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

4
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37

38 **Abstract**

39 Sexing bird species with monomorphic plumage is generally challenging, and sexual size
40 dimorphism (SSD) is often used to develop morphometric-based sexing tools, e.g., using
41 discriminant functions. Within species, local selection pressures, age-related and season-
42 related growth, may however induce geographical and temporal variations in body size and
43 SSD. Such variations may complicate the development of reliable morphometric-based sexing
44 methods at a broad scale.

45 We first investigated body size variations in a migratory shorebird species with wide breeding
46 and wintering ranges, the Sanderling *Calidris alba*, within the two breeding populations
47 (Greenland and Russia) and three staging/wintering populations (United Kingdom, Iceland
48 and Mauritania), which belong to the same flyway. Then, for samples from each region, we
49 tested whether site-specific (i.e., “regional”) functions performed better than functions

50 developed for birds from the other sites (i.e., “foreign” functions) or than an overall
51 (“flyway”) function that combined all sampled individuals.

52 We found minor variations in SSD between regions, but significant differences in body size
53 between sexes and regions. Females were larger than males and, for instance, breeders had
54 longer wings than staging and wintering birds. Regional functions had similar sexing
55 efficiency as any other functions applied to sample from each region, except for Western
56 Africa where the regional function performed slightly better than some of the other functions.
57 Furthermore, the flyway function developed after merging all subsamples had a similar
58 efficiency than the regional functions, i.e., from 75.4% to 90% of correct sex assignment
59 depending on the region.

60 Given the small or lack of benefit in using regional functions, we conclude that the flyway
61 function can be used reliably to sex Sanderlings measured at different sites, years or seasons
62 within the East Atlantic flyway. Our results may help to develop global sexing function for
63 other bird species.

64

65 **Keywords**

66 Discriminant function, Molecular sexing, East Atlantic flyway, Shorebird, Sanderling.

67

68

69 **Introduction**

70

71 Sex-related variations are commonly reported in ecological and evolutionary bird studies
72 (e.g., Remisiewicz and Wennerberg 2006; Saino et al. 2010; Carvalho Provinciano et al.
73 2018). In species which exhibit phenotypic differences between males and females,

74 determining the sex is an easy task. Conversely, in species with no or limited sexual
75 dimorphism and/or dichromatism, distinguishing between males and females can be
76 challenging. To solve this problem, molecular sexing is often the best and most accurate
77 option, but it requires the collection and preservation of biological samples, and comes with
78 economical and time constraints (Lessells and Mateman 1998; Morinha et al. 2012).

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

88 The accuracy of a sexing DF depends on the degree of SSD in the studied species (Kocijan et
89 al. 2011). In species with low to moderate SSD, i.e., with large overlap in morphometrics
90 between sexes, the risk of sex assignment errors is higher than in species with high SSD
91 (Baker et al. 1999; Remisiewicz and Wennerberg 2006). In addition, in species with a wide
92 geographical range, different populations may be exposed to local selection pressures
93 resulting in regional variations in body size and/or SSD (Badyaev et al. 2000; Helfenstein et
94 al. 2004; Santiago-Alarcon and Parker 2007; de Abreu 2018). In such cases, morphometric-
95 based sexing methods might be region-specific (Granadeiro 1993; Weidinger and van
96 Franeker 1998; Ellrich et al. 2010). For example, a sexing DF generated for Black Terns
97 (*Chlidonias niger*) in Wisconsin (USA) performed equally well on neighbouring populations,
98 whereas a sexing DF from the Oregon State performed poorly on the Wisconsin population

99 (Shealer and Cleary 2007). Moreover, individual measurements may change over time. Wing
100 length, like other plumage morphometric, can fluctuate during the year with moult and wear
101 (Low 2006; Fernández and Lank 2007), reducing the reliability of morphometrics-based
102 sexing tools developed and used during different seasons.

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

114 In the present study, our aim was to determine whether the efficiency of a morphometric-
115 based sexing tool is region-specific and time-specific, or if the development of a single
116 global-scale DF could be relevant. First, we compared body size and SSD of birds sampled in
117 different regions using morphometrics from the three published studies mentioned above as
118 well as from unpublished data collected in Greenland and Iceland, respectively additional
119 breeding and staging sites belonging to the same flyway. Second, we developed new sexing
120 DFs for (1) Greenland and Iceland and for (2) the East Atlantic flyway using all available
121 data. Finally, we cross-compared the respective efficiency of each of these six sexing DFs to
122 assess the extent to which geographical and temporal variations in body size and SSD affected
123 the predictive power of each sexing DF.

124

125

126 **Materials and methods**

127

128 **Study species**

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

134 Along East Atlantic flyway, most Sanderlings wintering in Western Europe and Western
135 Africa appear to belong to the Greenland breeding population and to stop in Iceland during
136 their northbound migration (Gudmundsson and Lindström 1992; Reneerkens et al. 2009,
137 2020).

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

141

142 **Data collection and morphometric measurements**

143 We collected morphometric data and genetic samples from 422 breeding adults (from June to
144 August during 2007-2021) at two study sites along the North-East coast of Greenland:
145 Hochstetter Forland (75.16666°N, 19.75000°W; 74 individuals) and Zackenberg
146 (74.46665°N, 20.56684°W; 348 individuals). Incubating adults were caught using a 40 cm
147 wide clap net (model BE40 from “Moudry traps”, Czech Republic, www.moudry.cz) placed
148 over the nest and automatically released by birds returning on nests when sitting on their

149 clutch. The same data and samples were also collected in Sandgerði, Iceland (64.04261°N,
150 22.71404°W; 560 adults and immatures birds trapped with canon nets), between 2007 and
151 2013, in May and early June (i.e., just before Greenland birds return to their breeding sites;
152 Reneerkens et al. 2020).

153 In addition, we made use of the morphometric and sexing data available from the three
154 previous studies mentioned above, (i) 71 breeding adults trapped from June to August in the
155 Knipovich Bay, Russia (76.08333°N, 98.53333°E), in 1990-1992 (Soloviev and Tomkovich
156 1995), (ii) 45 adults birds staging or wintering from September to May around the estuary of
157 the Tees river, United Kingdom (54.64162°N, 1.15293°W) in 1983-1984 (Wood 1987), and
158 (iii) 928 birds (all age classes) wintering in Iwik, Mauritania (19.87754°N, 16.30356°W),
159 trapped in November-December between 2002 and 2011 (ca. 75% of birds used to develop
160 Western Africa DF; Lourenço et al. 2016).

161 For morphometric traits, the multivariate approach recommended by Engelmoer et al. (1987)
162 was used in all sites and the following variables were measured with a single method : (1) *bill*
163 *length* (B), from the anterior edge of feathering to tip of culmen, (2) *total head* (TH), from the
164 back of the head to tip of culmen, (3) *tarsus length* (T), from the tarsal joint to the distal end
165 of the tarso-metatarsus (except in United Kingdom and Iceland), and (4) *wing length* (W),
166 straightened and flattened as described by Evans (1986). Bill length, total head and tarsus
167 length were measured to the nearest 0.1 mm with a calliper; wing length was measured with a
168 stop ruler to the nearest 0.5 mm in all sites but United Kingdom (to the nearest millimetre).

169

170 **Molecular and anatomical sexing**

171 In birds from Greenland, Iceland and Mauritania, sexes were determined molecularly with a
172 similar method. Three plucked pectoral feathers or one blood sample (with a capillary tube
173 after puncturing the brachial vein with a 25-gauge needle) were collected from each bird.

174 Blood from capillary tubes was transferred and stored in microtubes filled with absolute
175 ethanol. Cellular DNA was extracted following the protocol of Richardson et al. (2001) or
176 according to manufacturer's instructions with 96-Well Plate Genomic DNA Miniprep Kit
177 (Bio Basic). Sex of each bird was determined by polymerase chain reaction (PCR) analysis of
178 the extracted DNA using the primers 2602F (5' CAGATGGTGAGGATGCTGGAC 3') and
179 2669R (5' CCCTTTTATTGATCCATCAAGYCTCTRAAGAG 3') designed by van der
180 Velde et al. (2017). PCR reactions were carried out on a C1000 TouchTM thermal cycler (Bio-
181 Rad) in 10 µL reaction mixture containing 0.1 U of Taq polymerase (Quantabio or Roche),
182 1X buffer, 0.2 mM dNTPs, 0.2 µM of each primer and 2 µL of DNA template. The thermal
183 profile consisted of an initial denaturation for 1.5 min at 94°C, followed by 34 cycles of 30
184 sec at 94°C, 30 sec at 53°C or 62°C, 30 sec at 65°C, and a final extension at 65°C or 72°C
185 during 10 min. 4 µL of PCR products were separated on a 2% agarose gel and visualized by
186 UV-transillumination using GelDoc system (Bio-Rad) after ethidium bromide staining.

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

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

196

197 **Data analysis**

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

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

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

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

210

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

211

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

217 Second, we performed three linear discriminant analysis with the 'lda' function from MASS
218 package (Ripley et al. 2013) to determine functions which could distinguish sexes by the most
219 relevant morphometric characteristics for Sanderlings breeding in Greenland (Greenland DF),
220 staging in Iceland (Iceland DF), and using the East Atlantic flyway (flyway DF), including

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

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

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

246

247

248 **Results**

249

250 **Size variability between breeding, staging and wintering sites**

251 Multivariate analysis of variance indicated that body size varied significantly between sexes
252 ($F_{3,1976} = 397.254$, $P < 0.0001$) and among regions ($F_{12,5925} = 24.523$, $P < 0.0001$). Sex and
253 region, but not their interaction, had also significant effects on each morphometrical variable
254 (Table 2).

255 Females were larger than males and bill length was the most dimorphic trait across sites (from
256 5.9% to 8.1%). Sexual size dimorphism of each set of morphometrics was similar between all
257 sites, although the mean SSD was slightly higher in Russian birds (ca. 4.2% with very large
258 effect sizes) and lower in Icelandic birds (ca. 4.03% with smaller effect sizes) than in others
259 (Table 3).

260 Males differed in size among regions (MANOVA: $F_{12,2796} = 10.348$, $P < 0.0001$). Adult males
261 measured in Greenland and in United Kingdom had shorter bills than males measured in
262 Iceland (GL: $t_{282.47} = -2.424$, $P = 0.0159$, Cohen's $d = 0.27 \pm 0.21$; UK: ns), in Russia (GL:
263 $t_{57.76} = -4.324$, $P < 0.0001$, Cohen's $d = 0.61 \pm 0.37$; UK: $t_{33.40} = 2.525$, $P = 0.0164$, Cohen's $d =$
264 0.75 ± 0.57), and in Mauritania (GL: $t_{342.71} = -6.010$, $P < 0.0001$, Cohen's $d = 0.51 \pm 0.17$; UK:
265 $t_{22.45} = 2.340$, $P = 0.0285$, Cohen's $d = 0.55 \pm 0.43$). Males from Mauritania had shorter wings
266 than males from Greenland ($t_{442.21} = 2.620$, $P = 0.009$, Cohen's $d = 0.20 \pm 0.16$) and from
267 Russia ($t_{38.88} = -2.694$, $P = 0.0103$, Cohen's $d = 0.38 \pm 0.35$).

268 Females also differed in size among regions (MANOVA: $F_{12,3114} = 13.826$, $P < 0.0001$).
269 Females from Greenland had longer wings than females from Iceland ($t_{434.61} = 4.370$, $P <$
270 0.0001 , Cohen's $d = 0.37 \pm 0.16$) and from Mauritania ($t_{507.08} = 3.852$, $P = 0.0001$, Cohen's $d =$

271 0.32±0.17), and shorter bills than females from Russia ($t_{54.11} = -3.677$, $P = 0.0005$, Cohen's d
272 = 0.60±0.35) and from Mauritania ($t_{510.65} = -6.959$, $P < 0.0001$, Cohen's $d = 0.57±0.17$).
273 Females measured in Mauritania had shorter wings than females measured in Russia ($t_{51.18} = -$
274 4.038, $P = 0.0002$, Cohen's $d = 0.53±0.34$) and in the United Kingdom ($t_{24.63} = -2.583$, $P =$
275 0.0161, Cohen's $d = 0.57±0.42$).

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

278

279 **Discriminant functions for Greenland, Iceland and the East Atlantic flyway**

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

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

284 The Icelandic discriminant function, correctly classified the sex of 80.6% of the birds, with
285 92.4% of the females and 48.7% of the males by cross-validation.

286 The East Atlantic flyway discriminant function yielded correct assignment for 80% of the
287 males and 81.3% of the females, with 80.7% of overall accuracy, by cross-validation.

288 Misclassified birds had all D values ranged [-4 to 4.90] for Greenland, [-0.56 to 2.21] for
289 Russia, [-6.86 to 7.08] for Iceland, [-2.09 to 3.85] for UK, and [-9.36 to 6.57] for birds from
290 Mauritania (see Figure 2).

291

292 **Compared accuracy of the six discriminant functions**

293 When comparing overall (i.e., combining males and females) sex assignment success rates
294 obtained with each regional DF (using equations from Table 1), we found that regional DFs
295 showed similar success rates than foreign DFs. The only exception was for the Western Africa

296 DF which performed significantly better with Mauritanian birds than some of foreign DFs
297 did. At the sex level, some discrepancies in success rate appeared for both sexes for birds
298 from Greenland, Icelandic and Mauritania when using foreign DFs (Table 5).
299 DFs showed similar performances in sexing each foreign samples as their own sample at an
300 overall level, except Russia DF which performed better for Russian birds than for birds from
301 Greenland ($\chi^2_1 = 4.05$, $P = 0.0439$), United Kingdom ($\chi^2_1 = 5.02$, $P = 0.0249$), Iceland (χ^2_1
302 $=10.38$, $P = 0.0012$) and Mauritania ($\chi^2_1 = 5.25$, $P = 0.0219$).
303 Overall, the flyway DF provided similar sex assignment success rates to the regional DFs for
304 each regional sample. There were exceptions for birds from Iceland for which the flyway DF
305 performed better than the Iceland DF (McNemar's test: $\chi^2_1 = 4.82$, $P = 0.0279$), and for each
306 sex when applied to Mauritanian birds (McNemar's test: males $\chi^2_1 = 23.51$, $P < 0.0001$,
307 females $\chi^2_1 = 8.04$, $P = 0.0045$; Table 5, last column). The flyway DF was better to sex
308 mixed-origins birds than regional DFs (Table 5, last row).
309 Globally, Icelandic sample got the lowest sexing success rates ($\leq 75\%$) and Russian sample
310 got the highest ones (ca. 90%) with any DF, while for other samples the success rates were
311 similar (ca. 80%).

312

313

314 **Discussion**

315

316 In the Scolopacidae family (sandpipers and related species), most species present some SSD
317 with females larger than males (Jehl and Murray 1986), which allows the development of DFs
318 (e.g., Niemi et al. 2018; Meissner et al. 2018; Almeida et al. 2020; Witkowska and Meissner
319 2020; Pohlen et al. 2021). In this study, we investigated whether variation in SSD and body
320 size within a flyway could limit the efficiency of DFs to sex Sanderling on a large scale. We

321 found that, despite quite similar SSD between the regions, the body size may differ. Still, each
322 regional DF gave comparable results for any regional sample. The use of a sole flyway-scale
323 DF appears to be relevant and could facilitate future studies.

324

325 **Geographical and temporal size variations**

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

329 Sanderlings breeding in Greenland and in Russia belong to the same subspecies *C. alba alba*
330 (Engelmoer and Roselaar 1998) and show only a low genetic differentiation (Conklin et al.
331 2016), while they are separated by approximately 2000 km. Bill size discrepancies between
332 these two breeding populations could thus suggest a phenotypic or developmental plasticity in
333 Sanderling, and reflect differences in local environments experienced by adults or young birds
334 (Lafuente and Beldade 2019). The two regions have relatively similar climatic conditions
335 (Hijmans et al. 2005) but host different insectivorous avian communities, which might result
336 in different food competition pressures shaping divergent bill morphology (Kelly et al. 2024).
337 Interestingly, birds sampled in Mauritania and Iceland had on average longer bills than those
338 measured in Greenland. This could indicate that populations wintering in Western Africa are
339 composed not only by birds from North-East Greenland, but also include some birds from
340 Russia, while populations staging in Iceland also include larger birds from the North
341 American subspecies breeding in the Eastern Canadian Arctic (Reneerkens et al. 2008).

342 We also noted that wings were on average shorter in Mauritania and Iceland, where adults and
343 immatures were measured, compared to breeding sites in Greenland where only adults were
344 measured. Wing length varies seasonally due to wear and moult, as well as weather and
345 feeding conditions during the growth of the primaries (Hall and Fransson 2000; Milá et al.

346 2008). Also, immature Sanderlings wear their primaries faster than adults (Pienkowski and
347 Minton 1973), and replace them during their first summer at the earliest (Demongin 2016),
348 whereas adult Sanderlings moult their primaries on wintering grounds (Loonstra et al. 2016).
349 Immatures included in datasets could induce shorter wing lengths (for seabirds see e.g.,
350 Bugoni and Furness 2009). In Mauritania, for example, the proportion of immatures among
351 wintering birds is relatively high (Reneerkens et al. 2020).
352 However, unlike skeletal elements, for which standard measurements are reliably repeatable
353 (Rising and Somers 1989), wing length is difficult to calibrate and has limited accuracy
354 (measured to the nearest 0.5 mm at most). Most changes in wing length between seasons and
355 with age could be small relative to measurement errors and hence difficult to quantify
356 (Francis and Wood 1989). Furthermore, body size can “drift” over time (Anderson et al. 2019;
357 Zimova et al. 2023). Data used in this study were collected between the early 1980s for the
358 oldest and the early 2020s for the latest. Some morphometric changes may have occurred
359 among populations sampled 30 or 40 years ago, potentially adding a cohort effect.

360

361 **Morphometric-based sexing over large scale, any limits?**

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

368 Despite a moderate sexual dimorphism in Sanderling (ca. 4.6%), the DFs developed for this
369 species allowed for 67% to 100% of correct sex assignments, depending on the sex and origin
370 of the birds (Table 5). Such success rates might not be high enough for certain scientific

371 applications, e.g., sex-specific behaviour studies, which thus would require molecular
372 analysis.

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

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

382 To summarize, although we found significant geographical differences in body size (Table 1,
383 Fig. 1) and specific models were developed for each regional dataset, using regional DFs
384 provided almost no benefit to sex Sanderlings as compared with the use of foreign DFs.
385 Correct assignment rates varied between sexes and regions, reflecting small differences in
386 SSD between regions, but did not vary according to the functions used (all but one DFs being
387 equally reliable to sex regional or foreign individuals), likely as a result of large variability in
388 the measured populations (see above). As expected, the flyway DF did not improve sexing
389 efficiency at a regional scale, except for Icelandic birds, but performed equally well as
390 regional DFs, and could hence provide a more universal and practical tool than regional DFs.
391 However, as body size is predicted to change with ongoing climate change (Sheridan and
392 Bickford 2011; Gardner et al. 2014; Youngflesh et al. 2022), current discriminant functions
393 may require updates in the future.

394 Our results have important practical implications for the study of Sanderling populations
395 using the same flyway, regardless of the location and the season. In particular, they can be

396 useful to design or reframe scientific programs in the field, helping to target a sex or to
397 balance the sex-ratio in sampling for instance, without the need for molecular tools.
398 Moreover, similar methodological considerations might be valuable in sexing other bird
399 species with large geographical range.

400

401

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410

411

412 **Declarations**

413 **Conflict of interest**

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

416

417 **Ethical approval**

418 All data were collected by people who have licences for handling animals for scientific
419 purposes. All our fieldworks complied with the ethical standards and national laws on animal
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427

428 **Authors contributions**

429 TP and OG designed the study and wrote the first draft of the manuscript; TP and LE carried
430 out data analyses; MT helped with the genetic analysis; FXDM, GY and LB provided
431 methodological support and contributed to drafting the manuscript; GH, JH, JL, JM, JR, NS,
432 MS, JtH, PT, AW contributed to data collection and drafting the manuscript. All authors read
433 and approved the final manuscript.

434

435 **Data availability**

436 Data are available on request from the concerned authors.

437

438

439 **References**

440

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621 **Tables and figures**

622

623 **Table 1** Discriminant functions developed for birds from Greenland (present study), Russia (Soloviev and Tomkovich 1995), Iceland (present
624 study), United Kingdom (Wood 1987), Western Africa (Laurenço et al. 2016) and all sites combined (referred as East Atlantic flyway; present
625 study)

626

Location	Sample size	Age class	Trapping period	Statistical method ^a	Discriminant Function ^b
Greenland	411 (200M/211F)	Adults	Summer	DFA	$D = 110.28 - 0.397W - 0.919B - 0.748TH$ (male if $D > 0$)
Russia	47 (18M/29F)	Adults	Summer	DFA	$D = -166.059 + 0.932W + 0.645B + 0.625TH$ (male if $D < 0$)
Iceland	557 (150M/407F)	Adults + Immatures	Spring	DFA	$D = 98.38 - 0.359W - 0.764B - 0.67TH$ (male if $D > 0$)
United Kingdom	42 (22M/20F)	Adults	Autumn + Winter + Spring	DFA	$D = 0.375W + 1.13B - 75.5$ (male if $D < 0$)

Western Africa	990 (549M/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 (929M/1038F)	Adults + Immatures	All seasons	DFA	$D = 101.25 - 0.352W - 0.825B - 0.727TH$ (male if D>0)

627

628 ^a DFA: discriminant function analysis; GLM: generalized linear model.

629 ^b W: wing length; B: bill length; TH: total head; T: tarsus length.

630

631

632 **Table 2** Results of the univariate analysis of variance for factors contributing to variation in morphometric measures of Sanderlings in
633 Greenland, Iceland, Russia, United Kingdom and Mauritania. Values correspond to *F*-ratios for each factors, with associated probabilities
634

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)

635

636 ns = not significant at $P > 0.05$

637 **** = $P < 0.001$

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643 **Table 3** Descriptive statistics of morphometric measurements (in mm) of Sanderlings captured in Greenland (present study), Iceland (present
644 study), Russia (Soloviev and Tomkovich 1995), United Kingdom (Wood 1987) and Mauritania (Laurenço et al. 2016). Standard deviation (SD),
645 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
646 degree of sexual size dimorphism (SSD) between sexes are given for each morphometric

	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

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655 **Table 4** Statistical significances from the Sanderling morphometric variables selections in Greenland, Iceland and East Atlantic flyway (all
656 available samples combined) to determine the best sexing discriminant functions. Values correspond to Wilks' λ with associated Fisher F and *P*-
657 value

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		Wilks' λ	F	<i>P</i>
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

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660 W: wing length; B: bill length; TH: total head

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665 **Table 5** Success rates (percentage of correct sex assignments) of the six discriminant functions developed for Sanderling from Greenland,
 666 Russia, Iceland, United Kingdom, Western Africa and East Atlantic flyway (all samples pooled). Numbers in brackets indicate the difference
 667 (percentage point) to the success rate obtained with regional function by resubstitution (i.e., region were birds were captured, marked in grey).
 668 Values with significant differences ($P < 0.05$; McNemar's test) in success rate between the regional function and foreign functions for each
 669 sample are in bold

		<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)

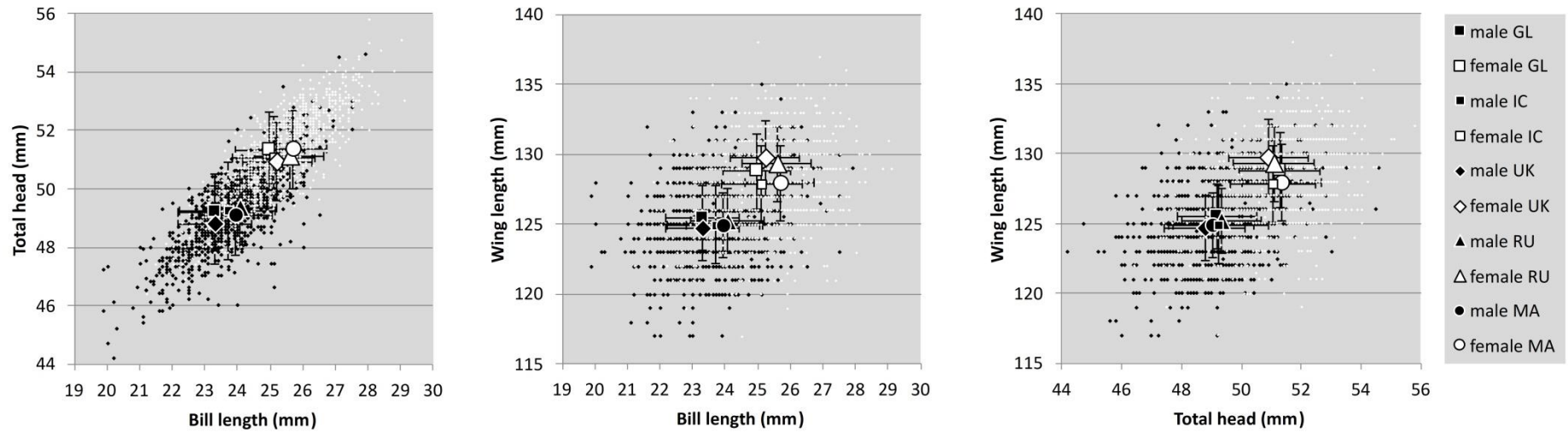
UK sample	M	22	81.8% (+4.5)	77.3%	86.3% (+9.0)	77.3%	–	81.8% (+4.5)
	F	23	78.3% (+4.4)	73.9%	69.5% (-4.4)	73.9%	–	78.3% (+4.4)
	All	45	80% (+4.5)	75.5%	77.8% (+2.3)	75.5%	–	80% (+4.5)
Mauritania sample	M	542	81.4% (-5.3)	87.6% (+1.1)	88% (+1.3)	83% (-3.7)	86.7%	81.7% (-5.0)
	F	372	81.4% (+3.7)	68.3% (-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%

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

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

673 **Fig. 1** Relationships between wing length, bill length and total head in Greenland (GL), Russia (RU), Iceland (IC), United Kingdom (UK) and
674 Mauritania (MA) Sanderlings males (black symbols) and females (white symbols). Small dots represent the individuals (symbols slightly larger
675 for males to show overlapping colours). Mean values + SD are indicated with large symbols.



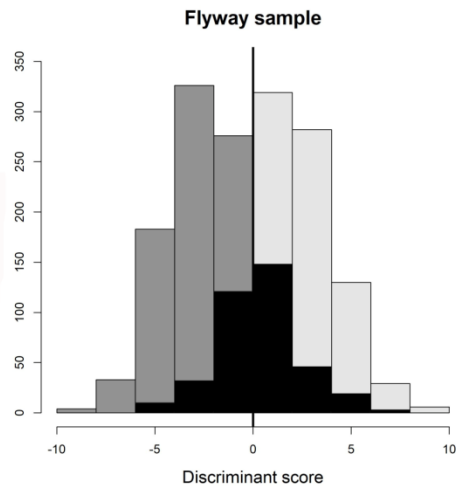
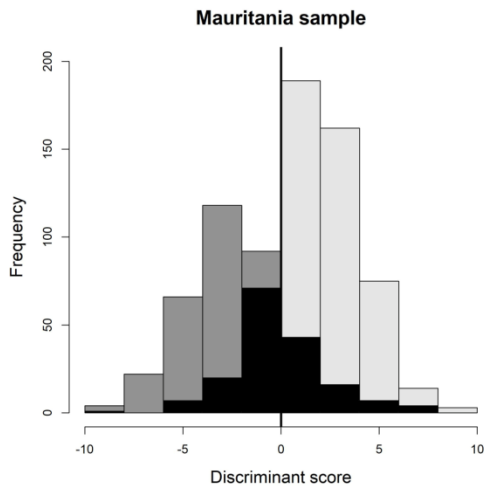
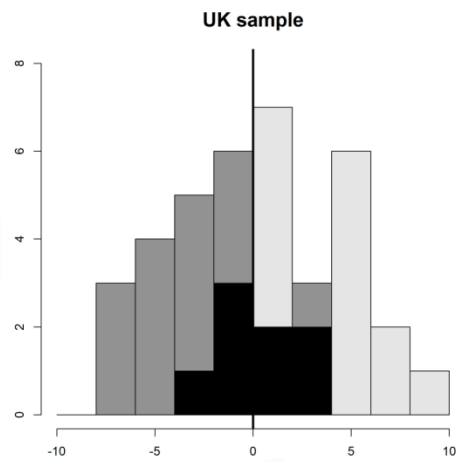
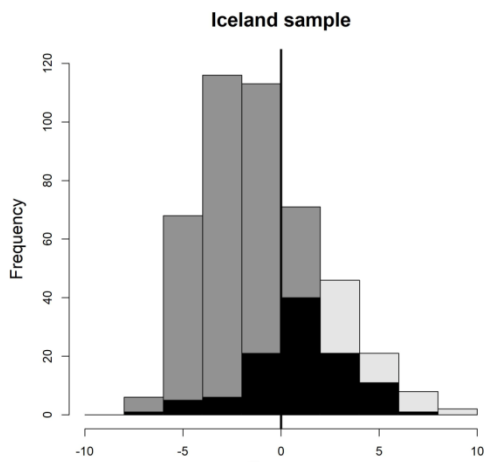
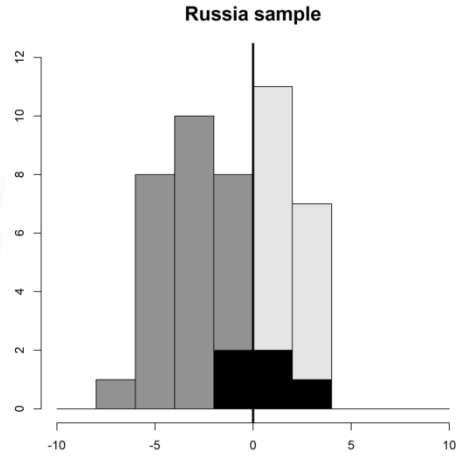
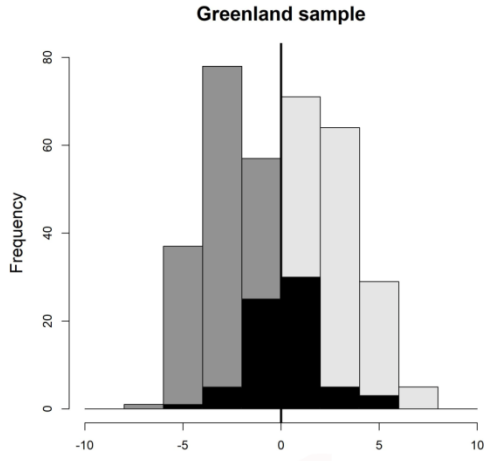
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680 **Fig. 2** Frequency distribution (number) of males (light grey) and females (dark grey)
681 Sanderlings measured for each regional sample and the pooled samples (flyway sample), as
682 assigned with the flyway discriminant functions. The black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