Sexual dimorphism and determination of sex of Yellow-legged gulls *(Larus michahellis lusitanius)* in southern Portugal

Dimorfismo sexual e determinação do sexo de gaivotas-de-patas-amarelas *(Larus michahellis lusitanius)* no sul de Portugal

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ABSTRACT

Male and female Yellow-legged gulls *Larus michahellis lusitanius* display identical plumage, making the determination of sex by observation virtually impossible. This study aims to provide a tool for determining the sex of *L. m. lusitanius* individuals of a gull population from the southern coast of Portugal based on biometric measurements. A discriminant function using the head length, bill depth and tarsus length was developed using data from dead gulls of known sex, as a tool to determine the sex of live gulls of the same population. The discriminant function obtained correctly classified the sex of 97.7% of the gulls used in the study. However, a limitation of this study is that the function cannot be used for other populations of the same sub-species, as there is significant variance between the biometric measurements of gulls of different populations.

Keywords: biometric measurement, *Larus michahellis lusitanius*, sex determination, sexual dimorphism, southern Portugal.

RESUMO

Machos e fêmeas de Gaivota-de-patas-amarelas *Larus michahellis lusitanius* apresentam plumagem idêntica, tornando a determinação do sexo por observação virtualmente impossível. Este estudo tem como objetivo fornecer uma ferramenta para determinar o sexo de de uma população de *L. m. lusitanius* da costa sul de Portugal, com base em medidas biométricas. Uma função discriminante usando o comprimento da cabeça, a altura do bico, e o comprimento do tarso foi desenvolvida usando dados de gaivotas mortas de sexo conhecido, como um método para determinar o sexo das gaivotas vivas da mesma população. A função discriminante obtida classificou corretamente o sexo de 97.7% das gaivotas amostradas neste estudo. No entanto, uma potencial limitação deste estudo é que a função não pode ser usada para outras populações da mesma subespécie, pois há uma variação significativa entre as medidas biométricas de gaivotas de populações differentes.

Palavras-chave: medidas biométricas, *Larus michahellis lusitanius*, determinação do sexo, dimorfismo sexual, sul de Portugal.

Introduction

The Yellow-legged Gull (*Larus michahellis*) is a resident species in Portugal, nesting along the littoral zones of mainland Portugal, as well as on the islands of Madeira and Azores archipelagos (Meirinho et al. 2014). The plumage of these gulls does not differ between males and females, rendering the process of determining the sex of individuals by observation difficult (Ingolfsson 1969). Although molecular testing of genes can be used to sex live individuals (Griffiths et al. 1998, Fridolfsson & Ellegren 1999), this method is invasive (requiring blood samples) and involves the use of laboratory resources (Meissner & Fischer 2017). A less invasive method that has been used in other studies is based on individual biometrics, as sexual size dimorphism is present in this gull species (Bosch 1996, Arizaga et al. 2008, Galarza et al. 2008). However, as biometric measurements vary between Yellow-legged Gull subspecies and even between different populations of the same subspecies, a discriminant function based on the measurements obtained from one population cannot be applied towards another (Evans et al. 1994, Galarza et al. 2008).

There are three different subspecies of *Larus michahellis*, spanning different distribution ranges. *Larus m. michahellis* is present in the Mediterranean basin, while *Larus m. lusitanius* resides on the Atlantic coast of the Iberian Peninsula and *Larus m. atlantis* resides in the Macaronesian islands and northwest African coastlines (Svensson et al. 2003, Olsen 2018).

This study took place at RIAS Wildlife Rehabilitation and Research Centre, located in Quinta de Marim, Olhão, southern Portugal. Each year, RIAS receives and releases thousands of wild animals, including gulls, birds of prey, passerines, hedgehogs, and chameleons, among many other wildlife species. Over the past eight years, the Centre has received over 200 adult *Larus m. lusitanius* gulls, which are part of the population breeding in the southern coast of Algarve.

This study aims to use data gathered from dead *Larus m. lusitanius* gulls which have been collected at RIAS, to test whether biometric measurements can be used to build a discriminant function allowing the accurate identification of the sex of live gulls of the same population.

Methods

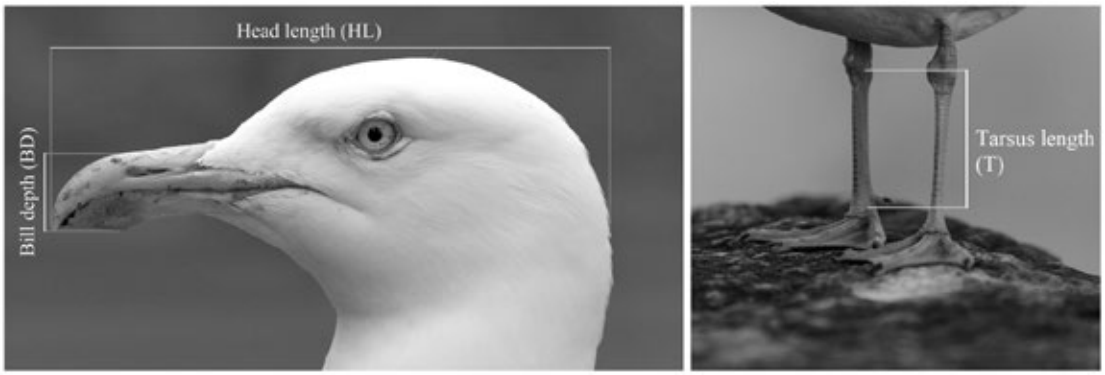
For this study, a sample of 88 adult gulls (4th year and older based on the plumage) of *L. m. lusitanius* were measured and dissected between June and October of 2019, at RIAS Centre. The 88 gulls were comprised of 51 females and 37 males, found within a 70-km radius from RIAS, and most likely belonging to the population of southern Portugal, as they were collected during the breeding season. Even though *L. michahellis* gulls are mostly sedentary, it is also possible that some individuals could have been visitors from the western coast of Portugal and the southern coast of Spain (Meirinho et al. 2014). The gulls had either entered the Centre already deceased or were too weak from their illness or injury and had died during rehabilitation. A vernier caliper was used measure (to the nearest 0.1mm) the head length, including the bill (HL), the bill depth, measured at the gonys (BD), and the tarsus length (T; Figure 1).

While wing measurements have been used in other studies (Arizaga et al. 2008, Galarza et al. 2008), over 30% of the individuals included in this study showed either damaged or molting primary feathers, preventing accurate wing measurements. Upon collection of the biometric measurements, necropsies were performed on the gulls to determine their sex. Each necropsy consisted of an incision across the abdomen of the specimen, followed by the removal of the liver, the stomach, and the intestines. The presence of eggs and oviduct within the reproductive system indicated that the gull was female, while the presence of two testes proved that the gull was male. Although the majority of the gulls were kept in a freezer between the time of death and the necropsy procedure, this method of preservation would not have altered the dimensions of the non-fleshy body parts required for the study, as previously established by Bosch (1996).

Two-sample t-tests were conducted to compare the differences in the means of the three

Figure 1- Measurements used to create a discriminant function to determine the sex of Yellow-legged Gulls (*Larus michahellis lusitanius*) in southern Portugal (Original images by Scott Carroll and Hermes Rivera, respectively).

Figura 1 - Medições utilizadas para criar uma função discriminante para determinar o sexo de Gaivotas-de-patas-amarelas (*Larus michahellis lusitanius*) no sul de Portugal". (imagens originais de Scott Carroll e Hermes Rivera, respetivamente).



measurements between the two sexes, providing a t-value for each measurement. Furthermore, the dimorphism index (% D) between the two sexes for each measurement was calculated using Storer's index (1966) as % D = 100*[(Xm - Xf)] / (Xm + Xf)*0.5, where Xm and Xf represent the measurement's mean value for males and females respectively, as used in a similar study by Galarza et al. (2008). The measurements of the three variables for the 88 gulls were used to resemble a variance-covariance matrix. This matrix was used to perform a Principial Components Analysis (PCA), visualizing the difference between males and females. A jacknife (leave-one-out) statistical method was used for the discriminant analyis, where each individual gull was classified using a discriminant function derived from the total sample excluding the individual being classified (Zuur et al. 2007), allowing for unbiased analysis of the effectiveness of the discriminant function. All analyses were performed using Past 4.03 software (Hammer et al. 2001).

Results

Male gulls were overall larger than females in all three biometric variables, as shown by the differences in mean and range values (Table 1). However, extensive overlap between the two sexes was also observed for all three measurements.

The variation between males and females is portrayed by the PCA analysis with the two axes capturing 99.2% of the variation in the resemblance matrix. The PCA results show a clear separation between males and females, with the head length being the most discriminative variable of the three (Figure 2).

Stepwise discrimination analysis of the three biometric measurements resulted in the function X = (0.26033)*(HL) + (0.08839)*(BD) + (0.11371)*(T) – 40.590, where X > 0 identifies male gulls and X < 0 identifies female gulls (Wilk's lambda = 0.2197; degrees of freedom = 3; p-value < 0.001). Using this function, 86 of the 88 gulls (or 97.7%) were correctly classified (jackknifed) as male or female.

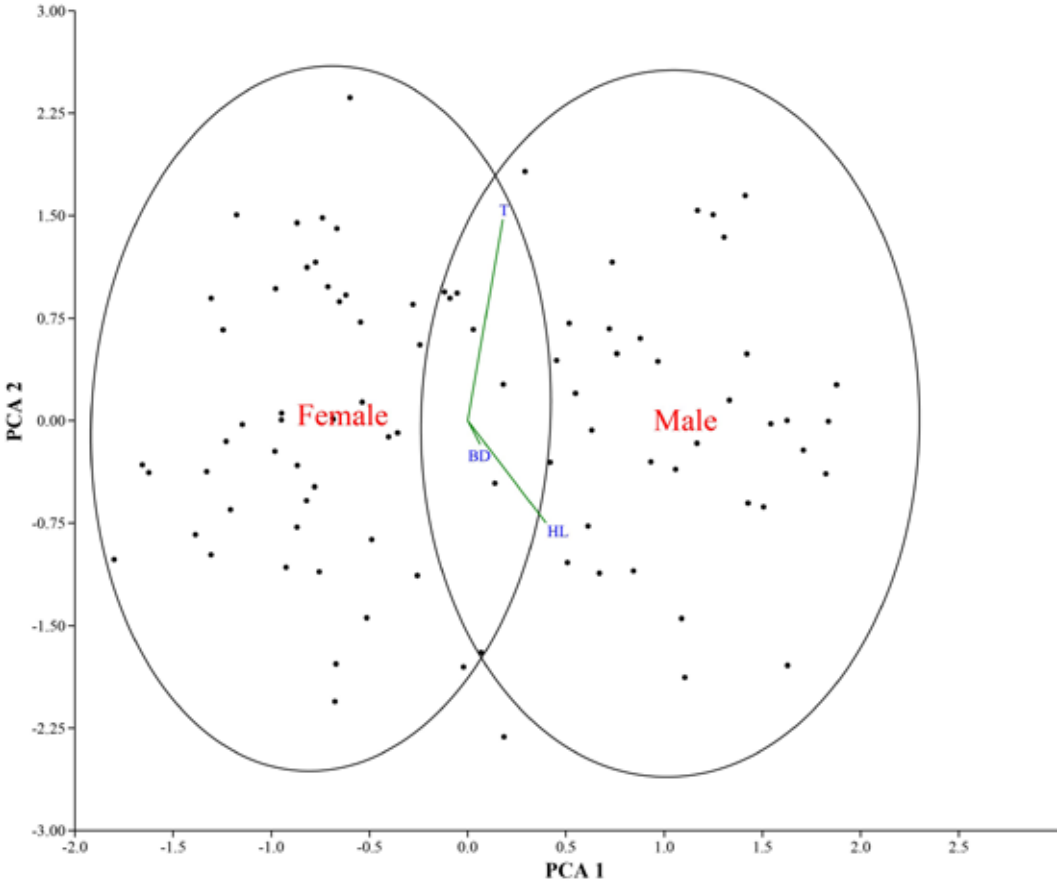
Table 1 - Metric measurements and dimorphism index (%D) of 88 Yellow-legged Gulls (*Larus michahellis lusitanius*) in southern Portugal. Head length (HL), bill depth (BD), tarsus length (T), standard deviation (SD), coefficient of variation (CV).

Tabela 1 - Medidas biométricas e índice dedimorfismo (%D) de 88 Gaivotas-de-patas-amarelas (*Larus michahellis lusitanius*) no sul de Portugal. Comprimento da cabeça (HL), altura do bico (BD), comprimento do tarso (T), desvio padrão (SD), coeficiente de variação (CV).

	MALES (n = 37)			FEMALES (n = 51)					
	Mean ± SD	Range	CV	Mean ± SD	Range	CV	t – value	p-value	% D
HL (mm)	127.5 ± 3.23	121.1 - 132.7	0.025	116.1 ± 3.04	110.4 - 122.7	0.026	16.92	<0.01	9.36
BD (mm)	19.1 ± 0.90	17.1 - 21.8	0.047	17.5 ± 0.73	15.8 - 19.5	0.042	9.45	<0.01	8.74
T (mm)	69.2 ± 2.40	62.4 - 73.4	0.035	63.4 ± 2.39	58.1 - 68.0	0.038	11.25	<0.01	8.758

Figure 1- Principal Component Analysis biplot demonstrating the separation between male and female *Larus m. lusitanius*, based on the three biometric variables used in the study (head length (HL), bill depth (BD) and tarsus length (T)).

Figura 1 - Biplot de Análise de Componentes Principais demonstrando a separação entre machos e fêmeas de *Larus m. lusitanius*, usando as três variáveis biométricas utilizadas no estudo (comprimento da cabeça (HL), altura do bico (BD) e comprimento do tarso (T)).



Discussion

In this study, the head length, bill depth and tarsus length were measured in 88 adult *L. m. lusitanius* individuals at RIAS Centre, in southern Portugal. These measurements were used to create a discriminant function which can be further used to identify the sex of *L. m. lusitanius* gulls in southern Portugal.

The *L. m. lusitanius* population sample used in this study showed similar measurements (differences of up to 1 mm) of the head length and bill depth to the population sample from Izaro Island, in northern Spain (Galarza et al. 2008). It is important to note, however, that the tarsus length showed great variation between the two populations, with the Izaro Island sample displaying approximately 5 mm larger tarsi in both female and male gulls. Individuals from the eastern Bay of Biscay were smaller in both head and tarsus length measurements than the gulls used in this study (Arizaga et al. 2008). Differences in biometric measurements can also be observed between different subspecies. Within this context, the gulls of southern

Portugal displayed smaller head and tarsus lengths to the sample used by Bosch (1996) of the subspecies *Larus michahellis michahellis*, located in Medes Island, northeastern Spain (Table 2).

The variation in biometric measurements between subspecies and different populations of the same subspecies demonstrates that to study the sex ratio of a gull population using biometric measurements, separate studies must be conducted for each population. Studying the proportion of male and female gulls in a population can help understand its behaviour and lifecycle. In this context, RIAS Centre will use the discriminating function presented in this study to analyse any patterns observed between the sex of *L. m. lusitanius* gulls received and released at the Centre and their susceptibility to infection, malnutrition, survival rate, and recovery rates.

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Table 2 - Comparison of the three biometric measurements (head length (HL), bill depth (BD) and tarsus length (T)) between four different population samples of male and female *Larus michahellis* subspecies. Each measurement is displayed as mean value ± standard deviation.

Tabela 2 - Comparação das três medidas biométricas (comprimento da cabeça (HL), altura do bico (BD), e comprimento do tarso (T)) entre quatro amostras populacionais de machos e fêmeas de subspecies de Gaivotas-de-patas-amarelas *Larus michahellis*. Cada medida é representada como média ± desvio padrão.

	RIAS	IZARO ISLAND	BAY OF BISCAY	MEDES ISLAND
MALES	*(Larus m. lusitanius)* n = 37	*(Larus m. lusitanius)* n = 31	*(Larus m. lusitanius)* n = 86	*(Larus m. michahellis)* n = 104
HL (mm)	127.5 ± 3.2	128.6 ± 3.1	123.5 ± 0.6	130.1 ± 2.6
BD (mm)	19.1 ± 0.9	20.1 ± 0.8	20.6 ± 0.1	19.0 ± .06
T (mm)	69.2 ± 2.4	74.2 ± 4.8	65.0 ± 0.3	71.0 ±2.0

	RIAS	IZARO ISLAND	BAY OF BISCAY	MEDES ISLAND
FEMALES	*(Larus m. lusitanius)* n = 51	*(Larus m. lusitanius)* n = 36	*(Larus m. lusitanius)* n = 69	*(Larus m. michahellis)* n = 77
HL (mm)	116.1 ± 3.0	115.5 ± 3.4	115.5 ± 0.6	119.0 ± 2.4
BD (mm)	17.5 ± 0.7	17.6 ± 0.7	18.5 ± 0.2	17.1 ± 0.5
T (mm)	63.4 ± 2.4	68.9 ± 2.2	60.7 ± 0.3	65.1 ± 2.2

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