

# SEXUAL DICHROMATISM IN MEDITERRANEAN STORM PETRELS *HYDROBATES PELAGICUS MELITENSIS*

## DICROMATISMO SEXUAL EN EL PAÍÑO EUROPEO *HYDROBATES PELAGICUS MELITENSIS*

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SUMMARY.—*Sexual dichromatism in mediterranean storm petrels* *Hydrobates pelagicus melitensis*.

We propose the use of a non-invasive technique for sexing the Mediterranean subspecies of the European storm petrel *Hydrobates pelagicus melitensis*. We found that this subspecies shows sexual dimorphism in rump band length and wing length. We found that storm petrels can be sexed using discriminant analysis and/or the product of wing length \* rump band length. Fast and cheap sex identification methods can improve conservation programs and population studies of this species.

*Key words:* *Hydrobates pelagicus*, non-invasive techniques, sex identification.

RESUMEN.—*Dicromatismo sexual en el paíño europeo* *Hydrobates pelagicus melitensis*.

Proponemos el uso de una técnica no invasiva para sexar la subespecie mediterránea del paíño europeo *Hydrobates pelagicus melitensis*. El tamaño de la banda del obispillo y la longitud del ala muestran un claro dimorfismo en esta subespecie. Los resultados obtenidos muestran que el paíño europeo puede ser sexado utilizando el análisis discriminante, o bien el producto de la longitud del ala por el tamaño de la banda del obispillo. Un método que permite identificar el sexo de forma rápida y económica, y que puede mejorar los programas de conservación y los estudios poblacionales de esta especie.

*Palabras clave:* identificación sexual, paíño europeo, técnicas no invasivas.

### INTRODUCTION

In studies of population dynamics, age and sex are vital components (Nisbet, 2001). Sex identification can be a difficult task in many

bird species particularly if they are not sexually dimorphic. The availability of molecular techniques for sexing birds has had a great impact on our understanding of avian behaviour, ecology and evolution (Lessells and Mate-

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man, 1996; Kalmbach *et al.*, 2009). Accurate sex identification can help ensure the success of conservation programmes during captive breeding, reintroductions and population monitoring (Lessells and Mateman, 1996). Molecular sex identification has also shown how birds can change the sex ratio of their offspring in response to resource availability (Ellegren *et al.*, 1996; Nager *et al.*, 1999).

Molecular sex identification has three disadvantages. The first is the economical cost, a limiting factor for some ecological studies. The second is that sex identification is required instantly for some experimental and behavioural studies. The last one is the disturbance caused to the individual to take the blood or feather samples. It is faster and less invasive to take some morphometrics than a blood sample from the vein. It is possible to avoid these problems in several bird species by using biometrical differences to determine sex. In the case of some species, such as cassin's auklets *Ptychoramphus aleuticus* (Nelson, 1981), and cory's shearwater *Calonectris diomedea* (Bretagnolle and Lequette, 1990), where the plumage is monomorphic, a pair can be captured and the relative size of the bill and tarsus can be used to identify sex (Nelson, 1981; Bretagnolle and Lequette, 1990). In the present study we have used the biometrics of plumage characteristics as a non-invasive method to identify the sex of the mediterranean storm petrel, *Hydrobates pelagicus melitensis*, a taxon distinguished both morphologically and genetically (Lalanne *et al.*, 2001; Cagnon *et al.*, 2004) which appears to show no sexual dimorphism. Results were corroborated by blood sampling and molecular sex identification.

## MATERIAL AND METHODS

The mediterranean storm petrel colony in **Marettimo Island**, Italy, has been monitored

since 1985, with about 6,000 individuals ringed (Lo Valvo and Massa, 2000; Sanz-Aguilar *et al.*, 2009; Sanz-Aguilar *et al.*, 2010). In 2008 and 2009 we captured 46 storm petrels and measured wing length, to the nearest 0.1 mm with a wing rule; tarsus, from the middle of midtarsal joint to distal end of tarsometatarsus; head plus bill, bill depth and width and rump band length (fig. 1), to the nearest 0.01 mm using Vernier callipers. Individuals were chosen from previously selected nests that were followed during most of the breeding season. Birds were captured while on the nest. From these 46 individuals we obtained blood samples. Blood spots, which were preserved by drying onto pieces of filter paper were then stored in separate labelled eppendorf tubes until analyses were carried out.

DNA for sex identification was extracted from the dried blood spots using Qiagen DNeasy® Blood and Tissue Kit following the

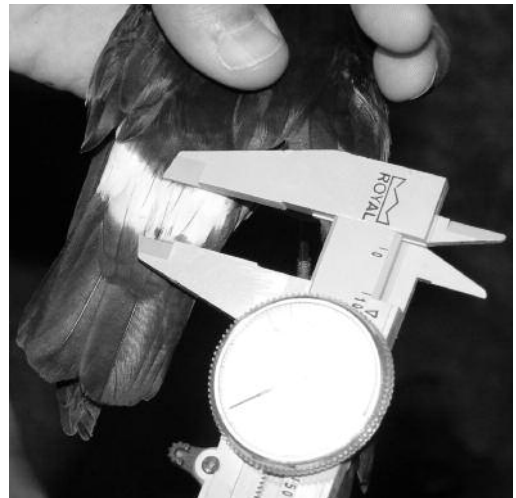


FIG. 1.—We measured the visible part of the rump band length for sexing storm petrels.

[Medida de la parte visible de la longitud de la banda del obispillo para sexar los paños europeos.]

recommended protocol. PCR amplification of selected regions of the sex linked CHD gene was performed using primers 2550F 5' GTT ACT GAT TCG TCT ACG AGA -3' (Fridolfsson and Ellegren, 1999) / 2757R 5' AAT TCC CCT TTT ATT GAT CCA TC -3' (R. Griffiths *pers. com.*). A reaction volume of 10ul was used with 1ul of genomic DNA as the template, 2ul 5x GoTaq® Flexi Buffer (Promega) 2mM MgCl<sub>2</sub> (Promega), 200uM of each dNTP, (Promega) and 0.4U GoTaq® DNA polymerase (Promega). PCR was carried out using a Biometra Thermocycler, with the following reaction conditions: initial denaturing step of 94 °C for 2 mins, followed by 30 cycles of 49 °C for 1 min, 72 °C for 1 min and 94 °C for 45 secs. Final annealing and extension temperatures of 49 °C for 1 min and 72 °C for 5 mins. PCR products were separated by gel electrophoresis using 2% agarose stained with ethidium bromide.

A t-test was carried out to test whether biometrics were a good predictor of the sex of an individual based on the known sexes of these birds determined from molecular analysis. We also compared the single biometrics between sexes. A discriminant analysis was carried out entering all the biometrics taken (head+bill, rump, tarsus, wing length and bill

length). We selected the variables with a P value < 0.05 and entered those in the model. We used SPSS 13 to carry out the analysis.

## RESULTS

We sexed 46 individuals using molecular techniques of which 21 were females and 25 were males. Sexes were confirmed using known sex individuals from studies in the Atlantic population as positive controls. Using morphometrics we found that females have a longer rump band than males (table 1). We also confirmed the larger size of females in comparison with males. There were no significant differences in either head plus bill length or tarsus measurements. To sex with a 95% confidence interval, we used the product wing length\*rump band length (females: 2042.08 ± 45.17, 95% confidence interval = 1948.15 – 2136.02; males: 1781.87 ± 45.71, 95% confidence interval = 1687.53-1876.21). The discriminant analysis correctly sexed 80.9% of the individuals, selecting wing-length ( $F_{1,41} = 29.9$   $P < 0.001$ ) and rump ( $F_{1,41} = 8.41$   $P < 0.01$ ); the discriminant function was  $D = 0.402 * \text{wing-length} + 0.294 * \text{rump} - 55.35$ .

TABLE 1

Mean ± Standard Error for different biometrics and rump according to sex. P values for differences between biometrics are indicated in the last column. In bold significant values.

[Media ± Error Estandard de varios parámetros biométricos y obispillo, de acuerdo con el sexo. Los valores de la P se indican en la última columna. En negrita los significativos.]

	Males	Range	Females	Range	<i>t</i>	P
Wing	125.24 ± 0.42	122-130	128.6 ± 0.43	124-134	291.75	<b>0.001</b>
Tarsus	23.8 ± 0.17	22-25-4	23.5 ± 0.18	22.1-24.9	0.171	0.41
Head+bill	32.6 ± 0.21	30.2-34.5	32.7 ± 0.24	30.5-34.9	-0.27	0.81
Rump	14.2 ± 0.34	11.2-16.9	16.0 ± 0.36	13.5-19.6	40.72	<b>0.001</b>

## DISCUSSION

The analysis was done based on individuals that were sexed genetically. We decided not to assign the sex to the partner because occasionally three different individuals were observed in the same nest over several days (unpublished data), probably because in our study site the nests were very close. Due to the peculiarity of the colony in **Marettimo**, we cannot assume the same would happen in other colonies where storm petrels nest on individual burrows.

When biometrics can be taken for both members of the pair, discriminant analysis can be a reliable method for sexing individuals (Hamer and Furness, 1991; Henderson, 1991; Granadeiro, 1993; Palomares *et al.*, 1997; Phillips and Furness, 1997), but in the case of storm petrel, biometrics do not consent a good discrimination except for wing length. Other Procellariiformes species can be sexed using vocalizations (e.g.: Ristow and Wink, 1980); however, for storm petrels it is a difficult task, as they do not emit calls when they are captured.

The results obtained in this study show that for sexing storm petrels, the use of wing length and rump band length together may increase the probability to separate sexes. Thus, the difference that we have reported in rump band length is an original contribution to identify sexes in this monomorphic species. Plumage patterns in storm petrels show that a white rump band is highly contrasted against the black of the rest of the feathers, and this could indicate the sex of an individual to other storm petrels, especially under low light conditions, in addition to the olfactory signals (de Leon *et al.*, 2003). Discriminant analysis provided a lower percentage of birds sexed correctly, but it is still a valid method when sex determination does not have to be 100% accurate.

This is the first reliable method that has been discovered for sexing storm petrels using

a visual, non-invasive technique. Using this method the probability of correct sexing individuals is very high (81%) and in the case of both parents present at the nest even higher. Furthermore using only two biometric measures, would speed up measuring operation reducing disturbance due to handling the birds. It would be interesting to confirm if this sexual difference is also present in the Atlantic subspecies *H. p. pelagicus*, which is smaller than the Mediterranean (Lalanne *et al.*, 2001; Cagnon *et al.*, 2004). The ability to accurately determine sex will provide opportunities to examine possible inter- and intrasexual differences in the behaviour and ecology of adults. Some adult storm petrels can be sexed while in the field, reducing logistical problems, the costs incurred using laboratory analysis, and furthermore speeding up data collection, data analysis and also possibly affect policy implementation.

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