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Morphological versus acoustic analysis: what is the most efficient method for sexing yelkouan shearwaters *Puffinus yelkouan*?

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Abstract Sexing monomorphic seabirds is particularly difficult, and available methods have various disadvantages, such as seasonal non-applicability or stress induction. The yelkouan shearwater *Puffinus yelkouan* is a poorly studied seabird endemic to the Mediterranean basin. Ecological and biological data are needed for this species, thus necessitating the development of appropriate field methods. Here, we tested two methods for sexing yelkouan shearwaters: the classical morphological method and a new acoustic method. Morphological analysis demonstrated sexual differences in head, leg and feather measurements, with a high degree of overlap resulting in only 87.2% accuracy. In contrast, acoustic analysis showed that the highest value of the fundamental frequency (FC) and duration of the clear note of calls did not overlap between males and females. Yelkouan shearwaters can be sexed with 100% accuracy by measuring FC. If FC < 678.4 Hz, the bird is female, while if FC > 678.4 Hz, the bird is male. Other advantages of this method are its simplicity and non-invasiveness, which are particularly important for a potentially threatened species.

Keywords Acoustic parameters · Morphometry · *Puffinus yelkouan* · Sexing methods · Sexual dimorphism

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Introduction

Sexing birds is crucial in many population dynamics studies because it allows: (1) assessment of sex effects on survival or dispersal, (2) evaluation of population dynamics, since female numbers are frequent model parameters (e.g., Cuthbert et al. 2001), and (3) identification of species threats such as sex ratio disequilibria or sexual differences in predation risk (e.g., Mougeot and Bretagnolle 2000). Sexing monomorphic species such as most seabirds is particularly difficult because the sexes do not have marked differences in plumage, size or other particular physical features. Some sexing methods for such species do exist, but with obvious disadvantages, such as time-consumption, cost (chromosome and hormone analyses), invasiveness, low suitability in the field (molecular analyses, laparotomy and laparoscopy; Edington 1989; Richner 1989; O'Dwyer et al. 2006), short-time applicability, and the need for specialized training and equipment (vent measurements and sexual behaviour; Serventy 1956; Edington 1989; Boersma and Davies 1987; Copestake et al. 1988; Swanson and Rappole 1992; O'Dwyer et al. 2006). Finally, external morphometric measurements have been developed as an alternative method because they are reliable, fast, inex-

pensive and usable during non-breeding seasons (Zavalaga and Paredes 1997). Characteristics generally measured are head and leg, feather lengths (wing and tail) and body mass. External morphometric measurements have been widely and successfully used to assist in sexing Procellariiformes (e.g., Ristow and Wink 1980; Van Franeker and Ter Braak 1993; Lorentsen and Rov 1994; González-Solís 2004).

The yelkouan shearwater *Puffinus yelkouan* was long considered to be a Mediterranean subspecies of the Manx shearwater *P. puffinus* (Brooke 1990; Warham 1990). A taxonomic revision, based on ecological, morphological and genetic traits, recently elevated the yelkouan shearwater to the rank of species, distinct from both the Manx shearwater and the Balearic shearwater *P. mauretanicus* (Bourne et al. 1988; Heidrich et al. 1998; Sangster et al. 2002a, b). This species is strictly endemic to the Mediterranean basin, and the world population probably does not exceed 15,000 pairs (Zotier et al. 1992). However, this data-deficient species has been little studied and many gaps remain in our knowledge of its ecology and status. Investigations on this species are thus needed, and methods for field studies must be specifically developed or derived from those used for closely relatedspecies.

In the Manx shearwater group, sexing by morphometry is generally considered inefficient. Previous morphological analyses have revealed a small number of sexually dimorphic measurements and a poor or fair accuracy of discriminant functions with <80% of birds correctly classified (Thompson 1987; Brooke 1990; Cuthbert and Davis 2002; Keitt et al. 2003; Bull et al. 2004, 2005), with the notable exception of the Balearic shearwater (Genovart et al. 2003). In a previous study, Zotier (1997) analysed how morphological measurements vary with sex in the yelkouan shearwater and concluded that measurement overlap was too high to allow accurate sex determination. However, statistical analyses used in this preliminary study were not detailed, sample sizes were small and the number of morphological measurements was limited. For these reasons, we considered that further investigations on this topic would be useful.

Breeding colonies of petrels and shearwaters show an intensive call activity in darkness. This is considered to be an adaptation for sexual advertisement, mate recognition and territorial defense of burrows in the dark (Brooke 1978; James 1985; James and Robertson 1985a, b; Warham 1990; Bretagnolle et al. 1998). Procellariidae generally have a poor vocal repertoire (Bretagnolle 1996). In some species, and particularly in the *Puffinus* group, there is just a major singular call composed of two parts, an inhalant note and an exhalant one (Bretagnolle 1996; Jouventin and Aubin 2000). In most petrels and shearwaters studied up to now, a sexual voice dimorphism has been demonstrated

(Ristow and Wink 1980; James 1984; James and Robertson 1985a, b; Brooke 1978, 1988; Taoka et al. 1989; Taoka and Okumura 1990); in the *Puffinus* group, female calls are generally harsher and lower pitched than male ones (Brooke 1978, 1990; James and Robertson 1985a; Warham 1990, 1996). While this vocal criterion is commonly used to sex petrels in the field (e.g., Bretagnolle and Lequette 1990; Bretagnolle and Thibault 1995), discriminant analysis on acoustic traits combined with molecular sexing has never been used for unequivocally sexing birds from their calls. To our knowledge, yelkouan shearwater calls have never been analyzed and vocal sexual dimorphism has never been verified.

In the present study, we investigate sexual dimorphism in the yelkouan shearwater from both morphological and vocal points of view and compare the ability of the two approaches to distinguish sex. Our final aim is to propose a simple and efficient method to sex yelkouan shearwaters in the field.

Methods

Molecular sexing

A blood sample (ca. 0.2 ml) was taken from the leg vein of each captured bird, using a 1-ml syringe. Samples were stored in absolute ethanol (0.5 ml) for several weeks at fresh temperature (ca. 4°C) before analysis. DNA was extracted from ethanol-preserved whole blood using a salting-out extraction protocol (Bruford et al. 1998). DNA concentration was quantified on a 0.8% agarose gel. In each reaction set, negative as well as positive controls were included to assess the reliability of the method. According to the method described by Fridolfsson and Ellegren (1999), we performed PCR using primers 2550F (5′GTTACTGATTCGTCTAC-GAGA3′) and 2718R (5′ATTGAAATGATCCGTGCTT G3′) to sex our samples. PCR products were separated by electrophoresis in 2% agarose gels stained using ethidium bromide and visualized under ultraviolet light. This method has proved successful on a wide range of bird species, including Procellariiforms (Fridolfsson and Ellegren 1999, 2000). Sex determination is based on the detection of a constant size difference between two homologous sex-linked genes, CHD1-W and CHD1-Z. The CHD1-W fragment is found in both sexes, while the CHD1-Z fragment is female-specific. Since PCR products vary in size, sex was determined as one band in males and two bands in females.

Morphological analysis

Adult yelkouan shearwaters were captured in theirburrows during the 2004 and 2005 breeding seasons in three colo-

nies on Port-Cros and Porquerolles islands (Hyères archipelago, south-east France, 43°00N, 6°23E and 43°00N, 6°12E). Eleven measurements were taken (Fig. 1): (1) head length: length from the cerebellum roof (=supraoccipital) to the edge of the feather implantation on the culmen; (2) bill depth at gonys: bill thickness of upper and lower mandibles at the gonys; (3) minimum bill depth: minimum bill thickness of upper and lower mandibles measured vertically; (4) bill length: exposed culmen from the tip of the hook to the edge of the feather implantation; (5) nalospi: exposed culmen from the tip of the hook to the nostril; (6) tarsus length: metatarsus length from the depression in the angle of the intertarsal joint to the base of the last complete scale before the toe diverges; (7) tarsus height: minimum metatarsus height; (8) toe length: middle toe length from the first scale of the middle toe to the base of the nail on this toe; (9) tail length: central feather length from the point of emergence from skin to tip; (10) wing length: maximum flattened chord from carpal joint to the tip of the longest primary; and (11) body mass. The five head and the three leg measurements (Fig. 1) were taken with a Dial caliper (±0.01 mm). The feathers (wing and tail) were measured with a ruler (±0.5 mm) and body mass was determined by using a 600-g Pesola balance (±5 g).

Duplicate measurements of 36 birds were taken on two occasions or successively to assess consistency of our results (Barrett et al. 1989; Genovart et al. 2003). We tested reli-
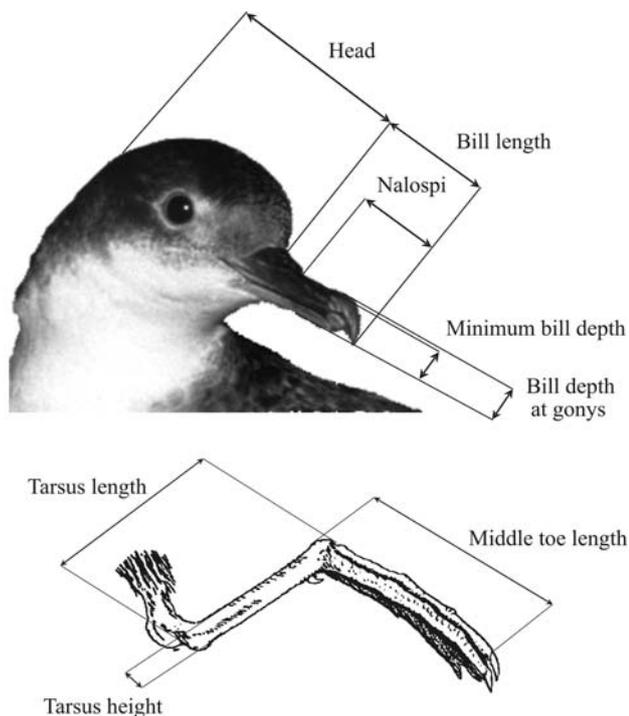

Fig. 1 Head and leg measurements taken on yelkouan shearwaters *Puffinus yelkouan*

ability of these two measurements by intraclass correlations with 1,000 iterations to obtain a mean value of the Pearson product-moment correlation coefficients for each parameter (Pearson r). We measured and molecularly sexed 88 adult yelkouan shearwaters to test inter-sexual differences using unpaired t-tests for each measurement. We calculated the percentage of sexual dimorphism for each measurement with the formula described by Holmes and Pitelka (1968): $100 \times (m–f)/m$, where m is the mean value of the male measurement and f the mean value of the female measurement. Discriminant function analyses (DFA) were used to determine firstly which measurements were most reliable, and secondly whether a yelkouan shearwater could be accurately assigned a sex using these measurements. Only birds for which we had all the measurements (i.e. 86 birds) were considered in stepwise DFA. We randomly selected 26 birds to form a validation sample. Three variables were not included in DFA: body mass, because this can vary greatly according to the time elapsed since the last food ingestion; and minimum bill depth and bill length because they were highly correlated to bill depth at gonys ($r = 0.81$) and to nalospi ($r = 0.87$), respectively (Kerry et al. 1992). Wilks' Lambda ratio was used as a criterion for variable selection. According to the discriminant function (D) obtained, an adult yelkouan shearwater was considered a male if $D > 0$ and a female if $D < 0$. The accuracy of the discriminant function obtained with the reduced sample (60 birds) was tested first in terms of the proportion of adults of known sex that were classified correctly using all individuals included in the analysis, second by a jackknife analysis (cross-validation; Lachenbruch and Mickey 1968), in which each individual in the same sample was classified using a function derived from the total sample excluding the individual being classified (e.g., Chardine and Morris 1989; Amat et al. 1993; Counsilman et al. 1994), and third by the classification of the additional validation sample of 26 molecularly sexed birds. We also compared measurements between the members of 25 breeding pairs using paired student t-tests and assessed how often the male was larger than the female. In order to apply a multi-measurement approach, we calculated the first axis of a principal component analysis based on all biometric measurements. We used this value as a proxy of the size of the bird and included this variable in the comparison of measurements between the members of breeding pairs.

Acoustic analysis

We molecularly sexed and recorded calls of 16 adult yelkouan shearwaters within the three colonies on Port-Cros and Porquerolles islands in March–April 2005 and 2006, during the pre-laying and incubation periods. Because this species is essentially vocal at night, the recordings were

performed during darkness, between 2100 and 0300 hours (French local time), using a Sennheiser MKH70 hyperdirectional microphone (frequency response: 30–20,000 Hz ± 1dB) connected to a numerical MARANTZ PMD 670 recorder (sampling frequency: 48 kHz). Each bird was recorded when alone in its nesting burrow, with the microphone placed on the ground near the burrow entrance. Calls were first under-sampled at 22,050 Hz, and then analysed with the SYNTANA analytic package (Aubin 1994) and Avisoft SAS Lab Pro (Specht 2004). We analysed 284 calls from the 16 recorded birds (181 calls from 10 males and 103 calls from 6 females with at least 10 calls per bird). A majority of individuals were recorded several times during the same session, the remaining birds being recorded on two different dates. The call of the yelkouan shearwater is composed of two notes: a noisy note with a harsh structure and a clear note with visible harmonic series (a fundamental frequency Fo and its multiples 2Fo, 3Fo...). In order to predict sex by acoustic analysis, we chose four easily measured parameters that were relatively insensitive to recording conditions (Fig. 2). In the temporal domain (envelope measurements), we measured (1) DN: the duration of the noisy note, and (2) DC: the duration of the clear note. In the frequency domain (spectrographic measurements: fast Fourier transform with a window size of 2,048 points), we measured: (3) FN: the highest frequency value of the fundamental of the noisy note, and (4) FC: the highest frequency value of the fundamental of the clear note. We also calculated the percentage of sexual dimorphism for each parameter.

Each bird was acoustically characterized by the means of the four parameters measured on its different recorded calls. For each parameter, sex comparisons were carried out using unpaired student t-tests. To analyze sexual variation in calls and to identify which acoustic parameters contributed the most to this variation, we performed a stepwise DFA on the mean values of the four acoustic parameters of the 16 birds tested.

All the required statistical assumptions were fulfilled and analyses were performed using Statistica 6.0.

Results

Morphological analysis

The two sets of measurements of birds measured twice were highly correlated (all Pearson $r > 0.79$, all $P < 0.001$), indicating that there were no systematic biases in field measurements (mean percentage of variation between the two measurements <3% for all parameters). Morphological measurements from the 88 molecularly sexed adults revealed a sexual dimorphism (Table 1). The means of all head measurements, tarsus length, middle toe length and wing length were significantly larger in males than females. The degree of sexual dimorphism was highest for bill depth measurements and lowest for feather measurements (wing and tail lengths).

The stepwise DFA applied to 8 variables of 60 adult yelkouan shearwaters (31 males and 29 females) indicated that bill depth at gonys ($F_{1,56} = 21.6$, $P < 0.001$), nalospi ($F_{1,56} = 12.7$, $P < 0.001$) and tarsus height ($F_{1,56} = 7.9$, $P < 0.01$) were the only significant variables and constituted the best predictive model (Wilks' Lambda = 0.52, $\chi^2 = 36.4$, $df = 3$, $P < 0.001$). The discriminant function (D_1) obtained was:

$$D_1 = -23.96 + 2.24 \text{ bill depth at gonys} + 0.55 \text{ nalospi} - 1.43 \text{ tarsus height.}$$

This discriminant function correctly classified 85.0% of the adults included in the analysis sample (80.6% of males, 89.7% of females). Cross-validation (Jackknife) classified adults with 80.0% accuracy (74.2% of males, 86.2% of females). When we applied the discriminant function to the validation sample (13 males and 13 females), 84.6% of the birds were correctly classified (76.9 % of males, 92.3% of females). The classification accuracies of the analysis sample, cross-validation and validation sample were similar, suggesting minimal sampling bias. The analysis and validation samples were combined and we performed a
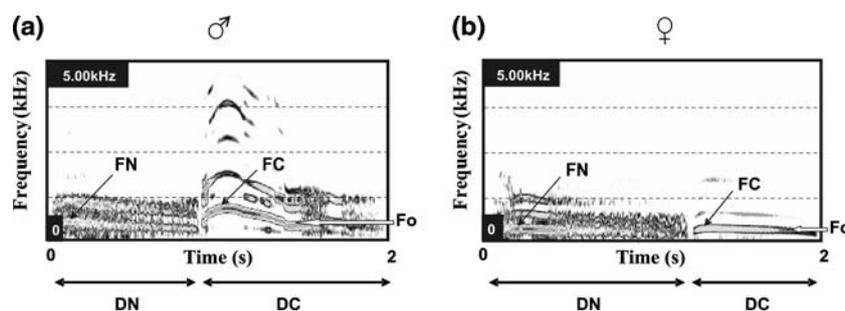

Fig. 2 Sonagrams of a male (**a**) and a female (**b**) yelkouan shearwater calls. *Fo* Fundamental frequency of the harmonic series, *DN* duration of the noisy note, *DC* duration of the clear note, *FN* highest frequency value of the fundamental of the noisy note, *FC* highest frequency value of the fundamental of the clear note

Table 1 Body measurements of 88 sexed, adult yelkouan shearwaters *Puffinus yelkouan* (in mm, except body mass in g) and the percentage sexual dimorphism for each measurement

	Males			Females				
	n	Mean ± SD	Range	n	Mean ± SD	Range	t	% Dimorphism
Head	44	49.00 ± 1.71	43.60–54.50	42	47.44 ± 1.52	44.35–50.50	4.44***	3.2
Bill depth at gonys	46	8.09 ± 0.45	7.00–9.25	42	7.55 ± 0.39	6.50–8.50	6.06***	6.8
Minimum bill depth	45	7.40 ± 0.57	5.80–8.80	42	6.80 ± 0.43	5.80–7.80	5.49***	8.1
Bill length	46	37.01 ± 1.24	34.30–39.75	42	35.46 ± 1.16	33.10–38.20	6.01***	4.2
Nalospi	46	29.67 ± 1.29	27.05–32.40	42	28.12 ± 1.14	25.50–30.50	5.24***	5.2
Tarsus length	46	48.46 ± 1.33	45.70–52.40	42	47.49 ± 1.27	44.40–50.40	3.48***	2.0
Tarsus height	45	6.65 ± 0.46	5.60–7.95	42	6.57 ± 0.49	5.25–7.40	0.81 NS	1.2
Toe	45	49.04 ± 1.55	46.40–52.20	42	47.46 ± 1.41	44.30–50.80	4.96***	3.2
Wing	46	242.29 ± 4.87	233.0–253.0	42	239.88 ± 4.86	229.0–250.0	2.32*	1.0
Tail	45	86.51 ± 4.29	75.0–97.0	42	85.80 ± 3.51	80.0–95.5	0.83 NS	0.8
Mass	45	441.33 ± 36.01	360.0–510.0	41	428.73 ± 34.13	355.0–495.0	1.66 NS	2.9

Unpaired *t*-test values and levels of significance are also indicated for comparison between sexes in each variable

NS not significant

* $P < 0.05$; *** $P < 0.001$

new stepwise DFA with the total sample (86 birds). This indicated that bill depth at gonys ($F_{1,81} = 17.7$, $P < 0.001$), nalospi ($F_{1,81} = 10.3$, $P < 0.01$), tarsus height ($F_{1,81} = 7.0$, $P < 0.05$) and toe length ($F_{1,81} = 4.1$, $P < 0.05$) were the only significant variables and constituted the best predictive model (Wilks' Lambda = 0.52, $\chi^2 = 52.9$, $df = 4$, $P < 0.001$). The discriminant function (D_2) obtained was:

$$D_2 = 29.82 + 1.68 \text{ bill depth at gonys} + 0.42 \text{ nalospi} - 1.02 \text{ tarsus height} + 0.23 \text{ toe length}.$$

This discriminant function correctly classified 87.2% of the birds (84.1% of males, 90.5% of females). Cross-validation (Jackknife) classified adults with 83.7% accuracy (79.5% of males, 88.1% of females). The classification accuracies of the analysis sample and the cross-validation were similar, suggesting minimal sampling bias. The slightly greater accuracy and the larger sample size used to perform D_2 suggest it as the most useful function when sexing yelkouan shearwaters based on biometric measurements.

Measurement comparisons between the members of 25 pairs revealed that males were larger than females for only up to 84% of the pairs analyzed (bill depth at gonys; Table 2). The multi-measurements approach showed that the male global size was larger for only 81% of the 21 pairs for which we had all measurements.

Acoustic analysis

Vocalizations of all recorded yelkouan shearwaters ($n = 16$) had the same acoustic pattern: a repetition of a

Table 2 Percentage of males larger than females and comparison of variable means between sexes within pairs

	Males larger (%)	n	t
Head	78	23	2.43*
Bill depth at gonys	84	25	4.57***
Minimum bill depth	75	24	3.85***
Bill length	80	25	3.60***
Nalospi	76	25	3.81***
Tarsus length	68	25	2.33*
Tarsus height	63	24	1.47 NS
Toe	63	24	2.80**
Wing	56	25	2.12*
Tail	63	24	1.02 NS
Mass	52	23	0.67 NS
Size	81	21	4.09***

Paired *t*-test values and levels of significance are also indicated

n Number of pairs examined, *NS* not significant

* $P < 0.05$: ** $P < 0.01$; *** $P < 0.001$

simple unit (= the call) composed of a noisy note and a clear one (Fig. 2). The comparison of acoustic parameters between sexes showed that all measured parameters differed significantly (Table 3). The highest percentages of dimorphism were observed for parameters measured on the clear note: DC (53.3%) and FC (48.5%). The ranges (minimum and maximum values) of these parameters did not overlap between males and females.

The discriminant function obtained immediately at the first step classified birds with 100% accuracy (Wilk's lambda = 0.03) with FC as discriminant variable

Table 3 Acoustic parameters of recorded yelkouan shearwater calls (n = 16) and the percentage sexual dimorphism for each parameter

	Male (n = 10)		Female (n = 6)		t	% Dimorphism
	Mean ± SD	Range	Mean ± SD	Range		
Temporal analysis						
DN (s)	0.86 ± 0.13	0.64–1.01	1.13 ± 0.24	0.90–1.47	2.95*	17.8
DC (s)	1.40 ± 0.18	1.12–1.75	0.70 ± 0.14	0.55–0.89	–8.22***	53.3
Frequency analysis						
FN (Hz)	550.6 ± 62.0	434.5–668.3	433.7 ± 33.6	369.6–463.2	–4.22***	20.8
FC (Hz)	893.1 ± 42.5	842.6–953.4	467.3 ± 34.6	440.0–514.2	–20.68***	48.5

Unpaired t-test values and levels of significance are also indicated for comparison between sexes in each parameter

DN Duration of the noisy note, *DC* duration of the clear note, *FN* highest frequency value of the fundamental of the noisy note, *FC* highest frequency value of the fundamental of the clear note

* P < 0.05; *** P < 0.001

($F_{1,15}$ = 427.6, P < 0.001). On this basis we can propose a very simple acoustic method to unambiguously separate males and females by considering only FC range values. To separate sexes, we chose the middle value (678.4 Hz) between the maximum female FC value (514.2 Hz) and the minimum male FC value (842.6 Hz). Thus, if FC < 678.4 Hz, the call belongs to a female and, conversely, if FC > 678.4 Hz, the call belongs to a male.

Discussion

Morphological dimorphism

The sexual dimorphism in morphological measurements that we observed in this study contradicts the preliminary results obtained by Zotier (1997) and the sexually monomorphic status given to this species by Bull et al. (2004, 2005). This can be explained by the small sample size or the different geographic origins of samples in these studies. Morphometry of the yelkouan shearwater demonstrated that females have smaller bills, heads, legs and wings than males at both the colony and within-pair levels. Sexual differences in head, bill, leg and wing measurements are often observed in the Manx shearwater group (Thompson 1987; Brooke 1990; Cuthbert and Davis 2002; Genovart et al. 2003; Keitt et al. 2003). Differences were found for bill length (*P. puffinus*, *P. huttoni*, *P. mauretanicus*), depth and width (*P. huttoni*, *P. mauretanicus*), nalospi (*P. mauretanicus*), head + bill length (*P. puffinus*, *P. opisthomelas*, *P. mauretanicus*), tarsus length (*P. puffinus*, *P. huttoni*, *P. opisthomelas*, *P. mauretanicus*), middle toe and wing lengths (*P. puffinus*), head length and width, and shoulder width (*P. huttoni*). As in other *Puffinus* species, yelkouan shearwater sexual dimorphism was >5% only for bill depth measurements (Genovart et al. 2003; Bull et al. 2005), and nalospi. It should be noted that even if males are larger than females on average for some measurements within pairs, males are not systematically the

largest shearwater in a pair (≤84% of pairs according to the measurements and 81% of pairs for the global size).

Vocal dimorphism

Usually, in *Puffinus* species, female calls are, contrary to male calls, harsh and noisy (James and Robertson 1985a; Bretagnolle 1996; Brooke 1978, 1988) and do not present a clear part with visible harmonics (Brooke 1978). The yelkouan shearwater deviates from this rule since males and females share the same basic call structure: a noisy and a clear note. This characteristic (clear part in males only) cannot therefore be used to distinguish sexes in the species. Nevertheless, our acoustic analyses showed that a clear sexual vocal dimorphism does exist in this species. This sexual vocal dimorphism appears at the level of the temporal and the frequency domains. Effectively, we showed that the highest frequency of the fundamental and the duration of the clear note of female calls are lower than those of male calls. Similar results have been shown in other *Puffinus* species. For example, in the sooty shearwater *P. griseus* and the short-tailed shearwater *P. tenuirostris*, female calls have a lower frequency than male calls (Warham 1996). In the greater shearwater *P. gravis* and the little shearwater *P. assimilis*, the mean length of the unit call is higher for males than for females (Brooke 1988; James and Robertson 1985a).

Sex determination

Bill depth, nalospi, tarsus height, head and toe length were the best discriminant morphological measurements in the yelkouan shearwater. Combining these measurements, we obtained a discriminant function for sexing adults with 87.2% accuracy. The accuracy of this discriminant function is higher than those reported for other species of the Manx shearwater group (Brooke 1990; Cuthbert and Davis 2002), except for the Balearic shearwater (Genovart et al. 2003).

The use of the discriminant function reported in this paper for sexing adult yelkouan shearwaters with morphological measurements is easy, fast, inexpensive and minimally stressful. However, studies on several seabirds have noted geographic variation in morphometry (e.g., Bost et al. 1992; Granadeiro 1993; Guicking et al. 2004; Gómez-Díaz et al. 2006). Morphometry of yelkouan shearwaters is likely to vary geographically as this species is distributed throughout the Mediterranean basin. Applicability to populations other than the one from which the function was performed must be tested and the performance of a discriminant function for the entire breeding range of this species may be necessary (Van Franeker and Ter Braak 1993; Bertelloti et al. 2002; Arnould et al. 2004).

In the acoustic analysis, we showed that DC and FC are two key parameters that, even taken alone, allow the sexing of adult yelkouan shearwaters with 100% accuracy. It seems reasonable to assume that any investigator, after a short training period in the field, could learn to determine yelkouan shearwater sex by hearing alone. However, to free oneself from investigator aptitude, the most reliable way remains to proceed with recorded signals. Thus, the stages of the acoustic sexing method that we propose are as follows: (1) record the bird, (2) make a spectrogram of the recorded signal using any signal processing software providing a sonographic representation, (3) select a call which is composed of the two kinds of note, and (4) measure on the sonogram the highest frequency value of the fundamental of the clear note (FC). According to the FC value, the sex is determined (female: FC < 678.4 Hz and male: FC > 678.4 Hz). As this acoustic parameter is very easy to measure, this acoustic sexing method is particularly simple, efficient and can be used throughout the year. Finally, this is the first 100% reliable sexing method of a shearwater species without individual manipulation, which is particularly important for a potentially threatened species. However, it is not always possible to wait for the spontaneous calls and to record them.

Zusammenfassung

Morphologische versus austische Analysen: Welche Methode eignet sich am besten zur Geschlechtsbestimmung des Mittelmeersturmtauchers (*Puffinus yelkouan*)?

Die Geschlechtsbestimmung von monomorphen Seevogelarten bereitet erhebliche Schwierigkeiten und die etablierten Methoden haben diverse Nachteile, wie beispielsweise jahreszeitlich bedingte Nichtanwendbarkeit oder Stresserzeugung. Der Mittelmeersturmtaucher (*Puffinus yelkouan*) ist eine wenig untersuchte Seevogelart, die im Mittelmeerbecken endemisch ist. Ökologische und biologische Daten über diese Art sind dringend erforderlich und dafür ist die Entwicklung geeigneter Feldmethoden unabdingbar. Im vorliegenden Fall haben wir zwei Methoden zur Geschlechtsbestimmung des Mittelmeersturmtauchers gegeneinander getestet: Die herkömmliche morphologische und eine neue akustische Methode. Morphologische Untersuchungen zeigten geschlechtsspezifische Unterschiede in Kopf-, Bein- und Federmaßen mit einem großen Überschneidungsbereich und einer daraus resultierenden Genauigkeit von lediglich 87,2%. Im Gegensatz dazu zeigte sich bei bioakustischen Vergleichen der Rufe, dass der Maximalwert der Grundfrequenz (FC) und die Dauer der Basisnote sich bei Männchen und Weibchen nicht überschnitt. Damit lässt sich das Geschlecht des Mittelmeersturmtauchers durch Messen des Grundfrequenzwerts (FC) eindeutig bestimmen. Vögel mit einem FC < 678,4 Hz sind Weibchen, während Männchen in einer Grundfrequenz von > 678,4 Hz rufen. Zusätzliche Vorteile der bioakustischen Geschlechtsbestimmung liegen in ihrer Einfachheit und darin, dass sie keinen direkten Zugriff erfordert, was vor allem für eine potentiell gefährdete Vogelart von Bedeutung ist.

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