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Development of Sexual Dimorphism and Sexing of Baltic Herring Gull (*Larus argentatus argentatus*) in Successive Age Classes

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Abstract.—Discriminant functions based on external body size measurements are widely used to sex different gull species with great accuracy. However, all of them have been derived for adult birds, which puts into question their usefulness for sexing immatures due to possible changes in size as birds mature. To address this issue, discriminant functions that allow sexing of Herring Gulls in immature age classes with an accuracy of 88-100% were developed. In total, 247 males and 111 females of wintering Herring Gulls, including birds in the first, second and third winter plumages and individuals in adult plumage, were measured and sexed in the region of the Gulf of Gdańsk (southeastern Baltic coast). In all age classes, total head length and bill depth were the best traits for sexing Herring Gulls. However, bill depth, but not total head length, increased with age. Hence, in the first and second winter plumages, total head length made a much higher contribution to the discriminant function than bill depth. In the third winter plumage, bill depth became more important. For individuals in adult plumage, however, the contribution of total head length and bill depth were nearly the same. Hence, using discriminant equations derived for adults resulted in erroneous sexing of 4.5-8.9% of immature males, which were identified as females, and illustrates the importance of deriving age-specific discriminant functions. *Received 28 October 2016, accepted 17 November 2016.*

Key words.—Baltic Sea, discriminant analysis, Herring Gull, *Larus argentatus*, sexing, sexual dimorphism.

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Gulls (Laridae) are monomorphic with respect to plumage characters (del Hoyo *et al.* 1996), and one of the problems in studying free-living gulls is difficulty in determining the sex of caught individuals. Although DNA-based sexing methods (Griffiths *et al.* 1998; Kahn *et al.* 1998; Fridolfsson and Ellegren 1999) allow determination of a bird's sex with great accuracy, in the case of large samples this technique is costly and prolongs the stay of birds in captivity. Nowadays, non-invasive approaches based on discriminant functions using morphometric data from a sample of birds with known sexes is a standard procedure in many bird families (Huynen *et al.* 2003; Meissner and Pilacka 2008; Poisbleau *et al.* 2010; Wojczulanis-Jakubas and Jakubas 2011). This procedure can be very useful for sexing gulls of different body size and from different geographical regions. In the vast majority of gull species, bill depth and total head length or bill length are the most useful measurements for sex identification (e.g., Nugent 1982; Coulson *et al.* 1983; Hanners and Patton 1985; Mawhinney and Diamond 1999; Chochi *et al.* 2002). This also holds true for species of the Herring Gull complex (*Larus argentatus*) (Fox *et al.* 1981; Bosch 1996; Galarza *et al.* 2008; Hammouda and Selmi 2013). The discriminant functions derived in these studies allow sexing of gulls with at least 90% accuracy. However, these discriminant functions were invariably derived for adult birds. In large species of gulls, reaching sexual maturity takes 5 years (Glutz von Blozheim and Bauer 1982; Grant 1986). Moreover, in the Herring Gull, bill depth, one of the most important measurements for recognizing the sex of an individual, increases with age for at least 9 years of life (Coulson *et al.* 1981). Temporal changes in the size of traits included in discriminant function analyses may affect the predictive value of biometric sex-discrimination methods (van de Pol *et al.* 2009). Hence, the equations derived for adult gulls may fail to sex immatures correctly.

Our objectives were to: 1) determine whether the size of particular traits in the Herring Gull varies among the four identifiable age classes, which may have consequences for sexing birds according to these measurements; and 2) derive discriminant functions useful for in-hand sex determination for this species that take into account the age of an individual.

METHODS

In total, 337 Herring Gulls were caught between 2007 and 2015 in the Gulf of Gdańsk region (southeastern Baltic) and aged according to plumage characteristics (Malling Olsen and Larsson 2004). Three immature age classes, corresponding to the first, second and third winter plumages, were recognized. The fourth age class consisted of birds in definitive winter plumage, hereafter referred to as adult plumage. Herring Gulls in the fourth winter plumage were excluded from analyses because recognizing birds in this plumage is problematic (Glutz von Blozheim and Bauer 1982; Grant 1986) and the sample size was small.

The sex of captured birds was identified molecularly. About 20-50 µl of blood was taken from the 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). In the case of 105 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 and Ellegren 1999). PCR products were visualized with a 2% agarose gel stained with Midori Green (ABO Sp. z o.o.) following a 60-min long electrophoresis at 85 mA and 300 V. Blood samples from the three smallest males and five largest females were analyzed for the second time using another pair of primers, i.e., P2 and P8 (Griffiths *et al.* 1998); in all of these cases, the initial sex assessment was confirmed. Additionally, 42 freshly dead Herring Gulls were collected in the same area. These individuals were sexed by dissection.

Only Herring Gulls caught or found in the wintering period (November-March) were considered. The following measurements were taken for all birds: total head length and bill depth at gonys with dial caliper to the nearest 0.1 mm, and tarsus plus toe length and wing length (maximum chord) with a stopped rule to 1 mm (Busse and Meissner 2015). Bill length has been found to be a fairly good predictor of sex in gulls (Evans *et al.* 1993; Rodrigues *et al.* 1996; Torlaschi *et al.* 2000); however, it was not used in this study because it was highly correlated with the total head length, and inclusion of both measurements would have violated the multicollinearity assumption of independent variables. Moreover, in the Herring Gull, the end of the horny part at the bill base is partially covered by feathers and is poorly visible. Hence, this measurement is less repeatable than the total head length.

In total, 247 males and 111 females were measured and sexed. More than 90% of birds were measured by three researchers, and the accuracy and repeatability of measurements taken by the different researchers were checked as described by Busse and 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 sexual dimorphism in size was assessed by 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 the default minimum partial F to enter the model equal to 3.84 and maximum partial F to remove 2.71 (McLachlan 2004). *A priori* classification probabilities were set equal for both sexes ($P = 0.50$). Discriminant function analyses were also performed separately on selected measurements to assess utility of individual characters in separating sexes. Equations presented here are based on unstandardized discriminant function coefficients, where $D > 0$ indicates a male and $D < 0$ a female, but standardized coefficients were also given to assess the contribution of one predictor in the context of the other predictors in the model. All statistical analyses were performed using Statistica software with additional Statistica Macro File (SVB) for jackknife procedure (StatSoft 2014).

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 that particular individual has been removed from the data set. This procedure is preferred over two other commonly used methods, because it gives smaller variation of the mean estimate of the proportion of correctly classified individuals than the sample-splitting and unbiased estimated discriminant rate when compared with the resubstitution procedure (Dechaume-Moncharmont *et al.* 2011). To show outliers and the overlapping zone of the measurements of males and females, the prediction interval ellipses for two sexes were shown in the scatterplots. Each ellipse describes the area in which a single new observation can be expected to fall with a probability of 0.95 (Tracey *et al.* 1992).

RESULTS

In all age classes, males were significantly larger than females in all measurements (Table 1). Among single measurements, bill depth, followed by total head length, were the most sexually dimorphic traits across all age classes, whereas wing length had the lowest values in the dimorphism index.

In both sexes, there were significant differences in bill depth (AVOVA, $F_{3,182} = 19.44$, $P < 0.001$ and AVOVA, $F_{3,189} = 27.43$, $P < 0.001$ for males and females, respectively) and

Table 1. Sexual differences in mean linear measurements of the Herring Gull in successive age classes, and Storer's dimorphism index (DI).

Measurement (mm)	Males			Females			Result of Test		DI (mm)
	Mean	SD	*n*	Mean	SD	*N*	*t* or *t'*	*P*	
First winter plumage									
Total head length	130.65	3.77	50	119.84	2.75	59	*t'* = 16.82	< 0.001	8.63
Bill depth	18.59	0.62	50	16.90	0.66	59	*t* = 13.68	< 0.001	9.52
Tarsus plus toe length	142.50	4.90	50	132.30	4.50	59	*t* = 11.28	< 0.001	7.42
Wing length	450.60	10.6	50	429.60	9.70	59	*t* = 10.77	< 0.001	4.77
Second winter plumage									
Total head length	130.85	3.21	46	119.46	2.67	43	*t* = 18.13	< 0.001	9.10
Bill depth	18.84	0.73	46	17.05	0.37	43	*t'* = 14.78	< 0.001	9.97
Tarsus plus toe length	141.40	5.10	46	130.90	4.10	43	*t* = 10.78	< 0.001	7.71
Wing length	453.90	10.0	46	431.50	8.20	43	*t* = 11.54	< 0.001	5.06
Third winter plumage									
Total head length	130.56	4.17	34	120.10	1.97	30	*t'* = 13.08	< 0.001	8.35
Bill depth	19.09	0.65	34	17.49	0.53	30	*t* = 10.67	< 0.001	8.75
Tarsus plus toe length	142.30	6.40	34	131.90	3.90	30	*t'* = 7.88	< 0.001	7.59
Wing length	455.40	11.40	34	435.20	5.00	30	*t'* = 9.35	< 0.001	4.54
Adult plumage									
Total head length	131.04	4.17	56	120.79	3.00	61	*t'* = 15.14	< 0.001	8.14
Bill depth	19.54	0.66	56	17.77	0.62	61	*t* = 14.93	< 0.001	9.49
Tarsus plus toe length	141.60	4.90	56	133.10	4.90	61	*t* = 9.39	< 0.001	6.19
Wing length	458.00	10.50	56	438.20	9.20	61	*t* = 10.87	< 0.001	4.42

wing length (AVOVA, $F_{3,182} = 4.39$, $P = 0.005$ and AVOVA, $F_{3,189} = 11.29$, $P < 0.001$ for males and females, respectively), which appeared to increase with age (Fig. 1). However, mean total head length and mean tarsus plus toe length did not differ significantly among age classes both in males (AVOVA, $F_{3,182} = 0.14$, $P = 0.934$ and AVOVA, $F_{3,182} = 0.43$, $P = 0.734$ for total head length and tarsus plus toe length, respectively) and in females (AVOVA, $F_{3,189} = 2.31$, $P = 0.078$ and AVOVA, $F_{3,189} = 2.18$, $P = 0.093$ for total head length and tarsus plus toe length, respectively).

There was an overlap in bill depth and total head length between sexes in all age classes (Fig. 2), and the probability of correctly classifying the sex in that overlapping range was lower. The size of this overlap in bill depth in the samples of birds collected for this study varied from 0.4 mm (second winter plumage) to 1.2 mm (adult plumage) and in total head length from 0.6 mm (second winter plumage) to 7.2 mm (adult plumage) (Fig. 2). Hence, sexing Herring Gulls using only one of these measurements is quite effective and allows sexing of more than 88% of birds when using bill depth and more than 91% when using total head length (Table 2). In the overlapping zone in all age classes, exceptionally small males were less common than exceptionally large females (Fig. 2).

The best discriminant functions for sexing Herring Gulls in all age classes included total head length and bill depth, which allowed for correctly sexing 88-98% of males and 91-100% of females (Table 2). In birds in the second winter plumage, besides these two traits, wing length was selected by stepwise discriminant analysis, but the equation containing three measurements provided exactly the same sexing accuracy for males and females. Hence, the equation with only two measurements was retained.

In males, classification accuracy was lowest in birds in the first winter plumage and highest in individuals in adult plumage. In females, there was the opposite tendency with a higher proportion of correctly sexed birds in the first two age classes and a lower proportion of correctly sexed individuals in the third and adult plumages (Table 2).

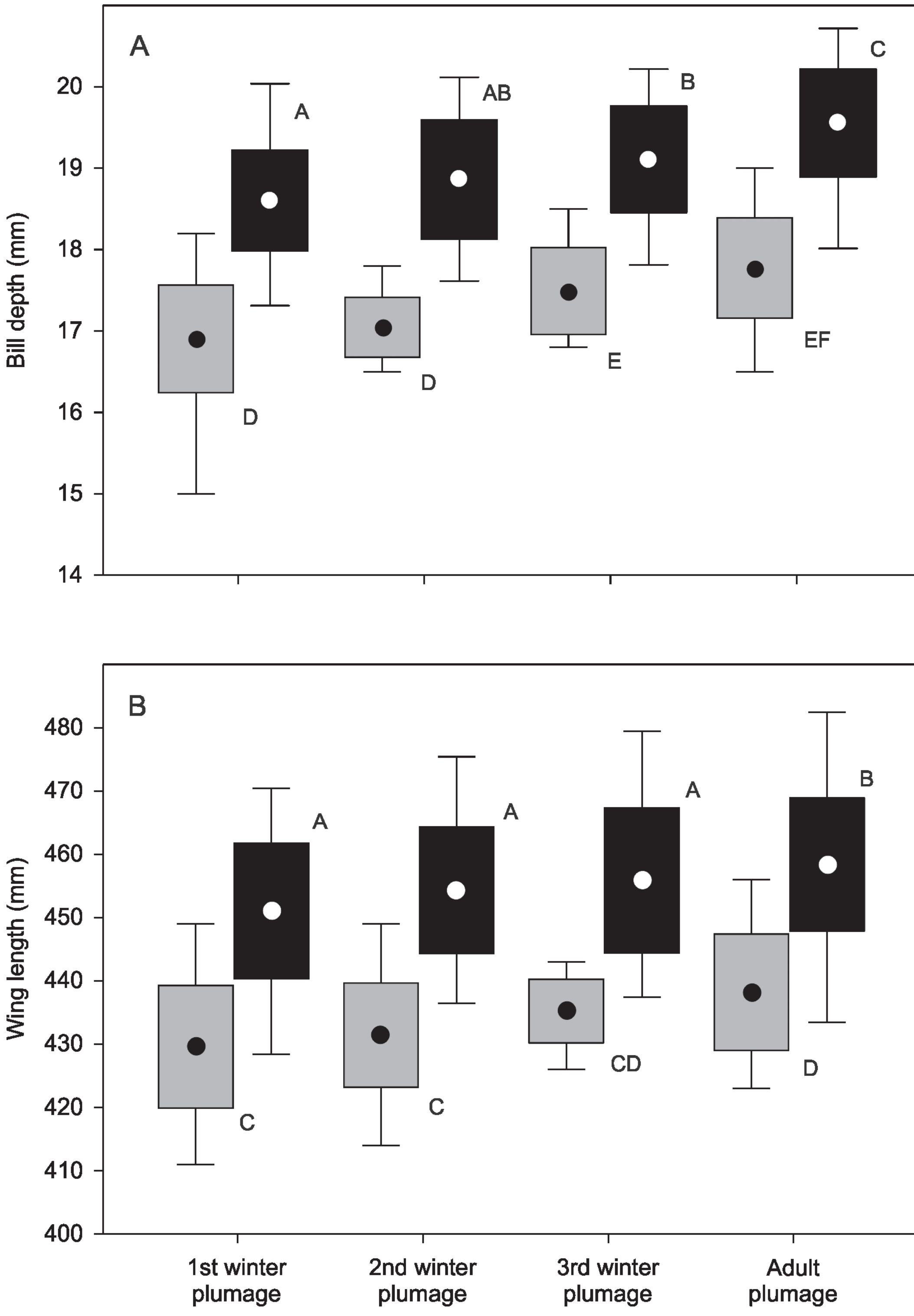

Figure 1. Changes in mean values of the bill depth (A) and wing length (B) in males (black rectangles) and females (gray rectangles) of the Herring Gull in successive age classes. Dot = mean value, rectangle = standard deviation, vertical line = range. Values with the same letters are not significantly different from each other (ANOVA and post-hoc Tukey test, $P > 0.05$).

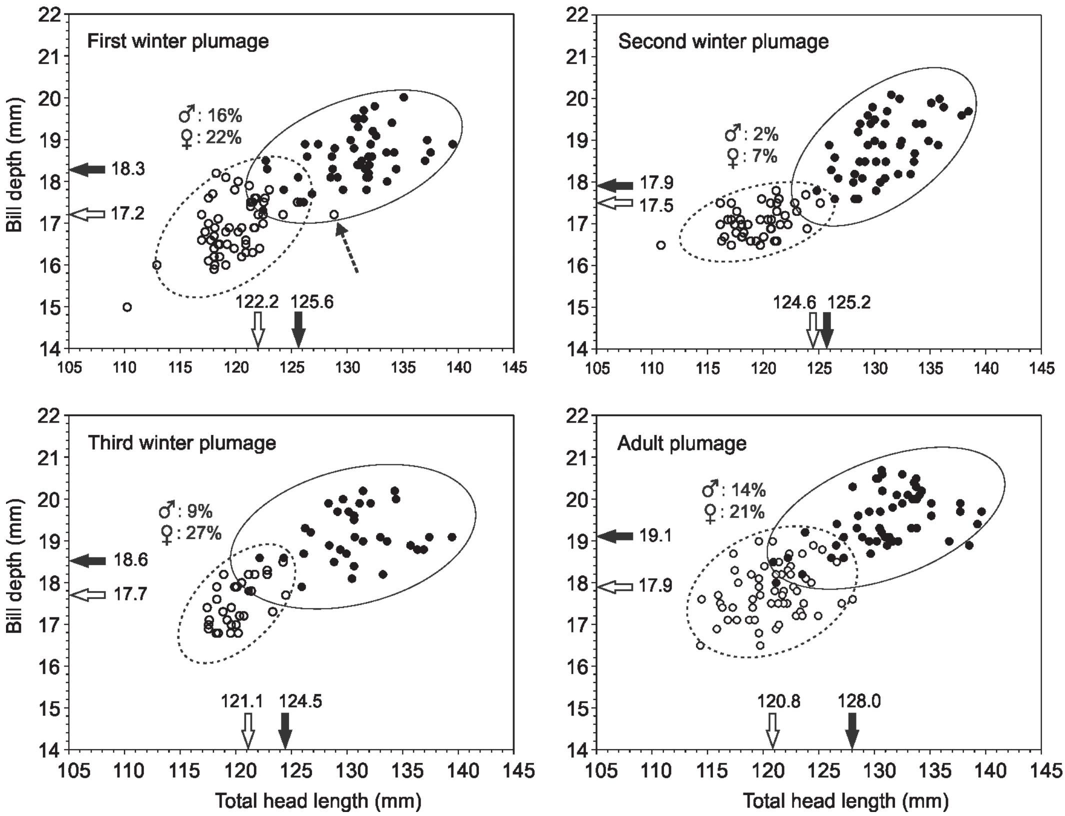

Figure 2. Relationship between the total head length and bill depth in male and female Herring Gulls in different age classes. Black dots = males, white dots = females. The ellipse shows the 95% prediction interval for a single new observation, given the parameter estimates for the bivariate distribution computed from the data for males (solid line) and females (dashed line). Arrows at axes show border values for sexing using only one measurement. The dashed arrow indicates the only individual that had the total head length larger than the border values of the overlapping zone and was not taken into account for presenting border values. Percentages of males and females found in the overlapping zone (including dots outside corresponding ellipse) are given.

Using a discriminant function derived for adults for sexing immature Herring Gulls resulted in 8.2%, 8.9% and 4.5% of erroneous sexing of birds in the first, second and third winter plumages, respectively. In all these cases, males were identified as females.

For birds in their first and second winter plumages, the standardized discriminant function coefficients for total head length were much higher than for bill depth (0.75 and 0.76 vs. 0.39 and 0.38 for the first and second winter plumages, respectively) indicating that total head length was a much better variable for sex discrimination in these two age classes. This difference became smaller in birds in the third winter plumage (0.71 vs. 0.50 for total head length and bill depth, respectively) and disappeared in Herring Gulls in adult plumage (0.62 vs. 0.58 for total head length and bill depth, respectively) (Fig. 3).

DISCUSSION

In all age classes, total head length and bill depth were the best traits for predicting the sex of Herring Gulls, as has been found in many other gull species. However, the contribution of these measurements in the discriminant function was not the same for all age classes. In younger birds, total head length provided a much higher contribution than bill depth. However, in the third winter plumage, bill depth became more important. For individuals in adult plumage, the

Table 2. Discriminant equations for sexing Herring Gulls in successive age classes. Classification accuracy was given according to jackknife procedure. THL = total head length, BD = bill depth at gonys.

	Correctly Sexed	
Discriminant Equation	Males	Females
First winter plumage		
$D_1 = 0.232 * THL + 0.616 * BD – 39.791$	88.0%	98.3%
$D_2 = 1.560 * BD – 27.579$	89.8%	92.0%
$D_3 = 0.307 * THL – 38.296$	96.6%	92.0%
Second winter plumage		
$D_1 = 0.255 * THL + 0.648 * BD – 43.633$	97.8%	100.0%
$D_2 = 1.710 * BD – 30.734$	89.1%	100.0%
$D_3 = 0.338 * THL – 42.307$	97.8%	97.7%
Third winter plumage		
$D_1 = 0.213 * THL + 0.835 * BD – 42.032$	96.7%	91.2%
$D_2 = 1.666 * BD – 30.553$	88.2%	93.3%
$D_3 = 0.301 * THL – 37.797$	91.2%	100.0%
Adult plumage		
$D_1 = 0.171 * THL + 0.908 * BD – 38.444$	98.3%	91.1%
$D_2 = 1.567 * BD – 29.163$	88.5%	89.3%
$D_3 = 0.277 * THL – 34.858$	95.1%	91.1%

contribution of total head length and bill depth were nearly the same. This is in agreement with the results of a previous study showing that the most rapid increase in bill depth in Herring Gulls occurs during the first year after fledging and continues up to at least 9 years of age (Coulson *et al.* 1981). The process of skull growth in birds is very rapid due to an early obliteration of the skull sutures (Ruprecht 1968), and this likely accounts for why we saw no difference in total head lengths among Herring Gulls in different age classes. As bill depth increases with no corresponding increase in total head length, this changes the cross-ratio of these measurements, and consequently leads to a higher contribution of this trait when sexing Herring Gulls in adult plumage. An increase in bill depth with age was also found in the Black-headed Gull (*Chroicocephalus ridibundus*) (Palomares *et al.* 1997).

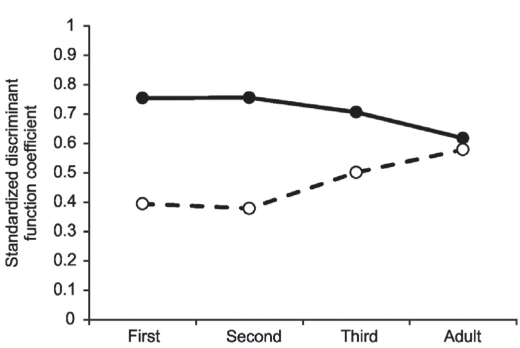

Figure 3. Standardized discriminant function coefficients assessing the contribution of total head length (solid line) and bill depth (dashed line) in D_1 equations containing these parameters in subsequent age classes.

Similar to other large species of gulls (Fox *et al.* 1981; Mawhinney and Diamond 1999; Arizaga *et al.* 2008; Herring *et al.* 2010), we noted fewer exceptionally small males than very large females in the Herring Gull sample. That is why only males were misclassified when using a discriminant function derived for adult birds to sex immature Herring Gulls. This may reflect different sex-specific selection pressures toward body size and especially bill size, as sexual size dimorphism is favored by selection acting during adult stages when differences in size provide higher reproductive success of both sexes (Teather and Weatherhead 1994; Badyaev 2002). During the nesting period, male gulls are engaged in more agonistic behavior than females, and males are also more aggressive throughout the season (Tinbergen 1960; Butler and Janes-Butler 1983; Kazama *et al.* 2011); hence, this behavior may promote selection toward larger dimensions in males. In shorebirds, gulls and alcids, changes in sexual dimorphism are attributable to male body size changing more than female body size, because females are under stronger natural selection constraints related to fecundity (Lindenfors *et al.* 2003). Moreover, there are sexual differences in foraging behavior in gulls (Camphuysen *et al.* 2015; García-Tarrasón *et al.* 2015), and different bill sizes of males and females may have evolved to reduce intraspecific competition for food, as presumably larger bills are more suitable for some feeding activities, such as predation, than thin bills (Harris and Hope Jones 1969). Coulson *et al.* (1981) also suggested that bill depth might be important as an intraspecific indicator of sex in gulls with no sexual difference in plumage and also might give information about social status. Indeed, foraging success and intraspecific dominance in gulls increase with age (Searcy 1978; Monaghan 1980; Greig *et al.* 1983; MacLean 1986), which corresponds to the bill depth enlargement in successive age classes observed in this study.

Apart from total head length and bill depth, wing length was sometimes used as one of the measurements included in the discriminant function developed for sexing gulls, but this measurement is usually the least dimorphic trait (Palomares *et al.* 1997; Coulson 2009; Hammouda and Selmi 2013; Dubiec *et al.* 2015). However, wing length varies seasonally due to progressive wear of the tip of the longest primary, and this can reduce the recorded wing length for an individual by several millimeters (Meissner 2007; Coulson 2009), and therefore this measurement is less repeatable than total head length and bill depth. Moreover, wing length in the Herring Gull increases with age (Fig. 1), which also was found in the Black-headed Gull (Palomares *et al.* 1997).

The discriminant functions obtained offer high classification accuracy and may be helpful not only in future research, but also to sex birds already measured during previous studies. However, their usefulness may be limited, because applying a discriminant analysis function derived from one gull population to populations of the same species in different geographic locations may be risky due to possible morphological differences between birds of different origin (Evans *et al.* 1993; Palomares *et al.* 1997; Aguirre *et al.* 2009; Hammouda and Selmi 2013). According to banding results in the study area, there is a mixture of wintering Herring Gulls from the northeastern Baltic populations, including birds mainly from Finland, Estonia, Latvia, and Lithuania, as well as Herring Gulls from a local sedentary population and to a lesser extent from Sweden (Kilpi and Saurola 1984; Fransson *et al.* 2008). Therefore, we recommend the equations provided in this study only be used for Herring Gulls that breed around the central, eastern and northeastern Baltic. However, these functions should be tested for their application to other populations beforehand as sometimes the discriminant function derived for Herring Gulls from one location is applicable on vast adjacent area (Robertson *et al.* 2016).

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