



Using a common morphometric-based method to sex a migratory bird along its entire flyway despite geographical and temporal variations in body size and sexual size dimorphism

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Abstract

Sexing bird species with monomorphic plumage is generally challenging, and sexual size dimorphism (SSD) is often used to develop morphometric-based sexing tools, e.g., using discriminant functions. Within species, local selection pressures, age-related and season-related growth may, however, induce geographical and temporal variations in body size and SSD. Such variations may complicate the development of reliable morphometric-based sexing methods at a broad scale. We first investigated body size variations in a migratory shorebird species with wide breeding and wintering ranges, the Sanderling *Calidris alba*, within the two breeding populations (Greenland and Russia) and three staging/wintering populations (United Kingdom, Iceland and Mauritania), which belong to the same flyway. Then, for samples from each region, we tested whether site-specific (i.e., “regional”) functions performed better than functions developed for birds from the other sites (i.e., “foreign” functions) or than an overall (“flyway”) function that combined all sampled individuals. We found minor variations in SSD between regions, but significant differences in body size between sexes and regions. Females were larger than males and, for instance, breeders had longer wings than staging and wintering birds. Regional functions had similar sexing efficiency as any other functions applied to sample from each region, except for Western Africa where the regional function performed slightly better than some of the other functions. Furthermore, the flyway function developed after merging all subsamples had a similar efficiency than the regional functions, i.e., from 75.4% to 90% of correct sex assignment depending on the region. Given the small or lack of benefit in using regional functions, we conclude that the flyway function can be used reliably to sex Sanderlings measured at different sites, years or seasons within the East Atlantic flyway. Our results may help to develop global sexing function for other bird species.

Keywords Discriminant function · Molecular sexing · East Atlantic flyway · Shorebird · Sanderling

Zusammenfassung

Die Geschlechtsbestimmung einer Zugvogelart anhand von Körpermaßen funktioniert trotz geografischer und zeitlicher Variationen der Körpergröße und des Geschlechtsdimorphismus entlang seiner gesamten Zugroute.

Die Geschlechtsbestimmung von Vogelarten mit monomorphem Gefieder ist in der Regel schwierig. Bei solchen Arten wird häufig der Größendimorphismus zwischen den Geschlechtern (SSD) z. B. unter Verwendung von Diskriminanzfunktionen zur Geschlechtsbestimmung verwendet. Innerhalb einer Art können jedoch lokale Selektionszwänge sowie alters- und saisonbedingtes Wachstum zu geografischen und zeitlichen Variationen der Körpergröße und des SSD führen. Solche Schwankungen können die Entwicklung zuverlässiger Methoden zur Geschlechtsbestimmung anhand morphometrischer Merkmale erschweren. Anhand einer Watvogelart mit großen Brut- und Überwinterungsgebieten, dem Sanderling *Calidris alba*, untersuchten wir auf derselben Zugroute die Variationen der Körpermaße innerhalb von zwei Brut- (Grönland und

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Russland) und drei Rast- bzw. Überwinterungspopulationen (Vereinigtes Königreich, Island und Mauretanien). Anschließend testeten wir für Proben aus jeder Region, ob standortspezifische Diskriminanzfunktionen besser abschnitten als solche, die für Vögel von anderen Standorten entwickelt wurden, oder als eine Gesamtfunktion, die alle beprobten Individuen kombinierte. Wir fanden geringe Unterschiede in der SSD zwischen den Regionen, aber signifikante Unterschiede in der Körpergröße zwischen den Geschlechtern und den Regionen. Weibchen waren größer als Männchen, und Brüter hatten beispielsweise längere Flügel als Rast- und Überwinterungsvögel. Die standortspezifischen Funktionen waren bei der Geschlechtsbestimmung ähnlich effizient wie alle anderen Funktionen, die auf die Stichproben aus jeder Region angewandt wurden, mit Ausnahme von Westafrika, wo die regionale Funktion etwas besser abschnitt als andere Funktionen. Darüber hinaus wies die nach der Zusammenführung aller Teilstichproben entwickelte Gesamtfunktion mit einer korrekten Geschlechtszuordnung zwischen 75,4% und 90% je nach Region eine ähnliche Effizienz auf, wie die jeweiligen standortspezifischen Funktionen. Angesichts des geringen oder fehlenden Nutzens standortspezifisch angepasster Funktionen kommen wir zu dem Schluss, dass die Gesamtfunktion zuverlässig zur Geschlechtsbestimmung von Sanderlingen verwendet werden kann, die an verschiedenen Orten, in verschiedenen Jahren oder Jahreszeiten auf der ostatlantischen Zugroute vermessen wurden. Unsere Ergebnisse können dazu beitragen, weitere umfassend geltende Methoden zur Geschlechtsbestimmung für andere Vogelarten zu entwickeln.

Introduction

Sex-related variations are commonly reported in ecological and evolutionary bird studies (e.g., Remisiewicz and Wennerberg 2006; Saino et al. 2010; Carvalho Provinciano et al. 2018). In species which exhibit phenotypic differences between males and females, determining the sex is an easy task. Conversely, in species with no or limited sexual dimorphism and/or dichromatism, distinguishing between males and females can be challenging. To solve this problem, molecular sexing is often the best and most accurate option, but it requires the collection and preservation of biological samples, and comes with economical and time constraints (Lessells and Mateman 1998; Morinha et al. 2012).

In many species, males and females differ slightly in some morphometric traits (Fairbairn et al. 2007). In birds, such sexual size dimorphism (SSD) mainly concerns wing, bill, tarsus, and head lengths (globally referred to as “body size”). Thus, based on morphological measurements, sexing tools can be developed. Discriminant functions (DF), allowing sex determination from a selection of measured morphometric data, are the most widely used statistical tools (Witkowska and Meissner 2020; Almeida et al. 2020). These methods have become popular among biologists because, after the initial effort in designing DFs, their further applications are cheap, easy-to-use, minimally invasive and time effective (Dechaume-Moncharmont et al. 2011; Yannic et al. 2016).

The accuracy of a sexing DF depends on the degree of SSD in the studied species (Kocijan et al. 2011). In species with low to moderate SSD, i.e., with large overlap in morphometrics between sexes, the risk of sex assignment errors is higher than in species with high SSD (Baker et al. 1999; Remisiewicz and Wennerberg 2006). In addition, in species with a wide geographical range, different populations may be exposed to local selection pressures resulting in regional variations in body size and/or SSD (Badyaev et al.

2000; Helfenstein et al. 2004; Santiago-Alarcon and Parker 2007; de Abreu 2018). In such cases, morphometric-based sexing methods might be region-specific (Granadeiro 1993; Weidinger and van Franeker 1998; Ellrich et al. 2010). For example, a sexing DF generated for Black Terns (*Chlidonias niger*) in Wisconsin (USA) performed equally well on neighbouring populations, whereas a sexing DF from the Oregon State performed poorly on the Wisconsin population (Shealer and Cleary 2007). Moreover, individual measurements may change over time. Wing length, like other plumage morphometric, can fluctuate during the year with moult and wear (Low 2006; Fernández and Lank 2007), reducing the reliability of morphometrics-based sexing tools developed and used during different seasons.

To assess the potential effects of geographical and temporal variations of body size and SSD on morphometrics-based sexing, we compared measurements from different populations using the same flyway and during different seasons in a migratory shorebird species with a wide geographical range, the Sanderling (*Calidris alba*). In breeding plumage, males of Sanderling often (but not always) have brown-spotted cinnamon feathers on the sides of the neck and upper breast, and tend to be slightly more colourful than females, while winter plumage is identical in both sexes (Pienkowski and Green 1976). As plumage cannot be reliably used for sexing, several sexing DF have already been proposed for this species along the East Atlantic flyway, from one breeding site (Russia; Soloviev and Tomkovich 1995), one staging-wintering site (United Kingdom; Wood 1987) and one wintering region (Western Africa; Mauritania and Ghana combined; Lourenço et al. 2016).

In the present study, our aim was to determine whether the efficiency of a morphometric-based sexing tool is region specific and time specific, or if the development of a single global-scale DF could be relevant. First, we compared body size and SSD of birds sampled in different regions using

Table 1 Discriminant functions developed for birds from Greenland (present study), Russia (Soloviev and Tomkovich 1995), Iceland (present study), United Kingdom (Wood 1987), Western Africa (Lau-

renço et al. 2016) and all sites combined (referred as East Atlantic flyway; present study)

Location	Sample size	Age class	Trapping period	Statistical method ^a	Discriminant function ^b
Greenland	411 (200 M/211F)	Adults	Summer	DFA	$D = 110.28 - 0.397W - 0.919B - 0.748TH$ (male if $D > 0$)
Russia	47 (18 M/29F)	Adults	Summer	DFA	$D = -166.059 + 0.932W + 0.645B + 0.625TH$ (male if $D < 0$)
Iceland	557 (150 M/407F)	Adults + Immatures	Spring	DFA	$D = 98.38 - 0.359W - 0.764B - 0.677TH$ (male if $D > 0$)
United Kingdom	42 (22 M/20F)	Adults	Autumn + Winter + Spring	DFA	$D = 0.375W + 1.13B - 75.5$ (male if $D < 0$)
Western Africa	990 (549 M/441F)	Adults + Immatures	Winter	GLM	$D = -0.5 + e^{(-6486.316 + 276.9894B + 128.3699TH + 50.50511W - 0.3635402T - 5.455647BTH - 2.148423BW - 0.9945474THW + 0.04216853BTHW)/1} + e^{(-6486.316 + 276.9894B + 128.3699TH + 50.50511W - 0.3635402T - 5.455647BTH - 2.148423BW - 0.9945474THW + 0.04216853BTHW)}$ (male if $D > 0$)
East Atlantic flyway	1967 (929 M/1038F)	Adults + Immatures	All seasons	DFA	$D = 101.25 - 0.352W - 0.825B - 0.727TH$ (male if $D > 0$)

^aDFA discriminant function analysis, GLM generalized linear model^bW wing length, B bill length, TH total head, T tarsus length

morphometrics from the three published studies mentioned above as well as from unpublished data collected in Greenland and Iceland, respectively additional breeding and staging sites belonging to the same flyway. Second, we developed new sexing DFs for (1) Greenland and Iceland and for (2) the East Atlantic flyway using all available data. Finally, we cross-compared the respective efficiency of each of these six sexing DFs to assess the extent to which geographical and temporal variations in body size and SSD affected the predictive power of each sexing DF.

Materials and methods

Study species

The Sanderling is a high Arctic shorebird species split into two subspecies. *C. alba alba* breeds in Greenland and Russia and winters along the Atlantic coasts of Europe and Africa (East Atlantic flyway), the east coast of Africa (East and South African flyway) and Western Asia (Southwest Asian flyway). *C. alba rubidus*, slightly larger than *C. a. alba*, breeds in Canada and Alaska and winters along the coasts of the Americas (Reneerkens et al. 2009).

Along East Atlantic flyway, most Sanderlings wintering in Western Europe and Western Africa appear to belong to the Greenland breeding population and to stop in Iceland during their northbound migration (Gudmundsson and Lindström 1992; Reneerkens et al. 2009, 2020).

To date, no DF has ever been published to sex birds breeding in Greenland and migrating through Iceland. The reliability of available DFs from other locations to sex these birds is also unknown.

Data collection and morphometric measurements

We collected morphometric data and genetic samples from 422 breeding adults (from June to August during 2007–2021) at two study sites along the North-East coast of Greenland: Hochstetter Forland (75.16666°N, 19.75000°W; 74 individuals) and Zackenberg (74.46665°N, 20.56684°W; 348 individuals). Incubating adults were caught using a 40 cm wide clap net (model BE40 from “Moudry traps”, Czech Republic, www.moudry.cz) placed over the nest and automatically released by birds returning on nests when sitting on their clutch. The same data and samples were also collected in Sandgerði, Iceland (64.04261°N, 22.71404°W; 560 adults and immatures birds trapped with canon nets), between 2007 and 2013, in May and early June (i.e., just before Greenland birds return to their breeding sites; Reneerkens et al. 2020).

In addition, we made use of the morphometric and sexing data available from the three previous studies mentioned above, (i) 71 breeding adults trapped from June to August in the Knipovich Bay, Russia (76.08333°N, 98.53333°E), in 1990–1992 (Soloviev and Tomkovich 1995), (ii) 45 adults birds staging or wintering from September to May around the estuary of the Tees river, United Kingdom (54.64162°N, 1.15293°W) in 1983–1984 (Wood 1987), and (iii) 928 birds (all age classes) wintering in Iwik, Mauritania (19.87754°N, 16.30356°W), trapped in November–December between 2002 and 2011 (ca. 75% of birds used to develop Western Africa DF; Lourenço et al. 2016).

For morphometric traits, the multivariate approach recommended by Engelmoer et al. (1987) was used in all sites and the following variables were measured with a single method: (1) *bill length* (B), from the anterior edge of feathering to tip of culmen, (2) *total head* (TH), from the back of the head to tip of culmen, (3) *tarsus length* (T), from the

Table 2 Results of the univariate analysis of variance for factors contributing to variation in morphometric measures of Sanderlings in Greenland, Iceland, Russia, United Kingdom and Mauritania.

Factors	df	Wing length	Bill length	Total head
Sex	1	561.27****	774.77****	926.45****
Region	3	12.63****	11.98****	10.62****
Sex * Region	4	1.87 (ns)	1.42 (ns)	1.48 (ns)

Values correspond to *F*-ratios for each factors, with associated probabilities

ns not significant at $P > 0.05$

**** = $P < 0.001$

tarsal joint to the distal end of the tarso-metatarsus (except in United Kingdom and Iceland), and (4) *wing length* (W), straightened and flattened as described by Evans (1986). Bill length, total head and tarsus length were measured to the nearest 0.1 mm with a calliper; wing length was measured with a stop ruler to the nearest 0.5 mm in all sites but United Kingdom (to the nearest millimetre).

Molecular and anatomical sexing

In birds from Greenland, Iceland and Mauritania, sexes were determined molecularly with a similar method. Three plucked pectoral feathers or one blood sample (with a capillary tube after puncturing the brachial vein with a 25-gauge needle) were collected from each bird. Blood from capillary tubes was transferred and stored in microtubes filled with absolute ethanol. Cellular DNA was extracted following the protocol of Richardson et al. (2001) or according to manufacturer's instructions with 96-Well Plate Genomic DNA Miniprep Kit (Bio Basic). Sex of each bird was determined by polymerase chain reaction (PCR) analysis of the extracted DNA using the primers 2602F (5' CAGATGGTG AGGATGCTGGAC 3') and 2669R (5' CCCTTTTATTGA TCCATCAAGYCTCTRAAGAG 3') designed by van der Velde et al. (2017). PCR reactions were carried out on a C1000 Touch™ thermal cycler (Bio-Rad) in 10 µL reaction mixture containing 0.1 U of Taq polymerase (Quantabio or Roche), 1X buffer, 0.2 mM dNTPs, 0.2 µM of each primer

Table 3 Descriptive statistics of morphometric measurements (in mm) of Sanderlings captured in Greenland (present study), Iceland (present study), Russia (Soloviev and Tomkovich 1995), United Kingdom (Wood 1987) and Mauritania (Laurenço et al. 2016)

	Males				Females				<i>t</i>	df	<i>P</i>	Cohen's <i>d</i> [CI95%]	SSD
	Mean	SD	CV	N	Mean	SD	CV	N					
	Greenland												
Wing length	125.5	2.3	1.8	203	128.8	2.8	2.2	216	− 13.50	410.8	<0.0001	1.31 [1.10–1.52]	2.6
Bill length	23.3	1.2	5.1	205	25	1.1	4.4	217	− 14.73	405.8	<0.0001	1.44 [1.23–1.66]	7.3
Total head	49.1	2.1	4.3	204	51.3	1.3	2.5	216	− 12.39	340.5	<0.0001	1.22 [1.02–1.43]	4.5
Tarsus length	24.8	0.9	3.6	205	25.7	1.1	4.3	215	− 9.22	409.9	<0.0001	0.90 [0.69–1.10]	3.6
	Russia												
Wing length	126	2.2	1.7	33	129.6	2.3	1.8	37	− 6.55	67.8	<0.0001	1.56 [1.02–2.11]	2.8
Bill length	24.1	0.8	3.3	33	25.6	1	3.9	38	− 7.39	69	<0.0001	1.74 [1.18–2.29]	6.2
Total head	49.3	0.9	1.8	20	51.1	1.2	2.3	30	− 6.01	47.2	<0.0001	1.64 [0.97–2.31]	3.6
Tarsus length	24.8	0.7	2.8	33	25.8	1	3.9	38	− 5.10	66.7	<0.0001	1.19 [0.67–1.70]	4.0
	Iceland												
Wing length	124.9	2.8	2.2	150	127.8	2.8	2.2	410	− 10.91	264.1	<0.0001	1.04 [0.85–1.24]	2.3
Bill length	23.7	1.5	6.3	150	25.1	1.2	4.8	410	− 10.48	232.3	<0.0001	1.08 [0.88–1.28]	5.9
Total head	49.2	1.7	3.4	150	51.1	1.4	2.7	407	− 12.00	233.1	<0.0001	1.23 [1.03–1.44]	3.9
Tarsus length	—	—	—	—	—	—	—	—	—	—	—	—	—
	United Kingdom												
Wing length	124.7	4.4	3.5	22	129.7	3.4	2.6	23	4.27	39.1	0.0001	1.28 [0.62–1.94]	4.0
Bill length	23.3	1.2	5.1	22	25.2	1.5	5.9	23	4.61	41.5	<0.0001	1.37 [0.70–2.04]	8.1
Total head	48.8	1.5	3.1	22	50.9	1.7	3.3	23	4.50	42.8	<0.0001	1.34 [0.67–2.00]	4.3
Tarsus length	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mauritania												
Wing length	124.9	2.9	2.3	545	127.9	3.3	2.6	372	− 14.03	724	<0.0001	0.97 [0.83–1.11]	2.4
Bill length	23.9	1.1	4.6	552	25.6	1.2	4.7	376	− 21.52	748.8	<0.0001	1.47 [1.32–1.61]	7.1
Total head	49	1.6	3.3	552	51.3	1.7	3.3	376	− 20.42	774.5	<0.0001	1.38 [1.23–1.53]	4.7
Tarsus length	24.6	1	4.1	549	25.7	1.1	4.3	376	− 14.53	747.8	<0.0001	0.99 [0.85–1.13]	4.5

Standard deviation (SD), coefficient of variation (CV) and sample size (N) are given for each sex. Student *t*-test with degree of freedom (df) and *P*-value, Cohen's *d* and degree of sexual size dimorphism (SSD) between sexes are given for each morphometric

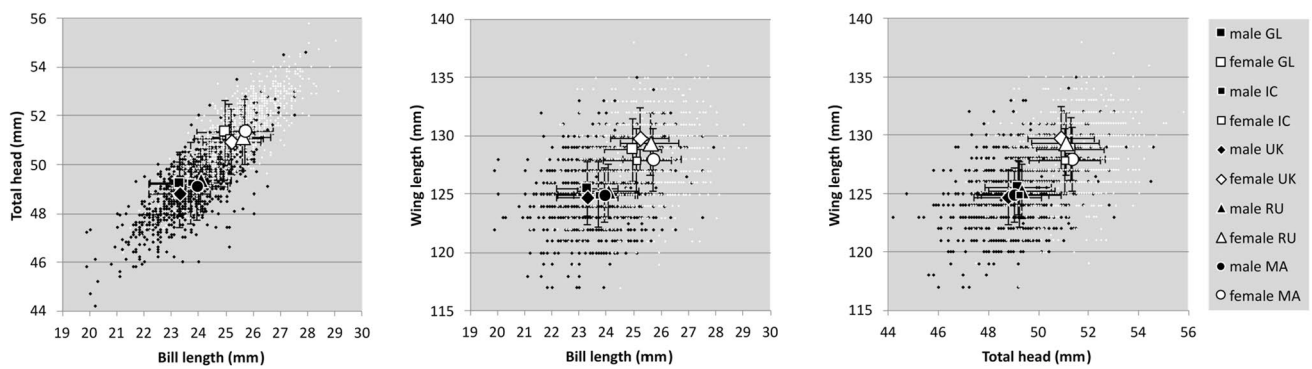


Fig. 1 Relationships between wing length, bill length and total head in Greenland (GL), Russia (RU), Iceland (IC), United Kingdom (UK) and Mauritania (MA) Sanderlings males (black symbols) and

females (white symbols). Small dots represent the individuals (symbols slightly larger for males to show overlapping colours). Mean values + SD are indicated with large symbols

and 2 μ L of DNA template. The thermal profile consisted of an initial denaturation for 1.5 min at 94 °C, followed by 34 cycles of 30 s at 94 °C, 30 s at 53 °C or 62 °C, 30 s at 65 °C, and a final extension at 65 °C or 72 °C during 10 min. 4 μ L of PCR products were separated on a 2% agarose gel and visualized by UV-transillumination using GelDoc system (Bio-Rad) after ethidium bromide staining.

Molecular sexing relied on the presence or absence of a sex-dependent DNA fragment, i.e., W chromosome fragment only found in females birds. The absence of this sex-dependent fragment is indistinguishable from a failure to amplify this fragment due to technical issues (Griffiths 2000). As in most comparable studies, we used sexes determined by this molecular method and assumed that they are correct (Baker et al. 1999), although we are aware that sexing errors could have happened (Roberston and Gemmell 2006).

Birds measured in England were sexed by gonadal examination after dissection. Birds from Russia were sexed either by gonadal examination, cloaca measurements, or by mating behaviour of colour-marked birds.

Table 4 Statistical significances from the Sanderling morphometric variables selections in Greenland, Iceland and East Atlantic flyway (all available samples combined) to determine the best sexing discriminant functions

		Wilks' λ	F	P
Greenland	W	0.697	177.4	<0.001
	B	0.632	237.8	<0.001
	TH	0.618	252.4	<0.001
Iceland	W	0.822	119.9	<0.001
	B	0.813	126.9	<0.001
	TH	0.768	167.2	<0.001
East Atlantic flyway	W	0.765	602.3	<0.001
	B	0.707	813.4	<0.001
	TH	0.631	1145.7	<0.001

Values correspond to Wilks' λ with associated Fisher F and P-value
W: wing length; B: bill length; TH: total head

Data analysis

All statistical tests were performed using R software version 4.2 (R Core Team 2022). Unless otherwise stated, the level of significance was set at $P < 0.05$.

All the assumptions required to run analysis were met, including normal distributions of the data, homogeneity of covariances (Box's M test) and homogeneity of variance (Levene's test), both for males and females.

We first tested variations in morphometrics according to site, sex and their interaction with multivariate and univariate analysis of variance (MANOVA/ANOVA). Then, we compared body size between sexes using unpaired Student's *t*-test and Cohen's *d* with its confidence interval 95% (effect size calculation; see Nagawaka and Cuthill 2007).

Morphometric means were given with standard deviation (SD) and coefficient of variation (CV). The degree of sexual size dimorphism (SSD) for a given trait was determined by the following equation (Almeida et al. 2020, modified from Lovich and Gibbons 1992):

$$SSD = \left[\left(\frac{\text{average of the larger sex}}{\text{average of the smaller sex}} \right) - 1 \right] \times 100$$

Next, we compared body size and SSD between regions. The overall differences in morphometric characters by sex between sites were determined with a MANOVA. Then, we used the same method as the comparison between sexes to compare morphometric measurements between sites. Tarsus length was not used in both multivariate analyses and in comparisons between sites, as this measurement is missing in UK and Iceland.

Second, we performed three linear discriminant analysis with the 'lda' function from MASS package (Ripley et al. 2013) to determine functions which could distinguish sexes by the most relevant morphometric characteristics for Sanderlings breeding in Greenland (Greenland DF), staging in

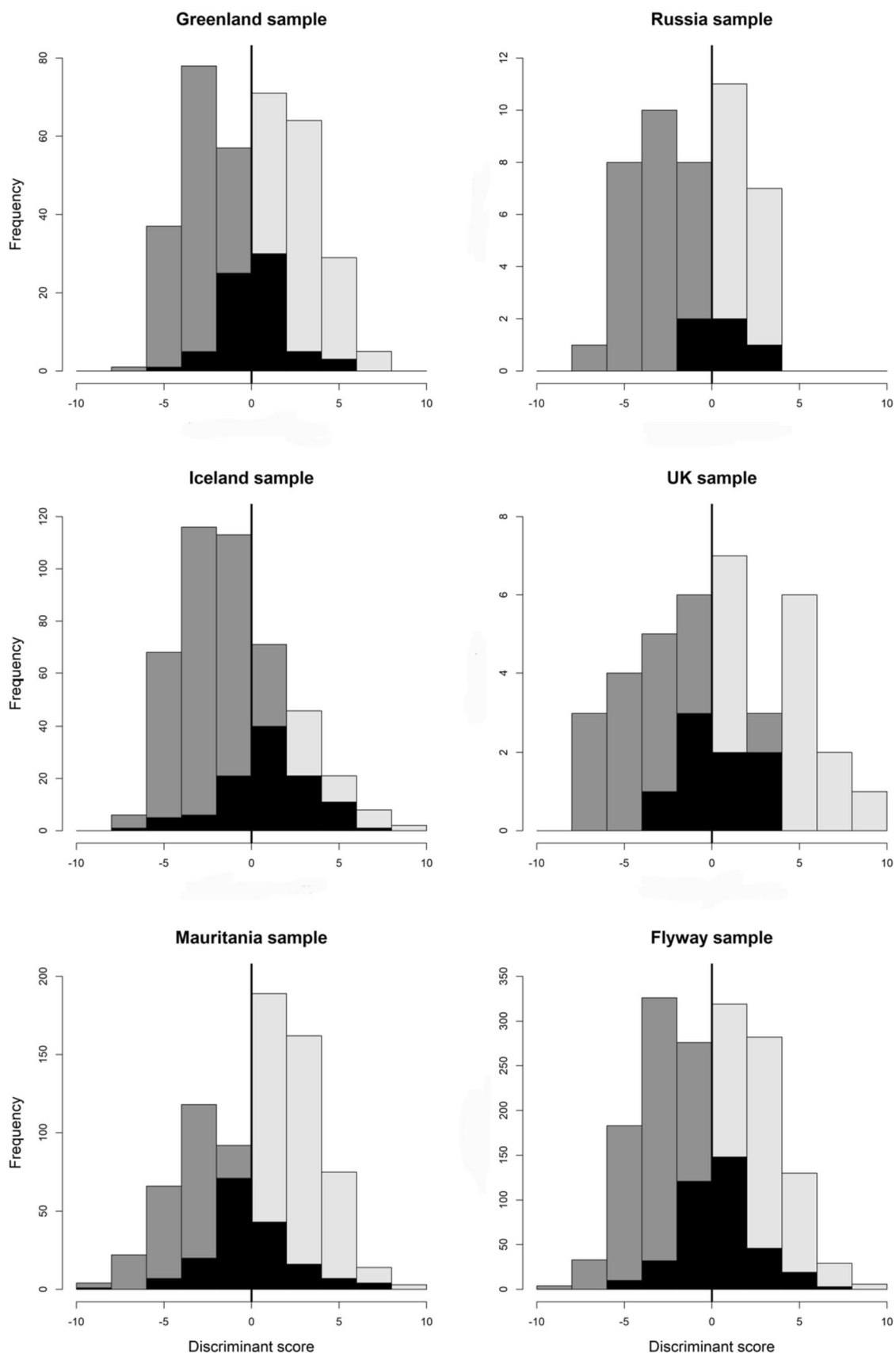


Fig. 2 Frequency distribution (number) of males (light grey) and females (dark grey) Sanderlings measured for each regional sample and the pooled samples (flyway sample), as assigned with the flyway discriminant functions. The black parts of the bars present the range of overlapping scores. Vertical lines present the discrimination value

Iceland (Iceland DF), and using the East Atlantic flyway (flyway DF), including datasets from all birds from all locations pooled. We used only data from birds having all measurements. Tarsus length data were only integrated in the process of discriminant analyses for Greenland. Potential outliers were initially detected by the Mahalanobis distances, using the `chisq.plot` and `aq.plot` functions from the `mvoutlier` package (Yannic et al. 2016). Detected outliers ($n=5$ from Greenland, $n=0$ from Iceland, $n=14$ from the flyway) were removed from the dataset due to uncorrectable mistakes in measurement or transcription. The final sample sizes and sex-ratios used to perform the discriminant analyses are indicated in Table 1. We estimated the discriminant rate of all combinations of morphometric variables using the leave-one-out cross-validation (LOOCV) method to determine the best model. This procedure predicts the sex of an individual after this individual has been removed from the data, instead of automated stepwise techniques which can capitalize on sampling error and lead to non-replicable results (Dechaume-Moncharmont et al. 2011).

Note that DFs previously developed in the United Kingdom and in Russia followed similar discriminant analyses as ours, whereas the DF developed for Western Africa opted for a generalized linear model (GLM) with binomial errors and a logit-link function to examine the relationship between the molecular sex, each morphometrics and their interactions (with sex as the binary response variable).

To test the ability of regional DFs (i.e., from Greenland, Iceland, Russia, United Kingdom and Western Africa) and the flyway DF to correctly assign sexes for birds measured in a given region, we compared result outputs between DFs using McNemar's test (Western Africa DF was not tested on UK, Iceland and all pooled samples because they do not include tarsus length measurements). To enable comparisons, success rates of DFs with their related samples were determined by resubstitution, i.e., the sex of each individual was predicted using the function obtained from the complete dataset and then compared with the sex identified using molecular or anatomical sexing.

Results

Size variability between breeding, staging and wintering sites

Multivariate analysis of variance indicated that body size varied significantly between sexes ($F_{3,1976} = 397.254$,

$P < 0.0001$) and among regions ($F_{12,5925} = 24.523$, $P < 0.0001$). Sex and region, but not their interaction, had also significant effects on each morphometrical variable (Table 2).

Females were larger than males and bill length was the most dimorphic trait across sites (from 5.9% to 8.1%). Sexual size dimorphism of each set of morphometrics was similar between all sites, although the mean SSD was slightly higher in Russian birds (ca. 4.2% with very large effect sizes) and lower in Icelandic birds (ca. 4.03% with smaller effect sizes) than in others (Table 3).

Males differed in size among regions (MANOVA: $F_{12,2796} = 10.348$, $P < 0.0001$). Adult males measured in Greenland and in United Kingdom had shorter bills than males measured in Iceland (GL: $t_{282.47} = -2.424$, $P = 0.0159$, Cohen's $d = 0.27 \pm 0.21$; UK: ns), in Russia (GL: $t_{57.76} = -4.324$, $P < 0.0001$, Cohen's $d = 0.61 \pm 0.37$; UK: $t_{33.40} = 2.525$, $P = 0.0164$, Cohen's $d = 0.75 \pm 0.57$), and in Mauritania (GL: $t_{342.71} = -6.010$, $P < 0.0001$, Cohen's $d = 0.51 \pm 0.17$; UK: $t_{22.45} = 2.340$, $P = 0.0285$, Cohen's $d = 0.55 \pm 0.43$). Males from Mauritania had shorter wings than males from Greenland ($t_{442.21} = 2.620$, $P = 0.009$, Cohen's $d = 0.20 \pm 0.16$) and from Russia ($t_{38.88} = -2.694$, $P = 0.0103$, Cohen's $d = 0.38 \pm 0.35$).

Females also differed in size among regions (MANOVA: $F_{12,3114} = 13.826$, $P < 0.0001$). Females from Greenland had longer wings than females from Iceland ($t_{434.61} = 4.370$, $P < 0.0001$, Cohen's $d = 0.37 \pm 0.16$) and from Mauritania ($t_{507.08} = 3.852$, $P = 0.0001$, Cohen's $d = 0.32 \pm 0.17$), and shorter bills than females from Russia ($t_{54.11} = -3.677$, $P = 0.0005$, Cohen's $d = 0.60 \pm 0.35$) and from Mauritania ($t_{510.65} = -6.959$, $P < 0.0001$, Cohen's $d = 0.57 \pm 0.17$). Females measured in Mauritania had shorter wings than females measured in Russia ($t_{51.18} = -4.038$, $P = 0.0002$, Cohen's $d = 0.53 \pm 0.34$) and in the United Kingdom ($t_{24.63} = -2.583$, $P = 0.0161$, Cohen's $d = 0.57 \pm 0.42$).

In all morphometrics and for each sex, we observed large intra-regional variability and large overlap between regions (Table 3, Fig. 1).

Discriminant functions for Greenland, Iceland and the East Atlantic flyway

The selection of variables that best predicted sex led to the combination of wing length [W], bill length [B] and total head [TH] for the three discriminant functions (Table 4).

For Greenland, the discriminant function correctly assigned 84% of the males and 84.4% of the females, with overall accuracy of 84.2%, by cross-validation (see Table 1).

The Icelandic discriminant function, correctly classified the sex of 80.6% of the birds, with 92.4% of the females and 48.7% of the males by cross-validation.

The East Atlantic flyway discriminant function yielded correct assignment for 80% of the males and 81.3% of the females, with 80.7% of overall accuracy, by cross-validation. Misclassified birds had all D values ranged $[-4$ to $4.90]$ for Greenland, $[-0.56$ to $2.21]$ for Russia, $[-6.86$ to $7.08]$ for Iceland, $[-2.09$ to $3.85]$ for UK, and $[-9.36$ to $6.57]$ for birds from Mauritania (see Fig. 2).

Compared accuracy of the six discriminant functions

When comparing overall (i.e., combining males and females) sex assignment success rates obtained with each regional DF (using equations from Table 1), we found that regional DFs showed similar success rates than foreign DFs. The only exception was for the Western Africa DF which performed significantly better with Mauritanian birds than some of foreign DFs did. At the sex level, some discrepancies in success rate appeared for both sexes for birds from Greenland, Iceland and Mauritania when using foreign DFs (Table 5).

DFs showed similar performances in sexing each foreign samples as their own sample at an overall level, except Russia DF which performed better for Russian birds than for birds from Greenland ($\chi^2_1 = 4.05$, $P = 0.0439$), United Kingdom ($\chi^2_1 = 5.02$, $P = 0.0249$), Iceland ($\chi^2_1 = 10.38$, $P = 0.0012$) and Mauritania ($\chi^2_1 = 5.25$, $P = 0.0219$).

Overall, the flyway DF provided similar sex assignment success rates to the regional DFs for each regional sample. There were exceptions for birds from Iceland for which the flyway DF performed better than the Iceland DF (McNemar's test: $\chi^2_1 = 4.82$, $P = 0.0279$), and for each sex when applied to Mauritanian birds (McNemar's test: males $\chi^2_1 = 23.51$, $P < 0.0001$, females $\chi^2_1 = 8.04$, $P = 0.0045$; Table 5, last column). The flyway DF was better to sex mixed-origins birds than regional DFs (Table 5, last row).

Globally, Icelandic sample got the lowest sexing success rates ($\leq 75\%$) and Russian sample got the highest ones (ca. 90%) with any DF, while for other samples the success rates were similar (ca. 80%).

Discussion

In the Scolopacidae family (sandpipers and related species), most species present some SSD with females larger than males (Jehl and Murray 1986), which allows the development of DFs (e.g., Niemi et al. 2018; Meissner et al. 2018; Almeida et al. 2020; Witkowska and Meissner 2020; Pohlen et al. 2021). In this study, we investigated whether variation in SSD and body size within a flyway could limit the efficiency of DFs to sex Sanderling on a large scale. We found that, despite quite similar SSD between the regions, the body size may differ. Still, each regional DF gave comparable results for any regional sample. The use of a sole

flyway-scale DF appears to be relevant and could facilitate future studies.

Geographical and temporal size variations

Regional variations occurred in body size. Birds from Russia were larger than any other birds, and birds on wintering/staging grounds had shorter wing lengths than birds on breeding grounds.

Sanderlings breeding in Greenland and in Russia belong to the same subspecies *C. alba alba* (Engelmoer and Roselaar 1998) and show only a low genetic differentiation (Conklin et al. 2016), while they are separated by approximately 2000 km. Bill size discrepancies between these two breeding populations could thus suggest a phenotypic or developmental plasticity in Sanderling, and reflect differences in local environments experienced by adults or young birds (Lafuente and Beldade 2019). The two regions have relatively similar climatic conditions (Hijmans et al. 2005) but host different insectivorous avian communities, which might result in different food competition pressures shaping divergent bill morphology (Kelly et al. 2024).

Interestingly, birds sampled in Mauritania and Iceland had on average longer bills than those measured in Greenland. This could indicate that populations wintering in Western Africa are composed not only by birds from North-East Greenland, but also include some birds from Russia, while populations staging in Iceland also include larger birds from the North American subspecies breeding in the Eastern Canadian Arctic (Reneerkens et al. 2008).

We also noted that wings were on average shorter in Mauritania and Iceland, where adults and immatures were measured, compared to breeding sites in Greenland where only adults were measured. Wing length varies seasonally due to wear and moult, as well as weather and feeding conditions during the growth of the primaries (Hall and Fransson 2000; Milá et al. 2008). Also, immature Sanderlings wear their primaries faster than adults (Pienkowski and Minton 1973), and replace them during their first summer at the earliest (Demongin 2016), whereas adult Sanderlings moult their primaries on wintering grounds (Loonstra et al. 2016). Immatures included in datasets could induce shorter wing lengths (for seabirds see e.g., Bugoni and Furness 2009). In Mauritania, for example, the proportion of immatures among wintering birds is relatively high (Reneerkens et al. 2020).

However, unlike skeletal elements, for which standard measurements are reliably repeatable (Rising and Somers 1989), wing length is difficult to calibrate and has limited accuracy (measured to the nearest 0.5 mm at most). Most changes in wing length between seasons and with age could be small relative to measurement errors and hence difficult to quantify (Francis and Wood 1989). Furthermore, body size can “drift” over time (Anderson et al. 2019; Zimova

Table 5 Success rates (percentage of correct sex assignments) of the six discriminant functions developed for Sanderling from Greenland, Russia, Iceland, United Kingdom, Western Africa and East Atlantic flyway (all samples pooled)

		<i>n</i>	Greenland function	Russia function	Iceland function	UK function	W Africa function ^a	Flyway function
Greenland sample	M	200	84.5%	88% (+ 3.5)	91.5% (+ 7.0)	90.5% (+ 6.0)	91% (+ 6.5)	84.5%
	F	211	82.5%	75.3% (− 7.2)	73.4% (− 9.1)	73% (− 9.5)	72.5% (− 10.0)	82% (− 0.5)
	All	411	83.4%	81.5% (− 1.9)	82.2% (− 1.2)	81.5% (− 1.9)	81.5% (− 1.9)	83.2% (− 0.2)
Russia sample	M	20	90% (− 10.0)	100%	100%	90% (− 10.0)	90% (− 10.0)	90% (− 10.0)
	F	30	93.3% (+ 3.3)	90%	80% (− 10.0)	90%	90%	90%
	All	50	92% (− 2.0)	94%	88% (− 6.0)	90% (− 4.0)	90% (− 4.0)	90% (− 4.0)
Iceland sample	M	150	78% (− 7.3)	85.3%	85.3%	82% (− 3.3)	–	78% (− 7.3)
	F	407	74.9% (+ 6.4)	67.1% (− 1.4)	68.5%	67.8% (− 0.7)	–	74.4% (+ 5.9)
	All	557	75.7% (+ 2.6)	72% (− 1.1)	73.1%	71.6% (− 1.5)	–	75.4% (+ 2.3)
UK sample	M	22	81.8% (+ 4.5)	77.3%	86.3% (+ 9.0)	77.3%	–	81.8% (+ 4.5)
	F	23	78.3% (+ 4.4)	73.9%	69.5% (− 4.4)	73.9%	–	78.3% (+ 4.4)
	All	45	80% (+ 4.5)	75.5%	77.8% (+ 2.3)	75.5%	–	80% (+ 4.5)
Mauritania sample	M	542	81.4% (− 5.3)	87.6% (+ 1.1)	88% (+ 1.3)	83% (− 3.7)	86.7%	81.7% (− 5.0)
	F	372	81.4% (+ 3.7)	68.3% (− 9.4)	75.8% (− 1.9)	76.3% (− 1.4)	77.7%	81.2% (+ 3.5)
	All	914	81.4% (− 1.6)	79.7% (− 3.3)	83%	80.3% (− 2.7)	83%	81.5% (− 1.5)
Flyway sample	M	929	82.2% (− 0.2)	87.8% (+ 5.4)	89% (+ 6.6)	85.1% (+ 2.7)	–	82.4%
	F	1038	79.7% (+ 0.5)	70.2% (− 9.0)	72.7% (− 6.5)	72.8% (− 6.4)	–	79.2%
	All	1967	80.9% (+ 0.2)	78.5% (− 2.2)	80.4% (− 0.3)	78.6% (− 2.1)	–	80.7%

Numbers in brackets indicate the difference (percentage point) to the success rate obtained with regional function by resubstitution (i.e., region where birds were captured). Values with significant differences ($P < 0.05$; McNemar's test) in success rate between the regional function and foreign functions for each sample are in bold

n denotes sample size for individuals genetically or anatomically sexed with all morphometrics measured

^aWestern Africa function was not tested on UK, Iceland and flyway samples because they do not include tarsus length measurements

et al. 2023). Data used in this study were collected between the early 1980s for the oldest and the early 2020s for the latest. Some morphometric changes may have occurred among populations sampled 30 or 40 years ago, potentially adding a cohort effect.

Morphometric-based sexing over large scale, any limits?

Morphometrics are usually effective in sexing birds from the *Calidrinae* subfamily (e.g., Meissner and Pilacka 2008; Almeida et al. 2020; Pohlen et al. 2021). Bill length is often the most discriminant morphometric amongst *Calidris* species, but appears seldom efficient alone for sexing because of substantial overlapping ranges between sexes (e.g., Niemc et al. 2018; Witkowska and Meissner 2020). Sexing requires the use of several other morphometrics in most of the cases, as for Sanderling.

Despite a moderate sexual dimorphism in Sanderling (ca. 4.6%), the DFs developed for this species allowed for 67% to 100% of correct sex assignments, depending on the sex and origin of the birds (Table 5). Such success rates might not be high enough for certain scientific applications, e.g., sex-specific behaviour studies, which thus would require molecular analysis.

All DFs performed better for males than for females. This difference is most likely related to sex-specific differences in size distribution, with many small females assigned as males and only a few large males assigned as females. This may weakly affect breeding population studies if both members of a pair are captured, but should be considered for staging and wintering population studies (Koloski et al. 2016).

Overall, foreign DFs performed equally well as regional DFs to assign sex for most regions. In Western Africa only, the regional DF performed slightly better than some of the foreign DFs. This may be due to the methodological difference (i.e., GLM compared to DFA) used to develop the Western Africa DF (Hallgrímsson et al. 2016).

To summarize, although we found significant geographical differences in body size (Table 1, Fig. 1) and specific models were developed for each regional dataset, using regional DFs provided almost no benefit to sex Sanderlings as compared with the use of foreign DFs. Correct assignment rates varied between sexes and regions, reflecting small differences in SSD between regions, but did not vary according to the functions used (all but one DFs being equally reliable to sex regional or foreign individuals), likely as a result of large variability in the measured populations (see above). As expected, the flyway DF did not improve sexing efficiency at a regional scale, except for Icelandic birds, but

performed equally well as regional DFs, and could hence provide a more universal and practical tool than regional DFs.

However, as body size is predicted to change with ongoing climate change (Sheridan and Bickford 2011; Gardner et al. 2014; Youngflesh et al. 2022), current discriminant functions may require updates in the future.

Our results have important practical implications for the study of Sanderling populations using the same flyway, regardless of the location and the season. In particular, they can be useful to design or reframe scientific programs in the field, helping to target a sex or to balance the sex-ratio in sampling for instance, without the need for molecular tools. Moreover, similar methodological considerations might be valuable in sexing other bird species with large geographical range.

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Data availability Data are available on request from the concerned authors.

Declarations

Conflict of interest The authors declare no conflict of interest. The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval All data were collected by people who have licences for handling animals for scientific purposes. All our fieldworks complied with the ethical standards and national laws on animal welfare.

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