Sexual dimorphism in adult Little Stints (*Calidris minuta*) revealed by DNA sexing and discriminant analysis

Aleksandra Niemc[1], Magdalena Remisiewicz[1,2], Joel Avni[3], Les G. Underhill[2]

[1]Bird Migration Research Station, University of Gdańsk, Faculty of Biology, Wita Stwosza 59, 80-308, Gdańsk, Poland

[2]Animal Demography Unit, Department of Biological Sciences, University of Cape Town, Rondebosch, 7701, South Africa

[3]Bird's-Eye View Productions, 23 Afrikaner Avenue, 7975, Kommetjie, South Africa

Corresponding Author:

Aleksandra Niemc

ul. Wita Stwosza 59/C306, Gdańsk, 80-308, Poland

Email address: aleksandra.niemc@phdstud.ug.edu.pl

Abstract:

Background. Sexual selection plays such an important role in a bird's life cycle that researchers must know the sex of an individual to interpret key aspects of its biology. Sexes of dimorphic species can be easily distinguished, but sexing monomorphic bird species often requires expensive and time-consuming molecular methods. The Little Stint (*Calidris minuta*) is a numerous species, monomorphic in plumage but showing a small degree of reversed sexual size dimorphism (RSSD). Females are larger than males but ranges of their measurements overlap, making Little Stints difficult to sex in the field. Our aim was to develop reliable sexing criteria for Little Stints in different stages of primary moult during their stay on the non-breeding grounds in South Africa using DNA-sexed individuals and discriminant function analysis.

Comentado [ALS1]: The observed SSD in the species is not due to sexual selection. Please review.

Methods. We caught 348 adult Little Stints in 2008–2016 at their non-breeding grounds at Barberspan Bird Sanctuary (26°33'S, 25°36'E, Northwest Province, South Africa) using mist nets and walk-in traps. To identify the birds' sex we isolated DNA from blood samples collected in the field using PCR with P2/P8 primers. We used Storer's Dimorphism Index to assess the degree of sexual size dimorphism. Then we divided our sample into two groups: before and after primary moult. For each group we developed two functions: one using wing length only and the other a combination of morphometrics. Then we used a stepwise procedure to check which combination of measurements best discriminated sexes. To validate our result we used a jack-knife cross-validation procedure and Cohen-kappa statistics.

Results. DNA-sexed females were larger than males in all the morphometric features we measured. Birds with fresh primaries had on average 2.3 mm longer wings than those in worn plumage. A discriminant function using wing length (D_1) correctly sexed 78.8% of individuals before moult, and a stepwise analysis revealed that a combination of wing length and tarsus (D_2) correctly identified the sex of 82.7% of the birds. For birds with freshly moulted primaries a function using wing length (D_3) correctly classified 83.4% of the individuals, and a stepwise analysis revealed that wing and total head length (D_4) classified 84.7%.

Discussion. Sexual size differences in Little Stints are connected with their breeding biology. Females are bigger, which increases their fecundity; males are smaller, which increases their manoeuverability during display flights and hence their mating success. Because of their extreme lack of breeding site fidelity we do not expect a geographical cline in their

biometrics despite their wide breeding range. Available sexing criteria for Little Stints in the literature were developed using museum specimens, which often shrink, leading to misclassification of live birds. The sexing criteria we developed can be used for studies on Little Stints in the future and can be used on past data.

Comentado [ALS2]: The authors should make it clear that the results should be used carefully because of the misclassification of sex (21.2 to 15.3%), according to the used FDA.

Introduction

An individual's sex is one of the most important factors shaping its biology. Male and female birds are subject to sex-specific selection pressures that entail differences in their biology, including migration strategies (Remisiewicz & Wennerberg, 2006; Jakubas et al., 2014), population structure (Nebel, 2006), foraging behavior (Mathot & Elner, 2004; Nebel, 2005), moult (Barshep et al., 2013) and physiology (Kulaszewicz, Wojczulanis-Jakubas & Jakubas, 2015). Differences in the biology of males and females leads to diverging body sizes (Fairbairn 2007), and are expected to emerge if selection for a character is stronger in one sex than the other (Székely, Lislevand & Figuerola, 2007). Sexing monomorphic birds is difficult in the field, but can be done with molecular methods (Dubiec & Zagalska-Neubauer, 2006) using DNA isolated from blood samples (Owen, 2011), feathers (Bello, Francino & Sánchez, 2001) or buccal swabs (Handel et al., 2006); however those methods are often stressful for the birds and expensive. For species in which males and females are monomorphic in plumage but show sexual size dimorphism (SSD) the sex can often be identified using morphometrics (Dechaume-Moncharmont, Monceau & Cézilly, 2011). In such cases the degree of SSD is crucial, because in some species measurements overlap between sexes, which might lead to misidentification (Jiménez, García-Lau & Gonzalez, 2015). Discriminant function analysis (DFA) enables observers to use a combination of morphological measurements to predict the sex of a bird with a certain probability (Tabachnick & Fidell, 2007). However, the efficiency of this method depends on the accuracy of the measurements, which suffers if researchers are not calibrated with each other or if wing length is measured on feathers with different wear (Dechaume-Moncharmont, Monceau & Cézilly, 2011). In our study we focus on Little Stints, which are monomorphic in plumage but exhibit a small degree of SSD, with the females slightly larger than the males (del Hoyo, Elliott & Sargatal, 1996). But the ranges of their measurements overlap, which impedes sexing them in the field (Prater, Marchant & Vuorinen, 2007). Sexing criteria for Little Stints in the literature are usually based on small samples or on museum specimens, which are known to shrink (Prater, Marchant & Vuorinen, 2007).

Excluído: sexual size dimorphism

We aim to develop reliable sexing criteria for Little Stints by discriminant functions that account for the stage of wear of their primaries using measurements of DNA-sexed individuals spending the non-breeding season in South Africa. We suggest that these discriminant functions serve as a useful tool, after adjustment to local measurement routines, for other researchers studying Little Stints at various stages of their life cycle.

Materials & methods

Species and study area

The Little Stint is a long-distance migrant wader, which breeds in the Eurasian tundra and spends the non-breeding season from Mediterranean coasts south throughout sub-Saharan Africa to South Africa in the south and southwestern Asia in the east (del Hoyo, Elliott & Sargatal, 1996; Tulp et al., 2002). Most Little Stints leave the non-breeding grounds and attempt breeding at the end of their first year; but a few stay in South Africa over the austral winter for a "gap year" (Underhill 1997; Underhill et al., 1999). Immature Little Stints can be distinguished from adults by the retained chestnut-fringed inner median coverts, which in adults are pale grey-brown tipped whitish (Prater, Marchant & Vuorinen, 2007). After arrival at the non-breeding grounds immature and adult birds undergo a complete moult, including the replacement of wing flight feathers. They complete this moult in January–March, after which the immatures become indistinguishable from the adults (Prater, Marchant & Vuorinen, 2007).

We caught Little Stints at Barberspan Bird Sanctuary (26°33′S, 25°36′E; North West Province, South Africa). This reserve is centered on a shallow alkaline lake whose area varies from 257 ha to 2000 ha, depending on rainfall (Milstein, 1975; Barnes, 1998). Lake Barberspan is fed by the Harts River. In the austral winter and during droughts it becomes the only permanent waterbody in the surrounding farmland, which makes it an important stopover, moulting and non-breeding destination for waterbirds, including Palearctic migrants (Barnes, 1998; Lipshutz et al., 2011; Remisiewicz & Avni, 2011). Barberspan Bird Sanctuary is a Wetland of International Importance in terms of the Ramsar Convention and an Important Bird Area according to BirdLife International (Cowan, 1995; Barnes, 1998; Remisiewicz & Avni 2011).

Comentado [ALS3]: It would be better represented on a map of the country with a study area

Data collection

During September–April in 2008–2016 we captured 348 adult Little Stints using walk-in traps (Busse & Meissner, 2015) and mist nets. We ringed and measured each bird. Measurements included: bill length (from the feather line to the bill tip), total head length (from the back of the skull to the bill tip) and tarsus length (from the tarsal joint to the distal end of the tarso-metatarsus), taken with callipers of 0.1 mm accuracy, and wing length (maximum flattened chord, as in Evans (1986)), tarsus-plus-toe (Piersma, 1984) taken to the nearest 1 mm with a ruler, and body mass to 1 g or 0.1 g in different years (weighed with an electronic scale). We used only morphological measurements taken by MR (SAFRING ringing permit 1454), as recommended in Henry et al. (2015) because measurements taken by different ringers can reduce the accuracy of sex discrimination. We also took blood samples from a brachial vein (Owen, 2011) from all ringed Little Stints and preserved the samples in 96% ethanol for DNA sexing. Only trained, experienced team members handled the birds and took the blood samples to ensure safety standards. All the procedures were approved by the management of Barberspan Bird Sanctuary, under permits from SAFRING and the Department of Rural, Environmental and Agricultural Development, North West Provincial Government, South Africa.

Comentado [ALS4]: low accuracy in body mass measurement

Molecular sex identification

We isolated DNA from blood samples using the Blood Mini kit (A&A Biotechnology, Poland). The next step was PCR with the pair of P2 and P8 primers (Griffiths et al., 1998) using a modified protocol. Total volume of PCR was 20 µl, the reaction mix included: 7.5 µl REDTaq® ReadyMix™ (Sigma Aldrich), 3.5 µl of water, 1 µl $MgCl_2$, 1 µl P2 primer (10 µM), 1 µl P8 primer (10 µM) and 2 µl of the DNA sample. For PCR amplifications we used an Eppendorf Mastercycler with this thermic profile: an initialisation at 94°C for 2 minutes, 40 cycles of denaturation at 94°C for 30 seconds, 40 cycles of annealing at 50°C for 30 seconds and 40 cycles of elongation at 72°C for 2 minutes, ending with a final elongation at 72°C for 5 minutes. This method is based on the amplification of chromo-helicase-DNA-binding (CHD) genes found on avian sex chromosomes. The CHD-Z gene (ca 370 bp) is located on the Z chromosome, therefore it is present in both sexes. The CHD-W gene (ca 390 bp) occurs only on chromosome W, therefore it is unique to females (Griffiths et al., 1998). We separated the products of PCR with electrophoresis on 3.5% agarose gel (75V, 120 min) stained with ethidium bromide (samples from 2008–2013) and Midori Green Advanced DNA

Stain (NIPPON Genetics, samples from 2013–2016). Products were visualised in UV light, one band was visible for males, which indicated ZZ chromosomes, and two bands for females (ZW chromosomes). The method enabled us to sex 100% of the birds we caught.

Statistical analysis

For each morphometric measurement we used Storer's dimorphism index ($DI_{storer's}$) to assess the degree of SSD in Little Stints (Storer, 1966), using the formula:

Excluído: sexual size dimorphism

$$\mathrm{DIstorer's} = \frac{\mathrm{mean}\,♀ - \mathrm{mean}\,♂}{\mathrm{mean}\,♂ + \mathrm{mean}\,♀} * 100$$

Comentado [ALS5]: (* 0,5)

Comentado [ALS6]: Results in table 1 are not accordig this formula.

We compared all the morphometrics we had measured of males and females using the two-sample *t*-test. Then we divided the birds into two groups: those before and those after primary moult, and compared the measurements of birds from these two groups. Birds caught in active moult were classified as "before moult" because they moult their outermost primary last. However, we did not measure wing length of any bird whose outermost primary was heavily damaged or if it was growing. For each group we used Discriminant Function Analysis to determine the best set of measurements for sexing Little Stints with a two-fold approach. First, we used wing length alone as a discriminant factor because this measurement differed the most between the sexes. The second approach used a stepwise method including other measurements after conducting pairwise correlation of all the measurements. We used only one of a pair of correlated measurements at a time in the stepwise procedure to avoid multicollinearity. We did not include the body mass of Little Stints, because it changes during the non-breeding season during pre-migratory fuelling. The aim of producing two different discriminant functions for each group was to make these functions applicable for different data sets, because wing length is the most commonly taken measurement, in contrast to tarsus and total head lengths. All the assumptions of discriminant function analysis were met (Tabachnick & Fidell, 2007), including the homogeneity of covariances (Box's M test), the homogeneity of variance (Levene's test) and the normal distributions of the measurements for males and females separately in each of the two groups, and we confirmed no multicollinearity of the selected measurements (r < 0.50 for all pairwise correlations). We computed prior classification probabilities from the group sizes because of the unequal number of males and females in our sample (Tabachnick & Fidell, 2007). To validate our models we used a jack-knife procedure to assess the percentage of correctly sexed individuals by discriminant function analysis (Dechaume-Moncharmont et al. 2011). This cross-

Comentado [ALS7]: But how, if bill lenght and Weight have a larger Storer index than wing lenght. So, the largest SSD was in bill and weight (I think body mass is not a good choice) validation technique predicts the sex of each individual using a discriminant function calculated for all the birds except the individual being classified (Hair et al., 1995). We had unequal samples of males and females, so we assessed the effectiveness of our proposed functions by calculating Cohen's kappa statistic (Titus & Mosher, 1984). It estimates the improvement made by the results of discriminant analysis over random chance: 0 = no improvement over chance, 1 = full compliance (Titus & Mosher, 1984). The optimal cutting score was calculated as a weighted average of the group centroids (Hair et al., 1995). Statistical analyses were performed in IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). All tests were two-tailed and the accepted level of significance was $P < 0.05$.

Results

Morphological differences between the sexes

We identified 187 males and 165 females using DNA sexing. Analysis of morphometrics and Storer's dimorphism index ($DI_{storer's}$) also revealed sexual differences. On average females were bigger than males in all morphological measurements (Table 1). In all our measurements, birds before and after wing moult, differed only in wing length ($t_{311} = 7.69$, $p < 0.001$), which was on average 2.3 mm longer in those with fresh primaries after moult than in those before moult with worn primaries. We therefore conducted the discriminant analyses separately for these two groups.

Comentado [ALS8]: 187 + 165 = 352
Methods and Data colletion = 348 birds. Where 4 birds not evaluated the morphometrics?

Some morphological measurements were correlated (correlation coefficients for males and females between tarsus and tarsus-plus-toe were r=0.73 and r=0.79 and for culmen and total head length r=0.81 and r=0.83). We chose wing length, total head length (because it is less prone to errors than bill length measured to the feather line), and tarsus length as the best factors for discriminant analysis.

Discriminant functions for adult Little Stints before primary moult

Using measurements of 156 adult Little Stints (70 females and 86 males) taken before they had moulted their primaries, and using only wing length as a discriminant factor, we obtained the equation:

$D_1 = -47.496 + 0.484$ (wing),

which allowed us to correctly classify 78.8% of the birds. A jack-knife cross-validation procedure yielded the same success rate, and our random chance-corrected procedure showed that our proposed classification was 56.8% better than chance (kappa = 0.568 ± 0.082 SE, $P < 0.001$). If $D_1 > 0.17$, the bird was classified as a female, and if $D_1 < 0.17$ as a male (Fig. 1)

In the stepwise procedure including three selected measurements (wing, tarsus and total head length), only the combination of wing and tarsus length was a significant discriminant factor. The best discriminant function we obtained was

$$D_2 = –50.428 + 0.421\ (\text{wing}) + 0.420\ (\text{tarsus}).$$

The percentage of correctly classified birds was 82.7%, more accurate than the previous equation. The cross-validation procedure correctly classified 80.8% of individuals and the classification was 65.1% better than chance (kappa = 0.651 ± 0.081 SE, $P < 0.001$). If $D_2 > 0.18$ then the individual was a female and if $D_2 < 0.18$ it was a male (Fig. 2).

Discriminant functions for adult Little Stints after primary moult

The second group we analysed was 159 adult Little Stints (82 females and 77 males) after their complete post-breeding moult, with all new primaries. The function obtained using only wing length was

$$D_3 = –52.184 + 0.520\ (\text{wing}).$$

This function correctly classified 83.4% of the individual birds, which was confirmed by the cross-validation procedure. The proposed classification was 66.9% better than chance (kappa = 0.669 ± 0.080 SE, $P < 0.001$). When $D_3 > –0.038$ the individual was classified as a female, when $D_3 < –0.038$ as male (Fig. 1).

The stepwise analysis revealed that the best discriminating combination of measurements was wing and total head length (THL), according to the equation

$$D_4 = –59.310 + 0.445\ (\text{wing}) + 0.377\ (\text{THL}).$$

This function correctly classified 84.7% of the individuals in the sample, which was the highest proportion of all the equations we present. The cross-validation procedure showed that the proposed equation was 84.1% correct and 69.4% better than chance (kappa = 0.694 ± 0.080 SE, $P < 0.001$). If $D_4 > –0.041$ a Little Stint was classified as a female and if $D_4 < –0.041$ as a male (Fig. 2).

Discussion

We showed that the female Little Stints were larger than the males, not only in wing length, as described earlier (Cramp & Simmons, 1983; Prater, Marchant & Vuorinen, 2007), but also in other body features. Thus we recommend a combination of several measurements in discriminant functions as a useful tool in studies of sexual differences of this species. Such size difference might benefit each sex in different ways, the females through increased fecundity and the males through higher mating success with smaller size, which we discuss.

Wing length was the most dimorphic morphometric, with females having longer wings than males. Mean wing lengths based on the sexing criteria in Prater et al. (2007) and established by Tree (1974) is 95.9 mm for adult males and 99.5 mm for adult females, 1.5 mm and 1.7 mm shorter than our results (Table 1). The sexing criteria presented in Prater et al. (2007) was developed using museum specimens, thus subjected to shrinkage (Jenni & Winkler, 1994). We compared our differences with the one obtained using a regression equation in Engelmoer et al. (1983), where: shrinkage = 0.006*fresh wing-length [mm] + 0.976, and predicted shrinkage for both sexes was 1.6 mm, similar to what we observe in our sample. Moult and feather wear are important considerations when taking feathered measurements like wing length (Meissner, 2005; Jiménez, García-Lau & Gonzalez, 2015), thus we propose different discriminant functions for birds before and after moult. During the non-breeding season all Little Stints should be carefully examined for moult because their outermost primary might still be growing. Measuring wing lengths of birds moulting P10 would underestimate wing length and could even cause misclassifications.

Comentado [ALS9]: That is not true. If bill lenght and Weight have a larger Storer index than wing lenght. So, the largest SSD was in bill length and body mass.
The Storer's index indicates how much SSD you have in each measure.

Excluído: ere

Comentado [ALS10]: Bill length shows the largest Storer's index of all measurements. Please discuss why it did not prove to be a good measure for building a DFA.
Supporting literature:
- Nebel et al. 2011. The evolution of sexual bill-size dimorphism in shorebirds: a morphometric test of the resource partitioning hypothesis. Evolutionary Ecology Research 13:35–44.
- Scherer et al. 2014. Sexual dimorphism and body condition of wintering Whiterumped Sandpipers in southern Brazil.

Sexual differences in the size of Little Stints might be closely related to their breeding biology. Male mating success is a strong form of sexual selection that affects their morphometrics. During the breeding season male Little Stints perform display flights, favouring smaller size to increase manoeuvrability (Figuerola, 1999; Székely et al., 2000). The females' bigger size might be connected with increasing fecundity, because Little Stints exhibit successive bigamy of both sexes where females lay two clutches in a short period (Cramp & Simmons, 1983; Hildén, 1983) and a larger body size allows her to compensate better for the increased energy expenditure of egg production (Jönsson & Alerstam, 1990). Sexual differences in morphometrics cannot be explained by a division in parental care, because each clutch and brood is cared for by a single parent of either sex (Tulp et al., 2002). Small calidridine sandpipers, such as Western Sandpiper (Nebel, 2005) and Least Sandpiper

Comentado [ALS11]: Nebel and Thompson (2011) evaluated the evolution of sexual bill-size dimorphism in 151 shorebird species and found that SSD is more pronounced in bill length as a resource-exploiting trophic character than nontrophic traits such as tarsus and wing length and body mass. The authors suggest that the comparative approach reveals that these patterns are not strictly the result of contemporary natural or sexual selection but rather reflect the shared ancestry of extant species.

(Nebel, 2006), exhibit substantial sexual differences in bill length, which is not the most dimorphic morphometric in Little Stints (Table 1) and suggests that they do not exhibit sexual differences in their foraging niche.

Comentado [ALS12]: Excluding body mass, the largest SSD is in Bill length (4,48)! Please, revise our conclusions.

Measurement	Females			Males			t	p	$\mathrm{DI}_{storer's}$
	N	Mean (SD)	Range	N	Mean (SD)	Range			
Wing length (mm)	152	101.19 (± 2.34)	95 – 106	164	97.42 (± 2.19)	92 – 102	14.81	< 0.001	3.80
Total head length (mm)	163	39.30 (± 1.06)	36.0 – 42.3	185	38.26 (± 0.98)	35.8 – 41.1	9.62	< 0.001	2.69
Bill length (mm)	163	18.11 (± 0.92)	16.3 – 20.5	185	17.32 (± 0.81)	15.1 – 20.1	8.55	< 0.001	4.48
Tarsus length (mm)	162	22.09 (± 0.86)	19.7 – 24.2	185	21.55 (± 0.78)	19.4 – 24.4	6.16	< 0.001	2.47
Tarsus-plus-toe length (mm)	163	40.94 (± 1.27)	37 – 44	185	40.15 (± 1.34)	37 – 46	5.69	< 0.001	1.96
Weight (g)	162	24.48 (± 3.66)	19 – 42	185	21.98 (± 2.43)	17 – 33	7.42	< 0.001	10.78

A wide breeding range, such as that of the Little Stint, often results in a geographical variation in biometrics (Zwarts et al., 1996; Dmitrenok et al., 2007), which might distort a DFA. We do not expect such differences in our study because Little Stints are an opportunistic species that show no natal philopatry or breeding-site fidelity and breed wherever they find favourable environmental conditions (Hildén 1983; Underhill et al. 1993; Tomkovich & Soloviev 1994). Their polygamous breeding system where males and females both frequently have two partners enhances gene-flow in the population, limiting geographical clines in the morphometrics of Little Stints.

Conclusions

DNA sexing remains the most reliable method for monomorphic species, but discriminant functions are useful when researchers cannot collect or process DNA samples, get permits or when sex identification is needed in field studies. The functions we developed can be applied on measurements collected in the past and can strengthen the analysis when individual birds' sex must be known. To make our functions more applicable for researchers we present equations that can be used with different types of morphological measurements that might be available and equations that consider the wear of primary feathers. Our results are based on relatively big samples and all of the measurement were taken by one ringer, but ringing teams should regularly calibrate all people taking measurements (Dechaume-Moncharmont, Monceau & Cézilly, 2011), which improves the accuracy of sexing by discriminant functions. The functions we suggest should be applied cautiously to data from other researchers, and should probably first be adjusted to each study, considering possible differences in measuring routines with those of our team. We suggest application of these or similar discriminant functions as a useful tool facilitating studies of differences in the biology of the sexes at different stages of the life cycle, in Little Stints and in other species of small sexual size dimorphism.

Comentado [ALS13]: The SSD and the Storer's index of the species was not discussed.
Studies have reported that sexual size dimorphism decreases with size when females are the larger sex (Rensch's rule), and is determined by a combination of sexual competition and male agility.

Acknowledgements

We thank the staff at Barberspan Bird Sanctuary for their help and for making us feel welcome. We are grateful to all volunteers who helped to collect the data we used.

References:

Barnes K. 1998. Important bird areas of the North-west Province. *The Important Bird Areas of Southern Africa*: 93–122. Johannesburg: BirdLife South Africa.

Barshep Y, Underhill LG, Balachandran S, Pearson DJ. 2013. Conditions on the Non-Breeding Areas Affect Primary Moult Strategy of the Curlew Sandpiper *Calidris ferruginea*. *Ardea* 101:13–22. DOI: http://dx.doi.org/10.5253/078.101.0114.

Bello N, Francino O, Sánchez A. 2001. Isolation of Genomic DNA from Feathers. Journal of Veterinary Diagnostic Investigation 13(2): 162–164.

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

Cowan GI. 1995. *Wetlands of South Africa*. Pretoria: Department of Environmental Affairs and Tourism.

Cramp S, Simmons KEL. 1983. Tom: 3. In: *The birds of the Western Palearctic. Handbook of the birds of Europe, the Middle East and North Africa*. Oxford: Oxford University Press, 303–310.

Dechaume-Moncharmont F-X, Monceau K, Cézilly F. 2011. Sexing Birds Using Discriminant Function Analysis: A Critical Appraisal. *The Auk* 128:78–86. DOI: 10.1525/auk.2011.10129.

Dmitrenok M, Puglisi L, Demongin L, Gilbert G, Polak M, Bretagnolle V. 2007. Geographical variation, sex and age in Great Bittern *Botaurus stellaris* using coloration and morphometrics. *Ibis* 149:37–44. DOI: 10.1111/j.1474-919X.2006.00592.x.

Dubiec A, Zagalska-Neubauer M. 2006. Molecular techniques for sex identification in birds. *Biological letters* 43:3–12.

Engelmoer M, Roselaar K, Boere GC, Nieboer E. 1983. Post-mortem changes in measurements of some waders. *Ringing and Migration* 4:245–248. DOI: 10.1080/03078698.1983.9673814.

Evans PR. 1986. Correct measurement of the wing-length of waders. *Wader Study Group Bulletin* 48:11.

Fairbairn DJ. 2007. Introduction: the enigma of sexual size dimorphism. In Fairbairn, D.J., Blanckenhorn, W. & Székely, T. (eds) *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*:1–10. Oxford: Oxford University Press.

Figuerola J. 1999. A comparative study on the evolution of reversed size dimorphism in monogamous waders. *Biological Journal of the Linnean Society* 67: 1–18

Griffiths R, Double MC, Orr K, Dawson RJG. 1998. A DNA test to sex most birds. *Molecular Ecology* 7:1071–1075. DOI: 10.1046/j.1365-294x.1998.00389.x.

Hair, J. F., Jr., R. E. Anderson, R. L. Tatham, & W. C. Black. 1995. *Multivariate data analysis*. Prentice Hall, Upper Saddle River, NJ

Handel CM, Pajot LM, Talbot SL, Sage GK. 2006. Use of Buccal Swabs for Sampling DNA from Nestling and Adult Birds. *Wildlife Society Bulletin* 34:1094–1100. DOI: 10.2193/0091-7648(2006)34.

Henry L, Biquand V, Craig AJFK, Hausberger M. 2015. Sexing Adult Pale-Winged Starlings Using Morphometric and Discriminant Function Analysis. *Plos One* 10(9): e0135628. DOI: 10.1371/journal.pone.0135628.

Hildén O. 1983. Mating system and breeding biology of Little Stint *Calidris minuta*. *Wader Study Group Bulletin* 39:47.

del Hoyo J, Elliott A, Sargatal J. 1996. *Handbook of the birds of the world*. Barcelona: Lynx

Editions.

Jakubas D, Wojczulanis-Jakubas K, Foucher J, Dziarska-Pałac J, Dugué H. 2014. Age and Sex Differences in Fuel Load and Biometrics of Aquatic Warblers *Acrocephalus paludicola* at an Autumn Stopover Site in the Loire Estuary (NW France). *Ardeola* 61:15–30. DOI: 10.13157/aria.61.1.2014.15.

Jenni L, Winkler R. 1994. *Moult and Ageing of European Passerines*. London: Academic Press.

Jiménez A, García-Lau I, Gonzalez A. 2015. Determination of Least Sandpiper (*Calidris minutilla*) and Western Sandpiper (*Calidris mauri*): Comparing Methodological Robustness of Two Morphometric. *Waterbirds* 38:10–18. DOI: 10.1675/063.038.0103.

Jönsson PE, Alerstam T. 1990. The adaptive significance of parental role division and sexual size dimorphism in breeding shorebirds. *Biological Journal of the Linnean Society* 41: 301–314.

Kulaszewicz I, Wojczulanis-Jakubas K, Jakubas D. 2015. Variation of the Savi’s warbler (*Locustella luscinioides*) leucocyte profiles and body condition in relation to age, sex and moult. *Annales Zoologici Fennici* 52:325–338. DOI: 10.5735/086.052.0507.

Lipshutz S, Remisiewicz M, Underhill LG, Avni J. 2011. Seasonal fluctuations in population size and habitat segregation of Kittlitz’s Plover *Charadrius pecuarius* at Barberspan Bird Sanctuary, North West province, South Africa. *Ostrich* 82:207–215. DOI: 10.2989/00306525.2011.618245.

Mathot KJ, Elner RWR. 2004. Evidence for sexual partitioning of foraging mode in Western Sandpipers (*Calidris mauri*) during migration. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 82:1035–1042. DOI: 10.1139/Z04-080.

Meissner W. 2005. Sex determination of juvenile Dunlins migrating through the Polish Baltic region. *Journal of Field Ornithology* 76:368–372. DOI: 10.1648/0273-8570-76.4.368.

Milstein P le S. 1975. The biology of Barberspan, with special reference to the avifauna. Ostrich Supplement 10: 1–74.

Nebel S. 2005. Latitudinal clines in bill length and sex ratio in a migratory shorebird: A case of resource partitioning? *Acta Oecologica* 28:33–38. DOI: 10.1016/j.actao.2005.02.002.

Nebel S. 2006. Latitudinal clines in sex ratio, bill, and wing length in Least Sandpipers. *Journal of Field Ornithology* 77:39–45. DOI: 10.1111/j.1557-9263.2006.00021.x.

Owen JC. 2011. Collecting, processing, and storing avian blood: A review. *Journal of Field Ornithology* 82:339–354. DOI: 10.1111/j.1557-9263.2011.00338.x.

Piersma T. 1984. International wader migration studies along the East Atlantic Flyway during spring 1985. Final announcement of a Wader Study Group project. *Wader Study Group Bulletin* 42:5–9.

Prater T, Marchant J, Vuorinen J. 2007. *Guide to the identification and ageing of Holarctic waders*. Tring: BTO.

Remisiewicz M, Avni J. 2011. Status of migrant and resident waders, and moult strategies of migrant waders using African inland wetland habitats, at Barberspan Bird Sanctuary in South Africa. *Ibis* 153:433–435. DOI: 10.1111/j.1474-919X.2011.01112_1.x.

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.

Storer RW. 1966. Sexual Dimorphism and Food Habits in Three North American Accipiters. *The Auk* 83:423–436.

Székely T, Lislevand T, Figuerola J. 2007. Sexual size dimorphism in birds. In Fairbairn, D.J., Blanckenhorn, W. & Székely, T. (eds) *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*:27–37. Oxford: Oxford University Press.

Székely T, Reynolds JD, Figuerola J. 2000. Sexual Size Dimorphism in Shorebirds, Gulls, and Alcids: the Influence of Sexual and Natural Selection. *Evolution* 54:1404–1413. DOI: 10.1111/j.0014-3820.2000.tb00572.x.

Tabachnick BG, Fidell LS. 1996. *Using multivariate statistics*. 3rd ed. Harper Collins Publishers, New York, New York.

Titus K, Mosher JA. 1984. Chance-corrected Classification for Use in Discriminant Analysis: Ecological Application. *The American Midland Naturalist* 111:1–7.

Tomkovich PS, Soloviev MY. 1994. Site Fidelity in High Arctic Breeding Waders. *Ostrich*

65:174–180. DOI: 10.1080/00306525.1994.9639680.

Tree AJ. 1974. Ageing and sexing the Little Stint. *Safring News* 3:31–33.

Tulp I, Schekkerman H, Chylarecki P, Tomkovich P, Soloviev M, Bruinzeel L, Van Dijk K, Hildén O, Hötker H, Kania W, Van Roomen M, Sikora A, Summers R. 2002. Body mass patterns of Little Stints at different latitudes during incubation and chick-rearing. *Ibis* 144:122–134. DOI: 10.1046/j.0019-1019.2001.00014.x.

Underhill LG. 1997. Little Stint *Calidris minuta*. W: Harrison JA, Allan DG, Underhill LG, Herremans M, Tree AJ, Parker V, Brown CJ. *The atlas of southern African birds*. Vol. 1: Non-passerines: 420–421. BirdLife South Africa, Johannesburg.

Underhill LG, Prýs-Jones RP, Syroechkovski EE Jr, Groen NM, Karpov V, Lappo HG, van Roomen MWJ, Rybkin A, Schekkerman H, Spiekman H, Summers RW. 1993. Breeding of waders (Charadrii) and brent geese *Branta bernicla bernicla* at Pronchishcheva Lake, northeastern Taimyr, Russia, in a peak and a decreasing lemming year. *Ibis* 135: 277–292.

Underhill LG, Tree AJ, Oschadleus HD, Parker V. 1999. Review of ring recoveries of waterbirds in Southern Africa: 80–81. Avian Demography Unit, University of Cape Town.

Zwarts L, Hulscher JB, Koopman K, Zegers PM. 1996. Discriminating the sex of Oystercatchers *Haematopus ostralegus*. *Ardea* 84A:1–12.

Morphological features of adult male and female Little Stints.

t, p – results of t-test comparing the sexes. $DI_{storer's}$ – Storer's dimorphism index.

Measurement	Females			Males					
	N	Mean (SD)	Range	N	Mean (SD)	R			
Wing length (mm)	152	101.19 (± 2.34)	95 – 106	164	97.42 (± 2.19)	92	[illegible]	[illegible]	[illegible]
Total head length (mm)	163	39.30 (± 1.06)	36.0 – 42.3	185	38.26 (± 0.98)	35.	[illegible]	[illegible]	[illegible]
Bill length (mm)	163	18.11 (± 0.92)	16.3 – 20.5	185	17.32 (± 0.81)	15.1 – 20.1	8.55	< 0.001	4.48
Tarsus length (mm)	162	22.09 (± 0.86)	19.7 – 24.2	185	21.55 (± 0.78)	19.4 – 24.4	6.16	< 0.001	2.47
Tarsus-plus-toe length (mm)	163	40.94 (± 1.27)	37 – 44	185	40.15 (± 1.34)	37 – 46	5.69	< 0.001	1.96
Weight (g)	162	24.48 (± 3.66)	19 – 42	185	21.98 (± 2.43)	17 – 33	7.42	< 0.001	10.78

Comentado [ALS14]: The results are not according the Storer's index informated in the methods
Wing = (101,19 – 97,42)/ (101,19 + 97,42) *100
Wing = 1,89

$$DIstorer's = \frac{mean\,♀ - mean\,♂}{mean\,♂ + mean\,♀} * 100$$