Sexual size dimorphism and sex determination by external measurements in the Redshank *Tringa totanus*

Włodzimierz MEISSNER[1,*], Pavel PINCHUK[2], Natalia KARLIONOVA[2], Izabela FISCHER[1], Lucyna PILACKA[1,3]
[1]Avian Ecophysiology Unit, Department of Vertebrate Ecology and Zoology, University of Gdańsk, Gdańsk, Poland
[2]Scientific and Practical Center for Biological Resources, National Academy of Sciences of Belarus, Minsk, Belarus
[3]Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences, Gdańsk, Poland

Abstract: The Redshank is a wader species that shows no sexual dimorphism in plumage, but some dimorphism in size. To propose a method for sexing birds of this species by linear measurements, a discriminant function analysis was applied to a set of morphometric traits in birds captured during the spring migration in southern Belarus. In total, 113 males and 75 females were measured and sexed molecularly. On average, females were larger in all measurements than males. The most sexually dimorphic trait was wing length, which was included in the discriminant function analysis. Jackknife cross-validation showed that the provided equation allowed the correct sexing of 67% of birds (81% of males and 52% of females). When identifying birds with this discriminant function, values lower than −0.96 indicated males, while those larger than 1.17 indicated females. This allowed the correct sexing of 95% of birds of each sex. The presented discriminant equation may be successfully used for sexing Redshanks when blood sampling is not possible, and could be applied to samples collected in the past, providing the opportunity to re-examine results of previous analyses.

Key words: Sexing, sexual dimorphism, discriminant analysis, shorebirds

1. Introduction

The possibility of sex identification of birds has substantial importance for studies on different aspects of bird behavior (Mathot and Elner, 2004; Saino et al., 2010), migration phenology (Meissner and Krupa, 2017), and foraging strategies (Nebel, 2005). However, among waders, there are many species that exhibit very weak or even no plumage dimorphism between the sexes, but that differ in size, and this allows the efficient identification of sex by discriminant analysis (e.g., Meissner, 2005; Sikora and Dubiec, 2007; Hallgrimsson et al., 2008; Meissner and Pilacka, 2008). For the Redshank (*Tringa totanus*), this sexing method was performed according to data from a local breeding population from southern Sweden (Ottval and Gunnarsson, 2007), where birds could be sexed efficiently with an accuracy rate of 81%. However, the equation provided in this study included body mass, which exhibits substantial seasonal changes, varying greatly during migration (Zwarts et al., 1990; Meissner et al., 2011), moult (Zenatello et al., 2002; Fox and King, 2011), egg production, and incubation (Langseth et al., 2001; Jamieson, 2012), and which remains under the influence of weather conditions (Kelly et al., 2002; Robson

and Barriocanal, 2008). This is why body mass is usually omitted in discriminant analyses when data outside the breeding season is taken into account (e.g., Palomares et al., 1997; Sikora and Dubiec, 2007; Jiménez et al., 2015). Hence, in this study, we propose a method for sexing Redshanks by linear measurements only, which may be applied not only in future research, but also to sex birds already measured in past studies.

2. Materials and methods

2.1. Field work

Redshanks were caught in walk-in traps and mist-nets in the floodplain meadows of the Pripyat River in the vicinity of Turov, Gomel Region, Belarus (52°04′N, 27°44′E), which is an important stopover site for waders during seasonal migrations (Meissner et al., 2011; Pinchuk and Karlionova, 2011). The field studies were conducted over 4 spring seasons, from 2006 to 2009. The fieldwork was carried out from the end of March to the middle of May. This period covered almost the entire spring migration season of Redshanks in the area (Pinchuk and Karlionova, 2011). All birds caught were measured, and the following morphometrics were recorded: wing length (maximum

* Correspondence: w.meissner@ug.edu.pl

chord method), total head length, bill length, nostril length, tarsus length, and tarsus plus toe length (Busse and Meissner, 2015). Approximately 50 µL of blood was taken from the branchial vein and preserved in 70% ethyl alcohol for further laboratory analysis. In total, 188 birds were caught, but not all measurements were taken from each individual. Therefore, the sample sizes differed in specific measurements. More than 90% of all captured Redshanks were measured by 2 authors (PP and NK).

Blood samples were taken at the Turov Bird Ringing Station under permits from the Ministry of Natural Resources and Environmental Protection No. 700 from 2 June 2006.

2.2. Laboratory work
DNA was extracted from blood samples using a Blood Mini DNA kit (A & A Biotechnology, Gdynia, Poland) following evaporation of ethanol, and the W- and Z-linked sequences were amplified with 2550F and 2718R primers (Fridolfsson and Ellegren, 1999). The thermic profile included an initial denaturation step of 94 °C for 2 min; followed by 40 cycles of 94 °C for 30 s, annealing at 50 °C for 50 s, and 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). Final PCR products were visualized with a 2% agarose gel stained with Midori Green (ABO, Gdańsk, Poland) following 60-min-long electrophoresis at 85 mA and 300 V. In total, 113 males and 75 females were molecularly sexed.

2.3. Data analysis
Sex differences in mean measurements were checked by Student's t-test. The size dimorphism index (SDI) was calculated according to Lovich and Gibbons (1992) to provide the measure of sexual dimorphism, where greater sexual dimorphism was indicated by a larger SDI value, and a negative value was assigned to measure the larger males. Stepwise discriminant function analysis was conducted to determine which set of variables best classified the sex of the birds. The inclusion of a measurement in the model was based on the Wilk's lambda ratio with minimum partial F to enter the model equal to 3.84, and the maximum partial F to remove from the model was 2.71. Similar to other studies (Sikora and Dubiec, 2007; Ackerman et al., 2008; Gates et al., 2013; Meissner and Krupa, 2016), a priori classification probabilities were set as equal for both sexes (P = 0.50). Despite the presence of a slightly male-biased sex ratio in the sample, we did not adjust the prior probabilities for the analyses, because we did not have prior knowledge of the sex ratio in the population. Discriminant equations presented in this paper are based on unstandardized canonical discriminant function coefficients, where D < 0 indicates a male and D > 0 indicates a female. The validation of a given discriminant function was conducted with the jackknife procedure, where each single case is classified using a discriminant function based on all other cases except the given one. The statistical analyses were performed using Statistica 12 software with additional Statistica Macro File (SVB) for the jackknife procedure, downloaded from http://sdn.statsoft.com.

3. Results
Females were larger than males in all measurements except those related to the length of the leg. The most sexually dimorphic trait was wing length, followed by bill length, while the least dimorphic traits were tarsus and tarsus plus toe lengths (Table). However, considerable overlap even in the wing length occurs between sexes, and individuals with wings shorter than 156 mm can be determined as males and those with wings longer than 167 mm as females. Using this criterion, only 12.5% of Redshanks in the sample could be correctly sexed (Figure 1).

In the stepwise procedure, only the wing length (WL) was selected, producing the equation for calculating the discriminant score:

$$D = 0.267WL - 43.416$$

Table. Comparison of mean measurements (in mm) of males and females of the Redshanks captured in southern Belarus. The size dimorphism index (SDI) is given for each trait.

| Measurement	Males			Females			t-test results		SDI
	Mean	SD	N	Mean	SD	N	t	P	
Total head	74.31	2.12	112	75.16	1.85	75	2.85	0.005	0.011
Bill	41.80	1.87	103	42.50	1.53	70	2.60	0.010	0.017
Nostril	32.04	1.61	112	32.52	1.38	74	2.11	0.036	0.015
Tarsus plus toe	86.6	2.97	113	86.7	2.99	75	0.08	0.939	0.001
Tarsus	51.96	2.25	113	51.82	2.18	74	0.42	0.676	−0.003
Wing	161.2	3.73	111	164.4	3.77	73	5.69	<0.001	0.020
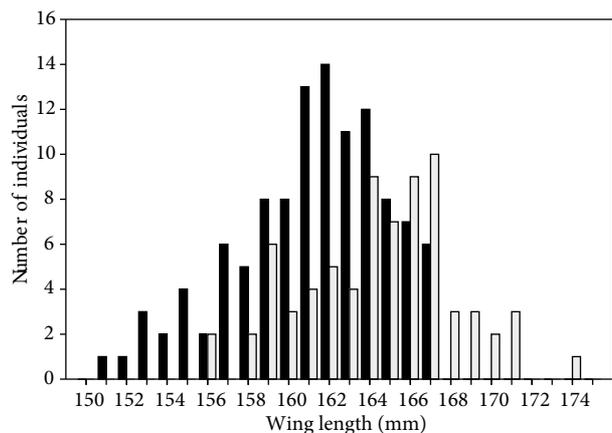
Figure 1. Wing length distribution of male (black bars) and female (gray bars) Redshanks caught in southern Belarus.

The jackknife cross-validation showed that this formula allowed the correct sexing of 67.6% of birds (81.1% of males and 52.1% of females). Using this equation for sexing Redshanks would produce a male-biased sex ratio, because 63% of birds from the overlapping zone would be classified as males. If identifying birds with D < –0.96 as males and those with D > 1.17 as females, only 5% of males and 5% of females will be misclassified (Figure 2). Hence, these 2 border values are recommended for further studies.

4. Discussion
In the Redshank, females are larger than males, but there is quite considerable overlap in the ranges of almost all measurements (Glutz von Blotzheim et al., 1977), which was also shown in this study. Only in the case of the 2 least dimorphic traits, i.e. tarsus and tarsus plus toe length, did the mean values not differ significantly between sexes, and this was also found in Redshanks breeding in southern Sweden (Ottval and Gunnarsson, 2007) and in other wader species, like Jack Snipe *Lymnocryptes minimus*, Eurasian Woodcock *Scolopax rusticola*, and White-rumped Sandpiper *Calidris fuscicollis* (Sikora and Dubiec, 2007; Scherer et al., 2014; Aradis et al., 2015). Redshank characteristics related to resource use (bill length, nostril) are more sexually dimorphic than those related to locomotion (tarsus and tarsus plus toe length). Hence, in this species we can expect sexual differences in the foraging niche, which might lead to spatial segregation of males and females during foraging, as in other wader species with similar differences in bill length between sexes (Nebel et al., 2000).

In the subfamily Tringinae, sexual dimorphism is rather weak, and considerable overlap of morphometrics occurs between the sexes. This overlap results in lower sex
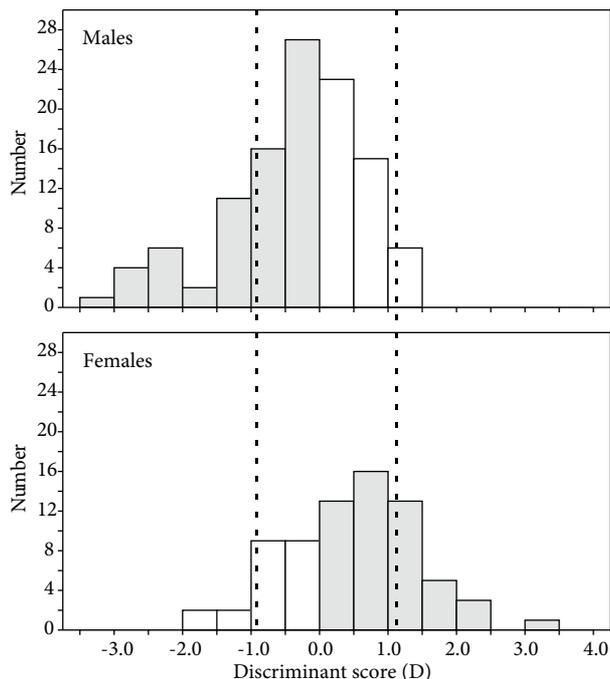
Figure 2. Distribution of the discriminant score D calculated for males and females sexed molecularly. Gray and white bars are for correct and incorrect classifications. Dashed lines show D border values of –0.96 and 1.17, which allowed for 95% of correct classifications of males and females.

classification accuracy rate of discriminant functions than in other shorebird families—for example, 76% in the Wood Sandpiper *Tringa glareola* (Remisiewicz and Wennerberg, 2006) and 77% in the Common Sandpiper *Actitis hypoleucos* (Meissner and Krupa, 2016). The Redshank in this study shows the lowest sexing accuracy rate of 67.6%, somewhat lower than the 71.3% found in the southern Swedish breeding population (Ottval and Gunnarsson, 2007). One reason for such a low sexing accuracy may be that the 2 groups of Redshanks exist within a breeding area inhabited by the subspecies *T. t. totanus*. Those from the northern part of the breeding area are smaller than others (Glutz von Blotzheim et al., 1977; Meissner, 1999). As individuals from both groups can be caught during the spring migration in southern Belarus, this may have resulted in lowering the effectiveness of sexing by the discriminant function, as was described in the case of a mixed group of different subspecies of Dunlins (*Calidris alpina*) (Gates et al., 2013).

The possibility of identifying the sex of Redshanks could be useful in different studies on ecology, behavior, migration, and conservation of this species, which in Europe has shown a moderate decline in the breeding population between 1980 and 2013 (EBCC, 2015).

However, to limit the misclassification error, individuals with a discriminant score D between –0.96 and 1.17 should be treated as unsexed. If using these recommended border D values for the 962 adult Redshanks captured and measured in spring in southern Belarus and 78 birds caught in spring 2012 near Odessa, Black Sea coast (authors' unpublished data), respectively 55% and 54% of individuals would remain unsexed. Hence, a large sample of measured Redshanks is needed to find significant differences between sexes, as was shown for the Common Sandpiper (Meissner and Krupa, 2017). Furthermore, second-year Redshanks usually have heavily worn

primaries in spring (Glutz von Blotzheim et al., 1977), which may lead to the underestimation of the wing length and a subsequent increase of incorrectly sexed birds. Thus, individuals with worn outermost primaries should be excluded from analysis and the proposed equations should be used with caution.

References

Ackerman JT, Takekawa JY, Bluso JD, Yee JL, Eagles-Smith CA (2008). Gender identification of Caspian Terns using external morphology and discriminant function analysis. Wilson J Ornithol 120: 378-383.

Aguirre JI, Arana P, Antonio MT (2009). Testing effectiveness of discriminant functions to sex different populations of Mediterranean Yellow-Legged Gulls *Larus michahellis michahellis*. Ardeola 56: 281-286.

Aradis A, Landucci G, Tagliavia M, Bultrini M (2015). Sex determination of Eurasian Woodcock *Scolopax rusticola*: a molecular and morphological approach. Avocetta 39: 83-89.

Busse P, Meissner W (2015). Bird Ringing Station Manual. Warsaw, Poland: De Gruyter Open Ltd.

EBCC. 2015. Pan-European Common Bird Monitoring Scheme. European Bird Census Council. Online at: http://www.ebcc.info/index.php?ID=587 (accessed 28 March 2017).

Fox AD, King R (2011). Body mass loss amongst moulting Pochard *Aythya ferina* and Tufted Duck *A. fuligula* at Abberton Reservoir, South East England. J Ornithol 152: 727-732.

Fridolfsson A-K, Ellegren H (1999). A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol 20: 116-121.

Gates HR, Yezerinac S, Powell AN, Tomkovich PS, Valchuk OP, Lanctot RB (2013). Differentiation of subspecies and sexes of Beringian Dunlin using morphometric measures. J Field Ornithol 84: 389-402.

Glutz von Blotzheim UN, Bauer KM, Bezzel E (1977). Handbuch der Vögel Mitteleuropas. VII. Wiesbaden, Germany: Akademische Verlag (in German).

Hallgrimsson GT, Palsson S, Summers RW (2008). Bill length: a reliable method for sexing Purple Sandpipers. J Field Ornithol 79: 87-92.

Jamieson SE (2012). Body mass dynamics during incubation and duration of parental care in Pacific Dunlins *Calidris alpina pacifica*: a test of the differential parental capacity hypothesis. Ibis 154: 838-845.

Jiménez A, García-Lau I, Gonzalez A, Acosta M, Mugica L (2015). Sex determination of Least Sandpiper (*Calidris minutilla*) and Western Sandpiper (*Calidris mauri*): comparing methodological robustness of two morphometric methods. Waterbirds 38: 10-18.

Kelly JP, Warnock N, Page GW, Weathers WW (2002). Effects of weather on daily body mass regulation in wintering Dunlin. J Exp Biol 205: 109-120.

Langseth I, Moe B, Bech C (2001). Reduction in body mass and basal metabolic rate in breeding female Black-legged Kittiwakes *Rissa tridactyla*: an adaptation to reduce maintenance costs? Atlantic Seabirds 3: 165-178.

Lovich E, Gibbons JW (1992). A review of techniques for quantifying sexual size dimorphism. Growth Dev Aging 56: 269-281.

Mathot KJ, Elner RW (2004). Evidence for sexual partitioning of foraging mode in Western Sandpipers (*Calidris mauri*) during migration. Can J Zool 82: 1035-1042.

Meissner W (1999). Biometrics of Redshank (*Tringa totanus*) caught in the region of the Gulf of Gdańsk during autumn migration. Vogelwarte 40: 110-116.

Meissner W (2005). Sex determination of juvenile Dunlins migrating through the Polish Baltic region. J Field Ornithol 76: 368-372.

Meissner W, Karlionova N, Pinchuk P (2011). Fuelling rates by spring-staging Ruffs *Philomachus pugnax* in southern Belarus. Ardea 99: 147-155.

Meissner W, Krupa R (2016). Identifying the sex of the Common Sandpiper (*Actitis hypoleucos*) by linear measurements. Ann Zool Fenn 53: 175-182.

Meissner W, Krupa R (2017). Sex-related differences in autumn migration timing of adult common sandpipers *Actitis hypoleucos* (Linnaeus, 1758) (Charadriiformes: Scolopacidae). The European Zoological Journal 84: 136-140.

Meissner W, Pilacka L (2008). Sex identification of adult Dunlins *Calidris alpina alpina* migrating in autumn through Baltic region. Ornis Fennica 85: 135-138.

Nebel S (2005). Latitudinal clines in bill length and sex ratio in a migratory shorebird: a case of resource partitioning? Acta Oecol 28: 33-38.

Nebel S, Piersma T, Van Gils J, Dekinga A, Spaans B (2000). Length of stopover, fuel storage, and a sex-bias in the occurrence of two subspecies of Red Knots (*Calidris c. canutus* and *islandica*) in the Dutch Wadden Sea during southward migration. Ardea, 88: 165-176.

Ottval R, Gunnarsson G (2007). Morphological and molecular sex identification of Redshanks *Tringa totanus*. Bird Study 54: 127-129.

Palomares LE, Arroyo BE, Marchamalo J, Sainz JJ, Voslamber B (1997). Sex- and age-related biometric variation of Black-headed Gulls *Larus ridibundus* in Western European populations. Bird Study 44: 310-317.

Pinchuk PV, Karlionova NV (2011). Influence of climate factors on phenology of spring migration of waders in the south of Belarus. Branta 14: 12-26 (in Russian).

Remisiewicz M, Wennerberg L (2006). Differential migration strategies of the Wood Sandpiper (*Tringa glareola*): genetic analyses reveal sex differences in morphology and spring migration phenology. Ornis Fennica 83: 1-10.

Robertson GJ, Roul S, Allard KA, Pekarik C, Lavoie RA, Ellis JC, Perlut NG, Diamond AW, Benjamin N, Ronconi RA et al. (2016). Morphological variation among Herring Gulls (*Larus argentatus*) and Great Black-backed Gulls (*Larus marinus*) in eastern North America. Waterbirds 39 (Special Publication 1): 253-268.

Robson D, Barriocanal C (2008). The influence of environmental conditions on the body mass of Barn Swallows (*Hirundo rustica*) during spring migration. J Ornithol 149: 473-478.

Saino N, Rubolini D, Serra L, Caprioli M, Morganti M, Ambrosini R, Spina F (2010). Sex-related variation in migration phenology in relation to sexual dimorphism: a test of competing hypotheses for the evolution of protandry. J Evol Biol 23: 2054-2065.

Scherer AL, Scherer JFM, Petry MV, Valiati VH (2014). Sexual dimorphism and body condition of wintering White-rumped Sandpipers in southern Brazil. Wilson Journal of Ornithology 126: 553-561.

Sikora A, Dubiec A (2007). Sex identification of Jack Snipe *Lymnocryptes minimus* by discriminant analysis of morphometric measurements. Ardea 95: 125-133.

Zenatello M, Serra L, Baccetti N (2002). Trade-offs among body mass and primary moult patterns in migrating Black Terns *Chlidonias niger*. Ardea 90 Special Issue: 411-420.

Zwarts L, Ens BJ, Kersten M, Piersma T (1990). Moult, mass and flight range of waders ready to take off for long-distance migrations. Ardea 78: 339-364.