuals with a low fuel load would be able to reach a stopover site located on average 200 ± 88 km (immatures) and 391 ± 75 km (adults) away (fig. 2).

Body size measurements

Immatures had longer wings than adults (tables 1 and 2) and males longer wings than females (table 2). Age × sex interaction was unrelated to wing length (table 1), but there was considerable overlap in wing length between males and females (81% in adults and 93% in immatures; fig. 3).

Bill length was significantly longer in adults than in immatures (tables 1 and 2). Bill length showed no relation to sex or age × sex interaction (table 1). Bill depth was significantly greater in adults than in immatures (table 2) and there was a significant sex × age interaction: bill depth in adult males (mean ± SD [*N*]: 3.00 ± 0.11 mm [28]) was significantly less than in adult females (3.08 ± 0.11 mm [11]) (fig. 4). Bill depth was not significantly related to sex (table 1). Tarsus and head-bill length were not related to sex, age or the sex × age interaction (table 1).

Immatures had longer third primaries than adults (table 2) and males had longer third primaries than females (table 2), and there was a significant sex × age interaction (table 1); the third primary length differed among all compared groups (HSD tests, $P < 0.007$) except for immatures ($P = 0.42$) (fig. 4).

Claw length was significantly related only to trapping date (ANCOVA, $F_{1, 161} = 6.34$, $P = 0.01$). No other factors, i.e. sex ($F_{1, 161} = 0.41$, $P = 0.52$), age ($F_{1, 161} = 0.33$, $P = 0.57$) or sex × age interaction ($F_{1, 161} = 2.16$, $P = 0.14$) affected claw length significantly.

Discriminant function

The best discriminant functions obtained for the aquatic warbler based on structural biometrics included wing length (immature and adults) and bill depth (adults).

The function for adults is as follows: D_{adults} = wing length × (0.757) – bill depth × (5.009) – 31.889.

This equation (Wilks' Lambda = 0.672, $\chi^2 = 14.300$, $P = 0.001$) assumes an *a priori* probability of being male of 0.72 and of

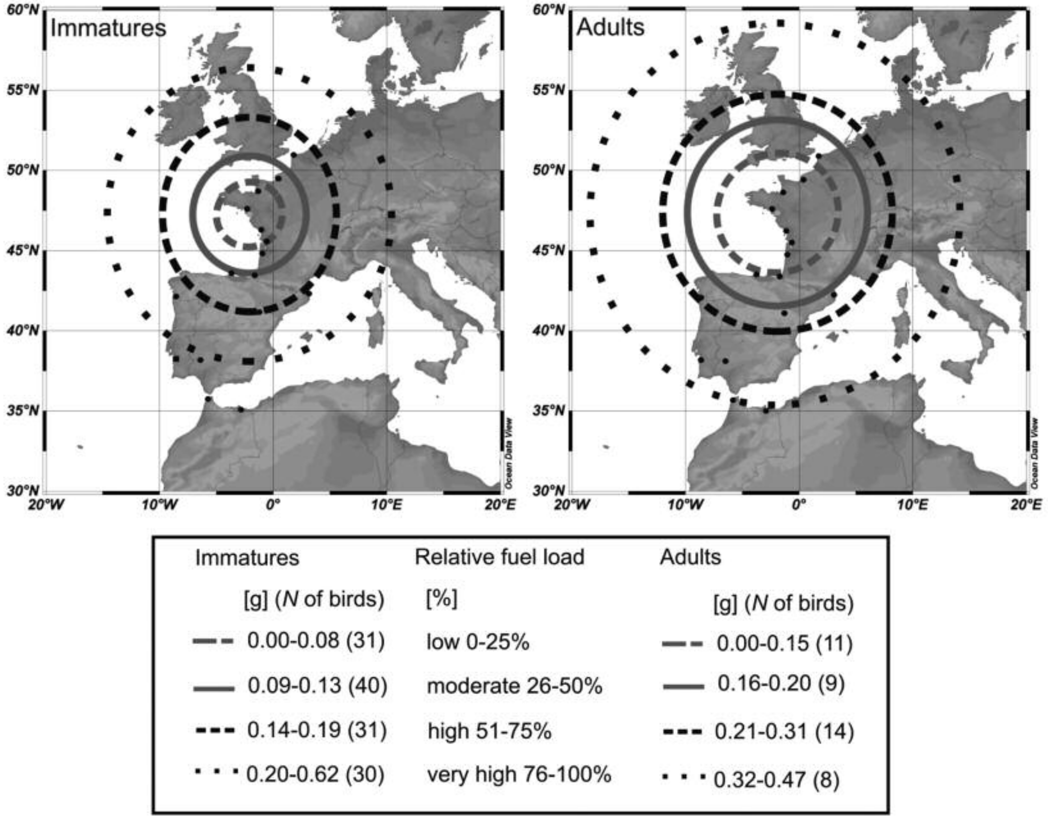

FIG. 2.—The estimated average non-stop flight ranges of adult (left) and immature (right) aquatic warblers (both sexes combined) with various fuel load levels (concentric circles). Light grey central dot indicates Donges. Black dots indicate stopover sites within range of calculated non-stop flights where considerable numbers of aquatic warblers were captured during autumn migration in France (> 20; Le Névé *et al.*, 2011), Spain (> 10; Atienza *et al.*, 2001), Portugal (> 10; Neto *et al.*, 2010) and north Morocco (all; Schäffer *et al.*, 2006).

[Rango estimado de vuelo sin paradas para adultos (izquierda) e inmaduros (derecha) de carricerín cejudo (ambos sexos combinados) con varios niveles de reservas corporales (círculos). El punto central gris claro indica Donges. Los puntos negros señalan sitios de parada migratoria dentro del rango estimado de vuelo sin paradas en los que hubo un número de capturas considerables durante la migración otoñal: Francia (> 20; Le Névé et al.*, 2011), España (> 10; Atienza* et al.*, 2001), Portugal (> 10; Neto* et al.*, 2010) y norte de Marruecos (todas; Schäffer* et al.*, 2006).]*

TABLE 1

Relationships between age (immatures, adults), sex (male, female) and age × sex interaction and different structural variables of aquatic warblers caught at a stopover site (Loire estuary, NW France) during autumn migration. Differences were tested using ANOVA.
[Efectos de la edad (inmaduros, adultos), sexo (macho, hembra) e interacción edad × sexo en diferentes variables biométricas de carricerines cejudos capturados en un sitio de parada migratoria otoñal (estuario del Loira, NO de Francia). Las diferencias se comprobaron usando análisis de varianza.]

Variable		Intercept	Sex	Age	Sex × Age	Error
Wing length	*df*	1	1	1	1	172
	F	292461	26.4	91.3	2.7	
	P	0.47	<0.001	<0.001	0.10	
Tarsus length	*df*	1	1	1	1	171
	F	138263	0.02	0.21	0.004	
	P	<0.001	0.90	0.64	0.95	
Bill length	*df*	1	1	1	1	171
	F	69914	0.27	5.45	1.86	
	P	<0.001	0.61	0.02	0.17	
Bill depth	*df*	1	1	1	1	165
	F	68144	2.33	10.49	4.12	
	P	<0.001	0.13	0.001	0.04	
Head-bill length	*df*	1	1	1	1	171
	F	443463	1.81	2.86	1.06	
	P	<0.001	0.18	0.09	0.31	
Third primary length	*df*	1	1	1	1	171
	F	201939	18.1	88.1	5.54	
	P	<0.001	<0.001	<0.001	0.02	

being female of 0.28 (computed from group sizes) and results in the cut-off point of $D = -0.329$. This discriminant function correctly classified 87% of 39 adults (82% of 11 females and 89% of 28 males) that were sexed using molecular techniques. The result of the cross-validation test produced the same results. Chance-corrected procedure showed that classification was 69% (kappa = 0.692, SE = 0.190, $P < 0.001$) better than chance.

The function for immatures was as follows: $D_{immatures}$ = wing length × (0.824) – 52.473.

This equation (Wilks' Lambda = 0.922, $\chi^2 = 10.731$, $P = 0.001$) assumes an *a priori* probability of being male of 0.74 and of being female of 0.26 (computed from group sizes) and results in the cut-off point of $D = -0.157$. This discriminant function correctly classified only 75% of the 134 immatures (11% of 35 females and 98% of

Table 2

Values of different structural variables of aquatic warblers at a stopover site (Loire estuary, NW France) during autumn migration in relation to age and sex. Figures in bold type represent statistically significant sex and/or age differences (ANOVA/ANCOVA, $P < 0.05$).
[Valores de diferentes variables de carricerines cejudos capturados en un sitio de parada migratoria otoñal (estuario del Loira, NO de Francia) en relación con la edad y el sexo. Los valores en negrita representan diferencias estadísticamente significativas en sexo y/o edad (análisis de varianza/covarianza, P *< 0,05.]*

		Sex		**Age**	
Variable		Males	Females	Adults	Immatures
Wing length [mm]	Mean	**63.6**	**62.5**	**62.0**	**63.7**
	SD	1.38	1.47	1.36	1.26
	n	128	48	42	134
Tarsus length [mm]	Mean	20.5	20.5	20.4	20.5
	SD	0.56	0.58	0.60	0.55
	n	127	48	42	133
Claw length [mm]	Mean	6.2	6.2	6.2	6.2
	SD	0.29	0.24	0.37	0.25
	n	122	44	36	130
Bill length [mm]	Mean	13.5	13.5	**13.7**	**13.5**
	SD	0.52	0.58	0.51	0.53
	n	127	48	42	133
Bill depth [mm]	Mean	2.98	2.99	**3.02**	**2.97**
	SD	0.11	0.13	0.11	0.11
	n	125	44	39	130
Head-bill length [mm]	Mean	29.9	29.7	29.9	29.8
	SD	0.47	0.45	0.42	0.48
	n	127	48	42	133
Third primary length [mm]	Mean	**47.8**	**47.1**	**46.2**	**48.0**
	SD	1.27	1.46	1.24	1.09
	n	127	48	42	133
Body mass [g]	Mean	**11.2**	**11.0**	**11.7**	**11.0**
	SD	0.99	1.03	0.97	0.96
	n	126	48	42	132
Relative fuel load [g]	Mean	**0.18**	**0.16**	**0.23**	**0.16**
	SD	0.10	0.11	0.10	0.10
	n	126	48	42	132

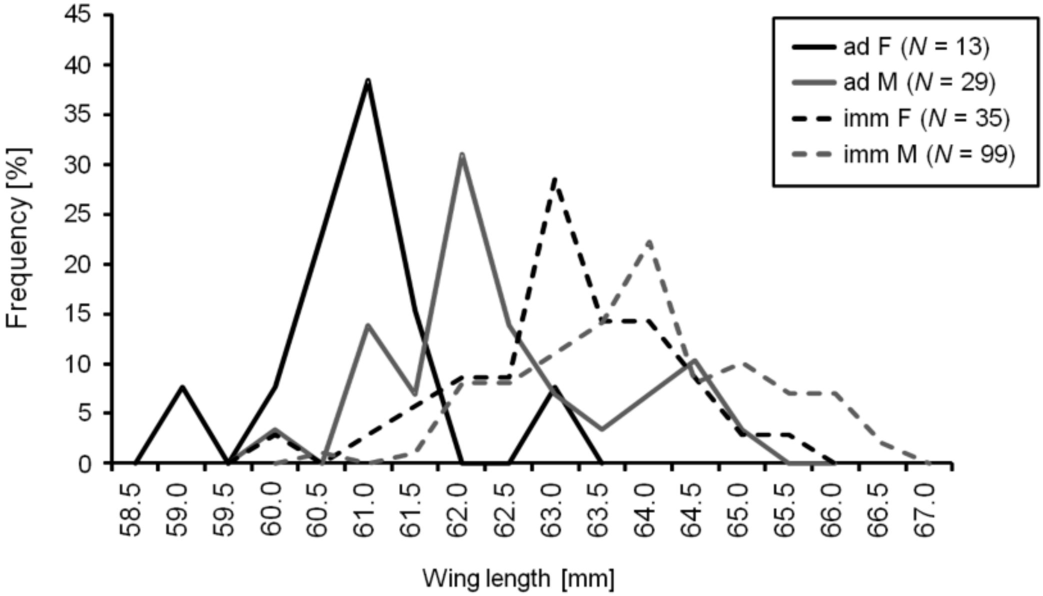

FIG. 3.—Frequency distribution of wing lengths in adult and immature male and female aquatic warblers.
[Distribución de frecuencias de las longitudes de ala de machos (M) y hembras (F) de carricerín cejudo en relación con la edad (adultos e inmaduros).]

99 males) that were sexed using molecular techniques. The result of the cross-validation test produced the same results. Chance-corrected procedure showed that classification was only 13% (kappa = 0.128, SE = 0.138), insignificantly ($P = 0.17$) better than chance.

DISCUSSION

Body mass, relative fuel loads and potential non-stop flight ranges

We found that adults had a higher body mass and relative fuel load than immatures. Similar patterns have been reported for sedge warblers *Acrocephalus schoenobaenus* and reed warblers *A. scirpaceus* at other stopover sites (Gyurácz and Bank, 1996; Yosef and Chernetsov, 2004; Zakala *et al.*, 2004; Jakubas and Wojczulanis-Jakubas, 2010). The lower mass of immatures may be due to 1) less efficient foraging, 2) their lower social status at stopover sites, 3) their benefiting from a decreased risk of predation since low fat load increases manoeuvrability, and 4) possible differences in migration routes or speeds, which affect their rate of fuel reserve accumulation (Gosler *et al.*, 1995; Woodrey and Moore, 1997). We found that body mass increased in aquatic warblers as the season progressed. Such a pattern has been described before for a number of species, including the present one (e.g. Neto *et al.*, 2008, 2010), and may reflect either a greater fattening rate at the end of the season or simply a lower proportion of lean birds as the time available for onward migration decreases.

The lack of differences in fat scores among all sex and age groups in the group of studied

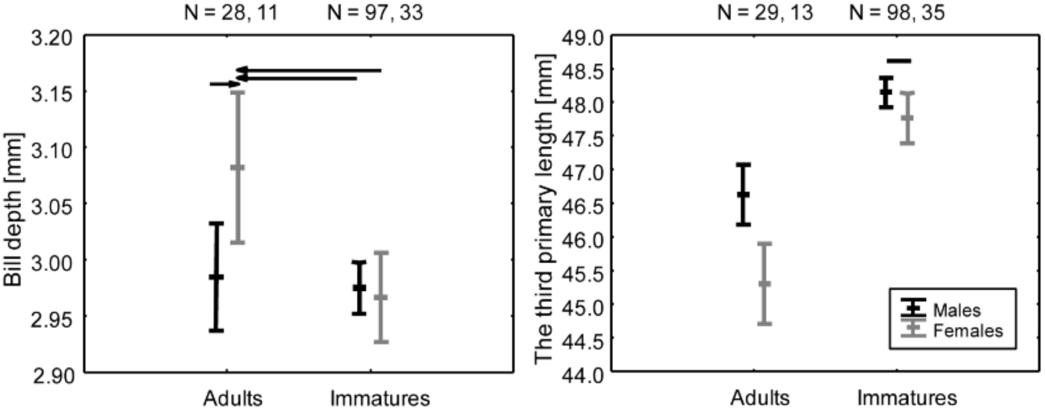

FIG. 4.—Means ± 95% confidence intervals of bill depth (left) and third primary length (right) in adult and immature aquatic warblers captured during autumn migration. Numbers above the boxes denote sample size (males, females). Arrows indicate significant differences (*post-hoc* Newman-Keuls test, *P* < 0.05). Line indicates lack of significant difference (*post-hoc* Newman-Keuls test, *P* > 0.05).
[Valores medios ± intervalos de confianza al 95% de la altura del pico (izquierda) y longitud de la tercera primaria (derecha) en carricerines cejudos adultos e inmaduros capturados durante la migración otoñal. En la parte superior están los tamaños muestrales (machos, hembras). Las flechas indican diferencias estadísticamente significativas entre muestras (prueba post-hoc *de Newman-Keuls,* P *< 0,05). La línea indica falta de diferencia estadísticamente significativa entre muestras (prueba* post-hoc *de Newman-Keuls,* P *< 0,05).]*

birds (Wojczulanis-Jakubas *et al.*, 2013) may suggest that recorded sex differences in body mass and relative fuel load were related to body size, not fuel deposits. However, body mass was not related to the surrogate for body size (head-bill length). The relatively low resolution of the fat scores scale used in this study (scores 1-4) might be a contributory reason for this discrepancy (higher fat scores usually coincide with higher body mass). Use of a wider-scale fat score system, such as the 0-8 scale proposed by Kaiser (1993), may provide a better indication of the accumulation of fat reserves in birds.

The fuel load obtained would permit aquatic warblers with different energy reserves to reach one of the known autumn stopover sites in France, Spain, Portugal or northern Morocco (fig. 2), including those situated to the north and west of Donges. Such 'back-migration' is known to have been undertaken by some aquatic warblers (Le Névé *et al.*, 2011). The maximal non-stop flight ranges of adults (1,396 km) and immatures (1,739 km) caught in Donges seem to contradict the hypothesis put forward by Julliard *et al.* (2006) that part of the population captured in autumn in France flies directly to winter quarters in Africa, without additional stops in Europe. Even the heaviest aquatic warbler captured in the Donges area (18.1 g; Foucher *et al.*, unpublished data), with estimated non-stop flight range of 2,321 km, would not be able to reach the Djoudj area in Senegal, the most northern known wintering site of the aquatic warbler (Flade *et al.*, 2011; Poluda *et al.*, 2012), 3,660 km from Donges. The hypothesis of non-stop flight across the Sahara is not plausible even in the case of aquatic warblers caught closer to the Sahara –for example in Portugal. Neto *et al*. (2010) claimed that considering the typical northerly winds occurring in the southern Mediterranean region during late summer/autumn and the maximum non-stop flight range of individuals captured in Portugal (1,998 km and 1,976 km for immature and adults, respectively), it is possible that some aquatic warblers stopping there would be able to reach the wintering quarters in Senegal without replenishing their fuel reserves. However, the shortest distance from southern Portugal to the Djoudj area in Senegal is 2,400 km, i.e. beyond the estimated flight range.

In this context, it seems that the migration strategy of the aquatic warbler is not that adopted by the sedge warbler (large fuel deposition before direct flight over Sahara; Bibby and Green, 1981; Schaub and Jenni, 2000a, b), as suggested by Julliard *et al*. (2006), Neto *et al*. (2010) and Kerbiriou *et al*. (2011). The aquatic warbler seems to gain body mass significantly only at few stopover sites in northern France or southern England (Kerbiriou *et al*., 2011) and to fly to the Iberian Peninsula or the southern Mediterranean region, but not directly to the wintering quarters in west Africa. In contrast to the reed warbler, which frequently breaks up the journey to refuel and then fuels up extensively just before reaching the Sahara (Bibby and Green, 1981; Schaub and Jenni, 2000a, b), aquatic warblers are able to forage within sub-Saharan Africa, at some staging sites along the Atlantic coast. This has recently been confirmed by data retrieved from aquatic warblers equipped with geolocators (Salewski *et al*., 2013), as well as observation records (Schäffer *et al*., 2006; Walther *et al*., 2007). Assuming non-stop flight to other stopover sites and acquisition of similar fuel loads there, aquatic warblers departing from Donges need to stop and refuel at one to three stopover sites before reaching the wintering ground in the Djoudj area, Senegal.

The sex ratio in the studied group of birds was male-biased. The most likely explanation for this bias is that using tape-luring may efficiently attract males, but not females (Wojczulanis-Jakubas *et al*., 2013). The sex ratio of the captures might be also influenced by differential behaviour or habitat use. It cannot be excluded that the observed pattern reflects a sex difference in migration routes. Females may more frequently have used an alternative, more direct migration route, as they have less time after breeding for migration than males (Wojczulanis-Jakubas *et al*., 2013). Further studies on the effects of sex on the migration behaviour of aquatic warblers would be valuable.

Knowledge of refuelling and migration strategies in this species is important with respect to its conservation. Given the reliance upon staging at only a very few stopover sites, the aquatic warbler is extremely sensitive to the degradation or loss of just one of a few important refuelling sites (Kerbiriou *et al*., 2011). The availability of wet grassland habitats (especially fen mires) near reedbeds appears to be a key requirement to supporting the rapid and efficient refuelling of migrating aquatic warblers during their autumn migration along the French Atlantic coast (Provost *et al*., 2010; Kerbiriou *et al*., 2011). All such habitats should be protected in some way.

Body size measurements

Male aquatic warblers had longer wings than females, as is the case in both immature and adult sedge and reed warblers (Jakubas and Wojczulanis-Jakubas, 2010). Females had deeper bills than males. Studies of European *Acrocephalus* warblers revealed that amongst polygynous and promiscuous species with a high degree of uniparental care, females had stronger bills, bred in habitats with more food and fed their nestlings on larger prey (Leisler and Catchpole, 1992). In the polygynous dusky warbler *Phylloscopus fuscatus*, females with deeper bills settled in territories with more food and received less male assistance in feeding their offspring. Females with stronger bills are better adapted to exploit the abundance of large food items in rich territories and thus to raise young on their own (Forstmeier *et al.*, 2001). As the promiscuous mating system in aquatic warblers involves exclusively maternal care (Dyrcz and Zdunek, 1993), their deeper bills may help females to exploit food-abundant breeding habitat more efficiently.

As found during the autumn migration in Portugal (Neto *et al.*, 2010), immature aquatic warblers had significantly longer wings than adults. This is surprising, because in the majority of passerines (including the closely related reed and sedge warblers; Norman, 1997; Jakubas and Wojczulanis-Jakubas, 2010), immatures have shorter wings than adults (e.g. Alatalo *et al.*, 1984; Schmitz and Steiner, 2006; Markovets *et al.*, 2008). Among 36 species of warblers studied by Norman (1997), only the immatures of both aquatic warbler and moustached warbler *Acrocephalus melanopogon* had longer wings than adults. However, those differences were not significant, probably because of the small sample size. Shorter wings in adults are probably attributed to greater wear of the old feathers (grown in the preceding winter) due to intense mechanical strain experienced in breeding habitat covered by dense vegetation. Bills were longer and deeper in adult aquatic warblers than in immatures. Similarly, in the great tit *Parus major*, despite the expectation that bill size should not change with age (bill size is usually fully grown six weeks after hatching), older individuals had deeper bills than younger ones (Gosler, 1987).

We found overall size differences in some measurements between male and female aquatic warblers. The discriminant function analysis amongst adults correctly classified more than 75% of studied birds. However, the function for immatures provided very poor levels of discrimination, as almost all females were classified wrongly. We could have obtained a similar proportion of correctly sexed birds (74%) by simply assuming that all individuals were males. The poorer discrimination power of the function proposed for sexing immatures may be a consequence of employing a larger sample size or to smaller sex differences in the studied variables. Although some birds may be discriminated on morphology, molecular methods, based on non-invasive feather collection or swab material, are recommended as the only reliable means of sexing aquatic warblers.

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