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Using wing length for sexing Eurasian Skylark *Alauda arvensis*: examples from a British population

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ABSTRACT
The Skylark *Alauda arvensis* is a common, yet rarely ringed species. Outside the breeding season, determining the sex of individual birds is problematic. While previous studies have provided sexually dimorphic ranges of wing length, those based on accurate DNA sexing focus on Continental populations, which are subject to seasonal influxes of migrants. In Britain, Skylark is largely a sedentary species, and Continental guidelines on in-the-hand sexing might be inappropriate. Here we present a comparative analysis of morphometrics and DNA sexing for 137 individual Skylarks, to assess the accuracy of using wing length to sex trapped birds. We suggest that birds with wing measurements of 107–109 mm be classified as of 'undetermined' sex, with those below this range being classified as female and those above as male.

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The Skylark *Alauda arvensis* is a Red-listed species in the UK (Stanbury *et al* 2021) and is reported to have suffered a significant decline of 58% across Europe from 1980 to 2021 (PECBMS 2022), a trend that has been attributed to several factors including increased winter mortality, agricultural intensification, and change in summer crops resulting in poor reproductive success (Geiger *et al* 2014).

One of the most effective and widely used methods of long-term monitoring of bird demographics and movements is bird ringing (BTO 2020). Skylarks are difficult to sex when caught, showing no sexual dimorphism in plumage (Hegemann *et al* 2012). Whilst females can be sexed within the breeding season by the presence of a brood patch, and males by the presence of a cloacal protuberance, these features regress at the end of the breeding season and feathers regrow. The species undergoes a complete moult in both adults and juveniles in August and September, precluding determination of sex based on brood patch. These features are therefore not available for sexing wintering birds.

Wing length has been used by other authors to classify ringed Skylarks by sex. Wing-length ranges quoted for the sexes vary, with Svensson (1992), Campbell *et al* (2020) and BTO (2023) providing ranges for females of 98–108, 99–112 and 100–110 mm respectively, and for males of 105–118, 108–123 and 109–119 mm respectively. Dougall (1997) provides ranges for wintering Skylark from a sedentary population in Scotland of 99–109 mm for females and 111–120 mm for males. Studies based on molecular sexing for birds caught in the Netherlands report wing ranges of 97–107 mm for females and 107–118 mm for males, with almost no overlap between the sexes (Hegemann *et al* 2012); these authors noted, however, that Skylarks caught during winter had longer wings than those in breeding season, with female wing lengths spanning 99–109 mm and males 106–122 mm, resulting in overlap in the range 106–109 mm at that season, likely due to the presence of migrants from visiting populations.

Unlike those in continental Europe, British Skylarks are considered to be relatively sedentary (Dougall 1996), with no significant migrations observable and ring recoveries indicating only short movements (Spina *et al* 2022). Whilst the arrival of some birds from the Continent cannot be ruled out, particularly in southern Britain, we would not expect wing measurements to be subject to much seasonal change,

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as the vast majority of birds are resident; sexing techniques based on morphometric ranges should therefore be applicable year round.

Developments in DNA sexing and its affordability have now allowed us to undertake a comparative analysis of male and female morphometrics from a sample of accurately sexed Skylarks in the West Midlands, central England, and to assess the efficacy of wing length alone to determine sex. The aim of our study was to provide wing-length ranges to aid the sexing of Skylarks caught for ringing in Britain, thus allowing more meaningful analyses of sex ratios in winter flocks. We hypothesise that our results will align with those for birds wintering in the Netherlands.

Methods

Catching, handling and data collection

Between October and December 2021, the West Midlands Ringing Group trapped Skylarks at two sites in the West Midlands, each comprising largely arable fields with winter stubble and associated hedgerows. During darkness, we deployed a Pulsar Helion XQ38F thermal imager, surveying each area to locate the heat signal of Skylarks roosting in the fields (Hughes *et al* 2022). By enabling surveyors to target Skylarks directly, the use of thermal imaging enabled surveyors to conduct targeted, standard dazzling and hand-netting (Macpherson 1897, Labisky 1959), using an Nightmaster NM1 XL white Pill torch and a 22-inch landing-net hoop, covered with three-ply, 235-denier, 15-mm nylon meshed netting, supplied by BTO, on a two-metre carbon-fibre landing-net pole. Catching did not take place during rain, in temperatures below freezing or during extended periods of inclement weather, fog or mist that might have disoriented birds upon release.

Once caught, individual birds were placed in cotton drawstring bags and transported to a ringing and processing area. Morphometric data were taken from all Skylarks caught, comprising weight, measured with a Brifit 500 × 0.01-g digital scale, and wing length, taken to the nearest mm using a 15-cm wing rule and the maximum-chord method (Svensson 1992, Redfern & Clark 2001). As all birds were captured at similar times after sunset, weight was not adjusted for time of capture. For consistency, all measurements were taken by the same two members of the field team, whose measurements had previously been cross-checked for standardisation. Birds were assigned a provisional sex based on wing length, as per Hegemann *et al* (2012), with those with wing lengths <106 mm being classified as females, those >110 mm as males and others as 'undetermined'. DNA samples were taken by extraction (under licence) of three flank feathers from each bird; these feathers were placed in a 70 × 100-mm resealable plastic bag, which was then sealed and marked with a unique reference number using an indelible pen, following laboratory procedure. These bags were then stored in an envelope in a dark, dry place until all feather samples had been collected.

Once processed, birds were released into the field in which they had been caught. Thermal imaging of previous releases of Skylarks at night had shown that they fly for a short distance before dropping back in to roost (Dolan, pers obs).

Laboratory procedures

DNA was isolated from 2–3 mm of the base of the calamus of each feather; we used the QIAGEN UCP DNA Micro Kit (QIAGEN, UK) according to the manufacturer's instructions, with a 20-minute Proteinase K digestion and resuspension in 80 µl of Buffer AUE. Genetic sexing was performed by PCR according to the protocol outlined by Chu *et al* (2015), using primers IntP2 (5'-GTCACTATCAGAT CCAGARTATCTTC-3') and IntP8 (5'-CTYCCAA GRATGAGRAACTGT-3') to amplify fragments of 297 base pairs (bp) of the CHD1-W genes and 259 bp of CHD1-Z. 25-µl PCR reactions were set up using MyFi DNA polymerase (Bioline) for 36 cycles, with an annealing temperature of 51.5°C. Bands were separated on a 2% agarose gel: one band at 259 bp indicated a male, and bands at 259 and 297 bp a female (Chu *et al* 2015).

Statistical methods

Comparative analysis of sexual variance in wing length, weight and a simple Body Condition Index (weight/wing length, BCI) was then undertaken (Labocha & Hayes 2012). Statistical analyses were conducted in R (R Development Core Team 2014) using RStudio (RStudio Team 2021). Outliers for wing length and weight were removed using the *rstatix* package (Kassambara 2022). Data were then subjected to Shapiro–Wilk tests for normal distribution, followed by Student's *t*-test to determine any significant differences in wing length, weight and BCI between the sexes.

Results

A total of 150 birds were captured, though two of these had to be excluded from the analyses as complete morphometric data had not been taken. Five DNA

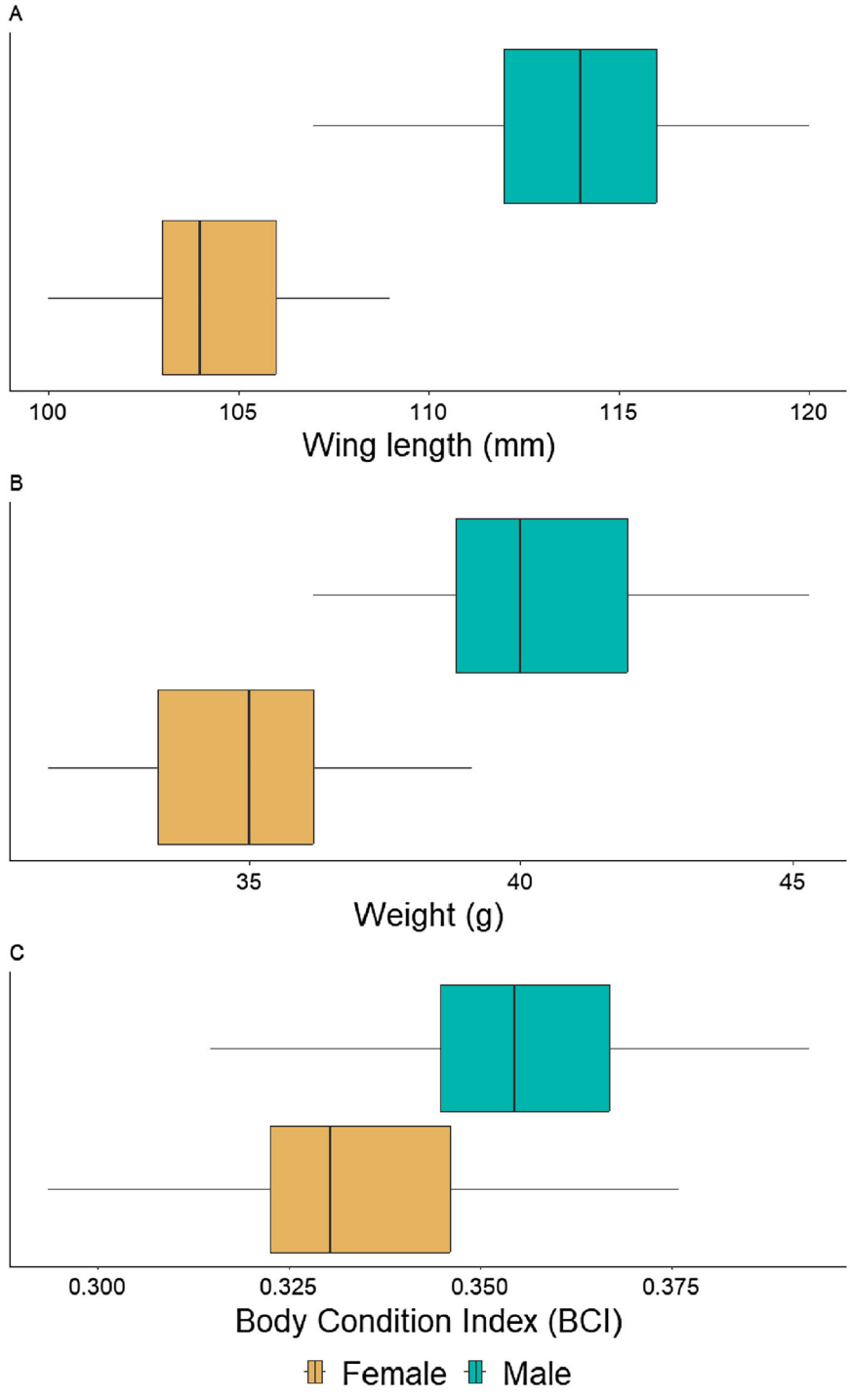

Figure 1. Boxplots illustrating sexual dimorphism in Skylarks for (a) wing length, (b) weight and (c) Body Condition Index.

samples failed to produce sufficient material to analyse and six birds were excluded as their measurements were clear outliers. The remaining sample size was 137 birds, comprising provisional sexing of 84 males, 33 females and 20 'undetermined'. DNA results determined that this sample actually contained 87 males and 50 females.

Female wing length was 100–109 mm (mean 104.4 mm) and male 107–120 mm (mean 113.8 mm). Weight ranges were 31.3–39.1 g (mean 34.88 g) for females and 36.2– 45.3 g (mean 40.52 g) for males. BCI values were 0.2935–0.3760 (mean 0.3342) for females and 0.3148–0.3929 (mean 0.3561) for males (Figures 1 & 2).

Morphometrics were normally distributed for wing length (male w = 0.9795, $P = 0.1835$; female w = 0.9668, $P = 0.1703$), weight (male w = 0.9796, $P = 0.1849$; female w = 0.9721, $P = 0.2807$) and BCI (male w =

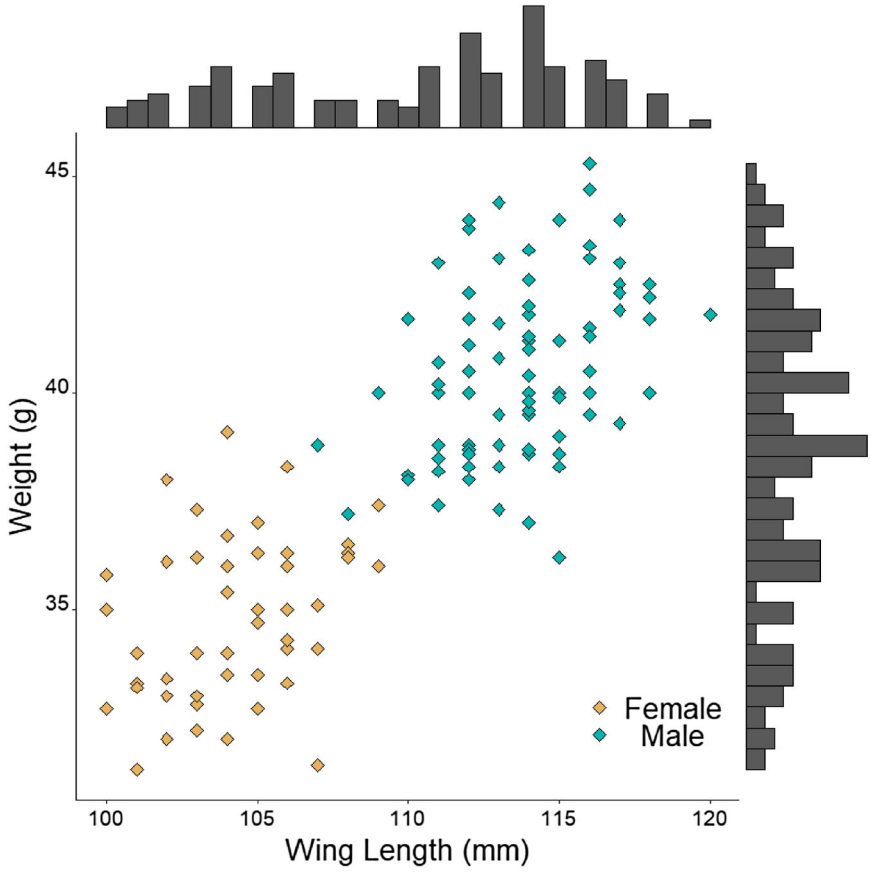

Figure 2. Scatter plot showing distributions of wing length (mm) and weight (g) for male and female Skylarks. Histograms for each variable are for the whole sample, showing a clearly bimodal distribution in wing length.

0.9731, $P = 0.0664$; female w = 0.9885, $P = 0.9041$). Subsequent student's *t*-test results showed that there are highly statistically significant differences between male and female Skylarks for wing length, weight and BCI (wing length $t(103.57) = -21.7$, $P < 0.0001$; weight $t(110.65) = -16.4$, $P < 0.0001$; BCI $t(101.92) = -7.23$, $P < 0.0001$).

Using the Netherlands winter parameters (Hegemann *et al* 2012) for wing length (individuals with wing length <106 mm being classified as females and those with a wing length >110 mm being classified as males), 15.8% of our UK birds lay between these two values and were classified as 'undetermined'. In our data set, provisional identifications of 96.6% of males and 65.3% of females were accurate, with no individuals being misidentified, resulting in a total accuracy of 84.2%. The range of overlap between male and female wing lengths in our data set, 107–109 mm, is similar to that for wintering birds trapped in the Netherlands (106–109 mm; Hegemann *et al* 2012). Moving our female maximum parameter from 106 mm to 107 mm, in line with our own data, would have increased our provisional identification of females to 81.6%, and our overall field accuracy to 91.2%, with no misidentifications, reducing 'undetermined' classifications to 8.8%.

Discussion

Analysis indicates that, in line with published data from the Netherlands (Hegemann *et al* 2012) and Scotland (Dougall 1997), wing length for Skylark in our data set is a significant predictor of sex, with males generally having longer wings than females, though a narrower margin of overlap between the sexes exists in our data. There is a natural relationship between wing length and weight, with larger birds weighing more; a logical inference from this would be that a significant corresponding difference in overall weight of female birds would also be present, and our data support that (Figure 2). Weight and wing length are proportional in both sexes, as demonstrated by an examination of Body Condition Index, with a mean BCI of 0.3342 for females 0.3561 for males.

Our data suggest that individual British Skylarks with wing lengths of 107–109 mm should not be sexed on this measurement alone, and that females will have wing lengths below these values and males above. Whilst

overlap between the sexes exists, and ringers cannot therefore always sex individual Skylarks in the hand with 100% confidence, our data show that accuracy in >90% of individuals can be reached using just wing length. We recommend that morphometric assessment guidelines should be specific to biogeographical populations, as earlier literature has demonstrated the geographical variability of European measurements (Svensson 1992, Campbell *et al* 2020, BTO 2023). The data presented here provide British-based ranges of wing measurements for the accurate sexing of most birds in the hand during winter though, especially in coastal and southern Britain, caution is needed due to the possible presence of winter immigrants. In summer, ringers should bear in mind that partly grown birds may not conform to these morphometric ranges.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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