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Sexing of common gull, *Larus canus*, using linear measurements

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Abstract. The common gull is a widespread species, occurring in almost the whole of Europe that shows no sexual dimorphism in plumage and some dimorphism in size. To propose a method for sexing birds from this species by linear measurements, a discriminant function analysis was applied to a set of morphometric traits in birds captured in northern Poland during the non-breeding season. In total, 138 males and 76 females were measured and sexed molecularly. The averages of measurements of males were larger than the average measurements of females. All individuals with a total head length shorter than 90.6 mm were females. However, the occurrence of larger individuals of the subspecies *heinei* among common gulls of the nominate subspecies *canus* wintering in Europe makes separating males according to a single border value of this measurement less efficient. The proposed discriminant function containing the total head length allowed for correct sexing of 95.7 % of males and 90.9 % of females. This discriminant equation may be used for sexing common gulls when DNA sampling is not possible, and could be applied to data collected in the past, providing the opportunity to re-examine the results of previous analyses. However the higher misclassification rate than in other gull species should be taken into account.

Key words: gulls, sexual dimorphism, discriminant functions

Introduction

Determination of sex is of considerable importance in studies on different features of bird morphology (Teather & Nol 1997, Scherer et al. 2014), behaviour (Mathot & Elner 2004, Saino et al. 2010, Quillfeldt et al. 2011), migration and wintering strategies (McCloskey & Thompson 2000, Nebel 2005, Müller et al. 2014), evolutionary ecology (Clutton-Brock 1986) and also different physiological processes (Bluhm et al. 2000, Moreno et al. 2001, O'Reilly & Wingfield 2003). However, sex differentiation is difficult in many species with monomorphic plumage due to a large overlap in the linear dimensions of males and females (Jakubas & Wojczulanis 2007, Meissner & Krupa 2016). Although the development of molecular sexing methods (Griffiths et al. 1998, Fridolfsson & Ellegren 1999) overcomes this problem, laboratory examination of many samples needed for comprehensive analyses is costly. Hence, approaches based on discriminant functions using morphometric data from a sample of birds with known sex became a standard procedure in studies of monomorphic bird species from different families (Huynen et al. 2003, Meissner 2005, Poisbleau et al. 2010, Wojczulanis-Jakubas & Jakubas 2011). This procedure can also be useful for sexing individuals captured and measured a long time ago, which enables the reanalysis of existing data with new aims of testing sex dependent differences (Burger & Gochfeld 1981, Meissner 2015, Meissner & Krupa 2017).

Discriminant functions are especially effective in the case of gulls (Laridae), allowing correct sexing from 90 to 100 % of individuals from different species (Mawhinney & Diamond 1999, Chochi et al. 2002, Galarza et al. 2008, Meissner et al. 2017). Such functions for sexing different species of European gulls have been already presented (e.g. Palomares et al. 1997, Galarza et al. 2008, Dubiec et al. 2015, Meissner et al. 2017), but there is no reliable method for sex recognition of the common gull (*Larus canus* Linnaeus, 1758), a widespread species occurring in almost the whole of Europe. There exist studies about the morphology (Hein & Martens 2002) and migration (Hauff 1984, Kilpi & Saurola 1985, Pedersen et al. 2000) of common gulls; however, in all those studies, authors were unable to sex birds, which limited interpretation of the results. Only Hein & Martens (2002) presented border values of the

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total head length for sexing common gulls, but they did not assess the effectiveness of this method using birds sexed molecularly. This paper aims to derive discriminant functions based on linear measurements of molecularly sexed birds, which could be useful for sex determination of this species in future studies, and also in analyses of already collected data. Moreover, the results of molecular sexing of common gulls were used in this study to verify the effectiveness of the method proposed by Hein & Martens (2002).

Material and Methods

In total, 201 common gulls were caught in 2007-2015 between November and March in the Gulf of Gdańsk region (south-eastern Baltic). Gulls were baited with pieces of bread into the loop-trap made of a fishing line placed on the ground. After pulling that line, it tightened on the bird's leg. Caught birds were aged according to plumage characteristics (Grant 1986) and only individuals in definite (adult) plumage were taken into account. The sex of these birds was identified molecularly using about 20-50 µl of blood taken from branchial vein and preserved in 70 % ethyl alcohol. DNA was extracted following evaporation of the ethanol and using a Blood Mini DNA kit (A & A Biotechnology, Gdynia, Poland). In case of 80 individuals caught in years 2007-2009, the W- and Z-linked sequences were amplified with primers 1237L and 1272H (Kahn et al. 1998), while in later years with 2550F and 2718R primers (Fridolfsson & Ellegren 1999). The length of the expected W- and Z- fragments were assessed to: CHD1W 400-450 bp and CHD1Z 600-650 bp as in other species from family Laridae (Fridolfsson & Ellegren 1999). The PCR protocol included an initial denaturation step of 94 °C for 2 min, followed by 40 cycles: 94 °C for 30 s, annealing at 50 °C for 50 s, elongation at 72 °C for 2 min. A final elongation step was performed at 72 °C for 5 min. Each 16.5 µl PCR sample contained: 3.2 µl of DNA, 0.8 µl of 25 mM $MgCl_2$, 3 µl of sterile-filtered water, 1 µl of 10 µM of each primer: 2550F and 2718R and 7.5 µl of Sigma REDTaq Ready-Mix (Sigma-Aldrich). PCR products were visualised with a 2 % agarose gel stained with Midori Green (ABO, Gdańsk, Poland) following 60 min long electrophoresis at 85 mA and 300 V. The sex of three exceptionally large females was confirmed by checking twice using both kinds of primers. Additionally, 13 freshly dead common gulls were collected in the same area. These individuals were sexed by dissection. Only gulls caught or found in the wintering period (November-March) were taken into account. From all these birds the following measurements were taken: total head length (THL) and bill depth at gonys (BD), with dial caliper to the nearest 0.1 mm, and tarsus plus toe length (TTL) and wing length (maximum chord) (WL), with stopped rule with accuracy 1 mm (Busse & Meissner 2015). Although the bill length was a fairly good predictor of sex in gulls (Evans et al. 1993, Rodrigues et al. 1996, Torlaschi et al. 2000), it was not used in this study, because it was highly correlated with total head length and inclusion of both measurements would have violated the multicollinearity assumption of independent variables. Moreover, in gulls the end of the horny sheath of keratin at the bill base is partially covered by small feathers, and this edge, which is crucial in bill length measuring, is poorly visible. Hence as this measurement is less repeatable than total head length, it was omitted from the analysis. Especially that total head length was commonly reported as a better predictor of the sex in gulls than bill length (e.g. Palomares et al. 1997, Chochi et al. 2002, Dubiec et al. 2015).

In total, 138 males and 76 females were measured and sexed. The accuracy and repeatability of measurements taken by different ringers were checked as described by Busse & Meissner (2015). Differences in morphological traits between males and females were tested with a two-sample t-test or Cochran-Cox test (t' statistic) when variances were not equal (Zar 1996). The degree of sexual dimorphism in size was assessed by the Storer's index, in which a larger value indicates greater sexual dimorphism (Storer 1966). A stepwise discriminant function analysis was used to determine which set of variables best classified the sex of birds. The inclusion of the measurement into the model was based on the Wilk's Lambda ratio with default minimum partial F to enter the model equal to 3.84 and maximum partial F to remove, 2.71. Despite the presence of a male-biased sex ratio in the dataset *a priori* classification probabilities were set as equal for both sexes (P = 0.50). The unbalanced sex-ratio among caught birds is rather a result of catching method and does not reflect unbalanced sex ratio in sampled population. Males are larger than females and perhaps they come first to the bait. Discriminant function analyses were also performed separately on selected measurements to assess the utility of individual characters in separating sexes. The equations presented in this paper are based on unstandardized discriminant function coefficients, where $D > 0$ indicates a male, and $D < 0$ indicates a female, but standardised coefficients were also given

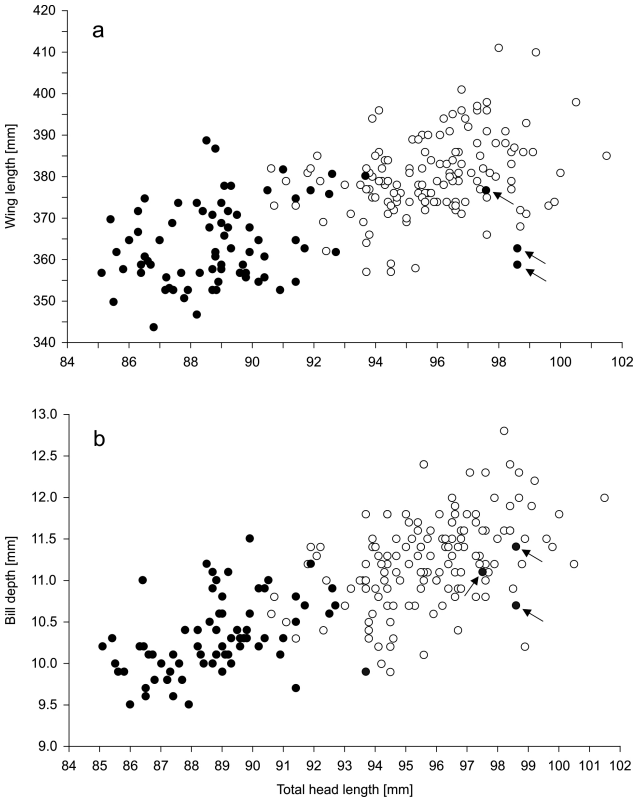

Fig. 1. Relationship between the total head length and wing length (a) and between the total head length and bill depth (b) in male and female adult common gulls. Black dots – females, white dots – males. Three exceptionally large females are indicated by arrows.

to assess the contribution of one predictor in the context of the other predictors in the model. Validation of developed discriminant functions was conducted with a Jackknife procedure, where the sex of each individual in the sample is predicted from the functions calculated after this particular individual has been removed from the data set. This procedure is preferred over other methods, because it gives smaller variation of the mean estimate of the proportion of correctly classified individuals (Dechaume-Moncharmont et al. 2011). All statistical analyses were performed using Statistica 12 software (StatSoft Inc.) with additional Statistica Macro File (SVB) for Jackknife procedure downloaded from http://sdn.statsoft.com.

Results

Males were larger in all measurements than females (Table 1). The most sexually dimorphic traits were bill depth followed by total head length, while the least dimorphic traits were tarsus plus toe length and wing length (Table 1). All individuals with total head length shorter than 90.6 mm were females (Table 1, Fig. 1). The presence of three very large females with total head length between 97.5 and 98.6 mm meant it was not possible to provide a single border value of this measurement, which would allow the separation of the majority of males. Common gulls with wing length above 389 mm may be sexed as males, but distributions of other measurements in both sexes overlapped to a great extent (Table 1).

Tarsus plus toe length was not included in any model. The equation containing total head length and wing length revealed the lowest misclassification rate and allowed the correct sexing of 96.4 % of males and 90.8 % of females. However, when taking into account only total head length, the misclassification rate of the

Table 1. Sexual differences in mean linear measurements of 138 males and 76 females of adult common gulls. Storer's dimorphism index (DI) is given.

Measurement	Males mean	SD	Range	Females mean	SD	Range	Result of test t or t'	*p*	DI
Total head length	95.79	2.06	90.6-101.5	89.12	2.65	85.1-98.6	t' = 19.02	< 0.001	7.2
Bill depth	11.25	0.54	9.9-12.8	10.33	0.47	9.5-11.5	t = 12.38	< 0.001	8.3
Tarsus plus toe length	102.8	3.96	92-114	97.2	3.64	86-109	t = 10.30	< 0.001	5.6
Wing length	380.3	9.38	357-411	364.0	9.70	344-389	t = 12.01	< 0.001	4.4

Table 2. Discriminant equations for sexing adult common gulls. Classification accuracy was given according to the Jackknife procedure. WL – wing length, THL – total head length, BD – bill depth at gonys.

Discriminant equation	Correctly sexed males	Correctly sexed females	Jackknife misclassification rate estimate
$D_1 = 0.379THL + 0.379WL - 47.402$	96.4 %	90.8 %	6.4 %
$D_2 = 0.397THL + 0.337BD - 40.744$	96.4 %	89.5 %	7.1 %
$D_3 = 0.438THL - 40.890$	95.7 %	90.8 %	6.8 %
$D_4 = 0.105WL - 39.434$	93.5 %	68.4 %	19.1 %
$D_5 = 1.931BD - 21.097$	87.0 %	75.0 %	19.0 %

discriminant equation was only slightly higher, with a similar proportion of correctly sexed males and females (Table 2). The inclusion of bill depth instead of wing length did not improve the accuracy of common gull sexing. As the wing length cannot be measured during outer primary moult, and decreases steadily during an inter-moult period due to primary wear (Pienkowski & Minton 1973), the equation D_3 containing only total head length is recommended for sexing of common gulls. It is worth to note that removing three exceptionally large females from the analysis did not improved the overall results as the Jackknife misclassification rate decreases only slightly to 5.6 %.

Discussion

The total head length was the best linear measurement allowing effective sexing of common gulls. This is in agreement with studies of many other gull species, where the total head length was the only measurement that is always presented in discriminant functions derived for sexing (e.g. Palomares et al. 1997, Torlaschi et al. 2000, Chochi et al. 2002, Galarza et al. 2008, Dubiec et al. 2015). According to the results of an extensive study conducted in northern Germany, common gulls with total head lengths shorter than 88.5 mm should be treated as females and those with total head lengths longer than 91.0 mm as males (Hein & Martens 2002). When applying this criterion to birds sexed molecularly in this study, 92 % of males and all females were sexed correctly, but 67 % of individuals remained unsexed. Moving the male border value of total head length (91.0 mm) towards a higher value results in increasing sexing accuracy, but also increases the number of unsexed individuals. All males will be correctly sexed when this border value was set at 98.6 mm, but in this case, as many as 89 % of common gulls remained unsexed. Hence, the discriminant equation presented in this study seems to be a better method for sexing common gulls in Europe than the border values of the total head length provided previously by Hein & Martens (2002).

The majority of common gulls appearing in central and western Europe belong to the nominate subspecies. Although individuals from the eastern subspecies *L. c. heinei* have occasionally been reported from different countries (Kuschert 1983, Kompanje & Post 1990, 1993, Šírek & Pohanka 2004), they may be more common, which is suggested by larger measurements of head and wing length of common gulls caught in winter (Schmitz & Degros 1990, Bengtsson & Pedersen 1998), as birds from *heinei* are larger than those of the *canus* subspecies (Glutz von Blotzheim & Bauer 1982). Despite low ringing activities in north-eastern Russia, some ringing recoveries were obtained from the westernmost part of the breeding range of the *heinei* subspecies (Bønløkke et al. 2006, Fransson et al. 2008, Bairlein et al. 2014, Valkama et al. 2014), that indicates that this subspecies regularly occurs in western and central Europe.

The presence of larger individuals of *L. c. heinei* in European wintering grounds makes sexing of the common gull according to linear measurements more complicated. Linear measurements of *heinei* females considerably coincide with *canus* males, as suggested by the case of the three exceptionally large females in this study. The presented discriminant equation to some extent takes into account the possibility of including individuals from *L. c. heinei*, and this is why it seems to be robust when sexing birds from the *canus* subspecies.

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