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1 Comparative biometrics of British Marsh Tits *Poecile palustris* and Willow Tits *P. montana*

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15 Running head: Biometrics of Marsh Tits and Willow Tits

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29 ABSTRACT

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31 Biometrics are commonly used to compare bird species. For British Marsh Tits and Willow
32 Tits there are few biometric data from birds of known age and sex, despite their value for
33 population analyses in estimating the proportion of males and females in samples.
34 Comparing measurements between the two species could also aid identification and the
35 monitoring of these declining species in Britain.

36 We present biometrics for a large sample of British Marsh Tits of known age and sex,
37 and new data for British Willow Tits, which act as reliable reference material. Overall, adults
38 of both species were larger than first-years and males were larger than females, but not
39 among first-year Willow Tits. Marsh Tits were slightly larger and heavier than Willow Tits, but
40 Willow Tits had proportionately longer tails. Discriminant analyses produced new equations
41 for separating the species based on wing length and the measurement between the shortest
42 and longest tail feathers. Probabilities were generated for estimating Marsh Tit population
43 structure from samples of ringing data, but there was a greater overlap between sexes in
44 Willow Tit measurements. We conclude by discussing issues of measurement accuracy and
45 consistency in the collection and analysis of biometric data.

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47 Keywords: measurements, subspecies, taxonomy, wing length

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56 Descriptive taxonomy is a foundation of ornithology, comparing the physical measurements
57 of birds in the classification of species and subspecies. Standard reference works give some
58 biometrics for all bird species that occur regularly in Europe (e.g. for the tits (Paridae) see
59 Cramp & Perrins 1993, Glutz von Blotzheim & Bauer 1993, Gosler & Clement 2007). In
60 many instances, however, these texts also highlight a lack of data, even for some common
61 and widespread species. The bird measurements reported in these reference works typically
62 include wing length, which is the most consistent measurement of body size of passerines
63 (Gosler et al. 1998), and also tail length and body mass (weight). Sample sizes are often
64 small (fewer than 30), with little information on the age class of samples (adults or juveniles).
65 In addition, many such measurements were taken from skins, which can differ from those of
66 live birds due to shrinkage (Haftorn 1982, Svensson 1992).

67 Published reference data for biometrics are particularly inadequate for two formerly
68 common, but now much declined, species in Britain that are each represented by a localised
69 subspecies: the Marsh Tit *Poecile palustris dresseri* and Willow Tit *P. montana kleinschmidti*
70 (Broughton 2009). Both species are 'red-listed' and of conservation concern in Britain due to
71 respective declines in abundance of 74% and 92% between 1967 and 2013 (Robinson et al.
72 2015). Cramp & Perrins (1993) contains full summary statistics (mean and standard
73 deviation and/or range) for the length of the wing, tail, bill and tarsus of just 12 male and ten
74 female British Marsh Tits, all taken from skins, with the range of body weights for seven
75 birds. For British Willow Tits, Cramp & Perrins (1993) gives biometrics from the skins of up to
76 12 males and 6-50 females, but the average body weight of only 20 birds (all unsexed). For
77 both species no distinction was made between juveniles and adults, despite age being an
78 important factor in wing length (Broughton et al. 2008a, Hogstad 2011).

79 Further summary data of Marsh Tit wing lengths have since been published,
80 including 89 sexed birds from Nottinghamshire (du Feu & du Feu 2014) and 230 birds aged
81 and sexed birds in Cambridgeshire and Oxfordshire (Broughton et al. 2008a). Summary
82 statistics of wing and tail length have also been published for 1147 unsexed Marsh Tits from
83 across Britain, which demonstrated that there was no regional variation in these biometrics

84 (Broughton et al. 2016). For British Willow Tits, Scott (1999) reported wing length
85 measurements for nine birds and Robinson (2015) gives maximum chord values of 56-63
86 mm for 1418 birds in the BTO ringing database, with 59-66 mm for 2628 Marsh Tits, but du
87 Feu & du Feu (2014) have highlighted the large variation in this dataset which may indicate
88 some error.

89 Accurate reference biometrics for British Marsh and Willow Tits are particularly
90 valuable, as this species pair can be difficult to separate, even in the hand, and differences
91 in morphology may help ringers with identification (Redfern & Clark 2001, Broughton 2009).
92 Wing and tail lengths have been used to compare Marsh and Willow Tits of other
93 subspecies, either as a ratio ('tail/wing index', Eck 2006) or in discriminant analyses
94 (Markovets 1999). For British birds, Scott (1999) found that the wing lengths of 14 Marsh Tits
95 averaged significantly longer than nine Willow Tits, but there was substantial overlap.

96 Tail shape may also be helpful for identification, such as the 'tail tip difference'
97 between the tip of the shortest, outermost tail feathers (T6) and the longest inner feather that
98 forms the end of the tail (du Feu & du Feu 1996). This difference is generally greater for
99 Willow Tits than for Marsh Tits, but Scott (1999) reported an overlap of 36% for British birds
100 and Abe & Kurosawa (1984) found 54% overlap in Japan.

101 The number of tail feathers visible on the underside of the closed tail may also help
102 identification (du Feu & du Feu 1996, Scott 1999); on Marsh Tits the outermost T6 is the
103 shortest feather, with the longer T5 covering the inner feathers of almost equal length, giving
104 a 'tail tip score' of two feathers clearly visible on each side (T6 and T5). On Willow Tits the
105 tips of T6, T5, T4 and perhaps T3 are visible as a series of more evenly spaced steps (at
106 least 1 mm apart) on the underside of the tail, giving a tail tip score of 3 or 4. The error rate
107 for this test is unknown.

108 A further benefit of analysing the biometrics of British Marsh and Willow Tits is the
109 estimation of population structure. Proportions of male and female Marsh Tits of a given
110 wing length are reported in Broughton et al. (2008a), and such data could be used to
111 approximate the population composition in other samples of ringing data. This approach

112 could be used to identify any gender bias and offer clues to the causes of population
113 declines (Broughton & Hinsley 2015). Such information would be especially valuable for
114 British Willow Tits, for which data on sexing and population structure is generally lacking.

115 The aims of this paper were, firstly, to provide the most complete set of biometric
116 data yet available for live Marsh Tits of known age and sex, with a consistent data quality.
117 Additional biometric data were collected for British Willow Tits, including birds of known age
118 and sex, to test whether measurements are useful for sexing birds. We also compare
119 biometrics between the two species to test for differences which may aid ringers and
120 analysts with identification.

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123 METHODS

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125 **Marsh Tit biometrics**

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127 A total 559 handlings of 448 individual Marsh Tits of known age and sex were made during
128 all months of the year in 1993-2016. These comprised 386 birds from Cambridgeshire
129 (Monks Wood: 52°24'N 0°14'W, and five woods within 5 km), with 34 birds from Oxfordshire
130 (Wytham Woods: 51°46'N 1°20'W, Bagley Wood: 51°43'N 1°16'W), and 28 birds from
131 Suffolk (Bradfield Woods: 52°10'N 0°82'E).

132 Birds were caught in baited traps or mist-nets and ringed with a BTO alloy ring and a
133 unique combination of colour rings (Broughton et al. 2008a). Ageing as first-years (EURING
134 codes 3J, 3 or 5) or adults (EURING codes 4 or 6) was based on prior ringing history, extent
135 of moult, and the presence/absence of distinctive juvenile greater coverts or tail feathers
136 (Amann 1980, Broughton 2010). Sexing was based on behaviour observed during fieldwork
137 (including the use of playback), such as 'courtship feeding', singing and aggressive 'gargle'
138 calls (males), and food soliciting, nest-building and incubation (females), or being paired with
139 a territorial bird of known sex (Broughton et al. 2008a, 2010). No sexing decisions needed to

140 be reversed on later observation (e.g. no birds recorded as singing 'males' were later found
141 as breeding females).

142 This behavioural sexing method was also validated against DNA sexing of 55 birds
143 based on blood samples taken under a Home Office licence during 2008-2011. Total
144 genomic DNA was isolated from whole blood which was taken from the brachial vein using a
145 capillary tube and archived on Whatman FTA Classic Cards (GE Healthcare Life Sciences,
146 Maidstone). A single 1.25 mm disc was cut from the cards using a Uni-Core punch
147 (Whatman) and DNA extracted with FTA purification reagent (Whatman) and the ZR DNA-
148 Card Extraction Kit (Zymo Research, Irvine, California) according to the manufacturer
149 instructions. The sex identification test employed the P8 (5'-CTCCCAAGGATGAGRAAYTG-
150 3') and P2 (5'-TCTGCATCGCTAAATCCTTT-3') primers (Griffiths et al. 1998) and PCR
151 amplification was conducted in a total volume of 10 μ L using the PCR conditions reported by
152 Griffiths et al. (1998).

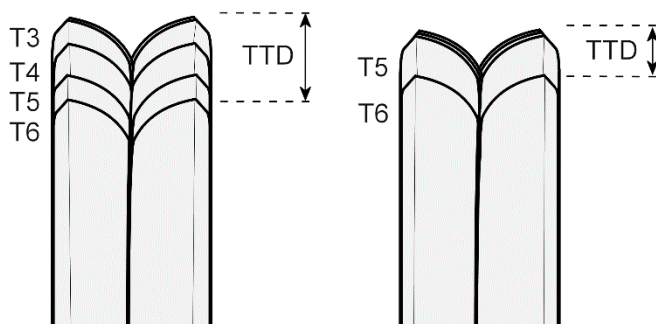
153 Measurements taken during handling of full-grown birds included wing length
154 (maximum chord to 1 mm precision), tail length (to 0.5 mm), the 'tail tip difference'
155 measurement between the ends of the longest and shortest tail feathers (to 0.5 mm, Fig. 1),
156 'tail tip score' as the number of tail feather tips visible \geq 1mm apart on the underside of the
157 closed tail (one side only, Fig. 1), body mass (to 0.1 g using a calibrated Pesola spring
158 balance or electronic balance), and maximum tarsus length (to 0.1 mm). Standard
159 measurements were taken as described by Redfern & Clark (2001). Sample sizes differed
160 for each variable as not all measurements were taken in all years. The dataset included 230
161 wing length measurements from 182 Marsh Tits previously reported in Broughton et al.
162 (2008a), and additional measurements of tarsus were available from 30 birds in 2015 that
163 were unsexed.

164 A total of 111 Marsh Tits measured originally as first-years and later as adults were
165 included in both age classes, because wing lengths differ significantly between these
166 plumages (Broughton et al. 2008). For each individual, only the first measurements taken in
167 each age class were included in analyses, and birds with obviously abraded flight feathers

168 (e.g. broken tips) were excluded. Measurements were highly consistent between sites, with
169 88% collected by a single experienced ringer (RKB), and comparisons between other ringers
170 (mostly trainers) were consistent.

171

172 **Figure 1.** Schematic of typical tail morphology of Willow Tit (left) and Marsh Tit (right), with
173 the end of the tail viewed from the underside. Measurements taken during fieldwork included
174 ‘tail tip difference’ (TTD), which was the measurement between the shortest tail feather (T6)
175 and the tip of the tail. ‘Tail tip score’ was recorded as the number of tail feather tips clearly
176 visible (emergent $\geq 1\text{mm}$ beyond other feathers) on one side of the underside of the closed
177 tail. In the schematic, the tail tip score for Willow Tit is 4 (T3, T4, T5 and T6) and for Marsh
178 Tit the score is 2 (T5 and T6).



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182 Willow Tit biometrics

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184 A total of 179 handlings of 149 individual Willow Tits were made during 2007-2015 at several
185 locations across England, including Berkshire (Combe Wood: 51°20'N 1°29'W, 17 birds),
186 Lincolnshire (Market Rasen area: 53°21'N 0°14'W, 48 birds), Cheshire (Woolston Eyes:
187 53°23'N 2°31'W, 38 birds, and two birds at other sites), Greater Manchester and adjoining
188 areas of Lancashire and Merseyside (within 9 km of Wigan: 53°32'N 2°37'W, 34 birds) and
189 Yorkshire (Potteric Carr: 53°30'N, 1°6'W, Fairburn Ings: 53°44'N 1°19'W and Allerthorpe:
190 53°55'N 0°48'W, ten birds).

191 Willow Tits were caught in all months of the year using cage traps or mist-nets and
192 fitted with a BTO alloy ring, with Berkshire birds also being colour-ringed. As with Marsh Tits,
193 ageing as first-years or adults was based on ringing history and plumage criteria (Laaksonen
194 & Lehtikoinen 1976, Broughton 2010), and 37 birds were sexed using behaviour or the
195 appearance of a brood patch (BP) or cloacal protuberance (CP) during the breeding period
196 (April-early July).

197 Measurement protocols followed those for Marsh Tits, with 20 birds (six of which
198 were sexed) originally caught as first-years and later as adults being included in analyses of
199 both age classes. Measurements taken comprised wing length, tail length, tail tip difference
200 and tail tip score (Fig. 1), and body mass. Data were pooled from 11 ringers, including six
201 trainers, with cross-checking for consistency between one author (RKB) and seven of the
202 contributing ringers or their trainers.

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205 **Statistical analyses**

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207 Biometric summary statistics for both species included the variables given by Cramp &
208 Perrins (1993), to allow for direct comparisons. Analyses of differences between age and
209 sex classes of Marsh Tits superseded previous work in Broughton et al. (2008) due to the
210 availability of much larger sample sizes and range of biometrics. We used non-parametric
211 tests throughout to accommodate some non-normal distributions (assessed using Anderson-
212 Darling tests) and to maintain consistency between all tests comparing groups. As birds
213 were caught and weighed throughout the day, reflecting the activity of typical ringers across
214 Britain, we made no adjustments to mass to account for time of day (as per Robinson 2015).

215 Marsh Tit wing lengths for birds of known age and sex were used in binomial logistic
216 regression, applying a logit link function and using age class as a factor. This was in order to
217 provide probability estimates for sexing birds in other samples, to indicate the sex of
218 unknown individuals or the proportion of males and females in different populations.

219 Willow Tit biometrics were compared between age and sex classes, and logistic
220 regression was attempted in order to produce probability estimates for sexing birds using
221 wing length, as per Marsh Tits. Wing length, mass and tail length measurements were
222 compared with those of Marsh Tits, including use of discriminant analysis with species as the
223 grouping factor. To investigate differences in tail shape, we calculated and compared the
224 tail/wing index for Marsh and Willow Tits (Eck 2006), and the tail tip difference and tail tip
225 score to test the separation methods of Redfern & Clark (2001) and du Feu & du Feu (1996).

226 All analyses were performed in Minitab 16, and usage followed methods in Dytham
227 (2011). In particular, discriminant analysis in Minitab produces a linear discriminant function
228 for each group, with the accuracy of classification given as a proportion of the sample.
229 Unknown individuals can be classified with this reported accuracy by applying the function
230 for each group and assigning the individual to the group which gives the highest value
231 (Dytham 2011).

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234 RESULTS

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237 **Marsh Tit biometrics**

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239 Summary statistics for Marsh Tit biometrics are given in Table 1, with results of statistical
240 comparisons between sexes. The DNA sexing of 55 birds (28 females and 27 males) agreed
241 completely with the sex assigned from behavioural observations in the field, and so all birds
242 were considered to be sexed correctly using the behaviour method.

243 Table 1 shows that the wing and tail lengths of males were significantly longer than
244 those of females in all age classes, and adults of both sexes had longer wings than first-
245 years (Mann-Whitney tests, males: $U = 29905.5$, $P < 0.001$; females: $U = 17057.0$, $P <$
246 0.001). Adults also had longer tails than first-years (males: $U = 3421.5$, $P < 0.001$; females U

247 = 3776.5, $P < 0.001$). Individuals with longer wings generally had longer tails (males:
248 Spearman's rank-order correlation, $r_s = 0.76$, $P < 0.001$; females: $r_s = 0.59$, $P < 0.001$).

249 Male Marsh Tits were typically heavier than females (Table 1), but adults were not
250 heavier than first-years (males: $U = 21386.0$, $P = 0.947$; females $U = 13906.5$, $P = 0.948$).
251 Within all age and sex classes, heavier birds tended to have longer wing lengths (all groups:
252 $r_s = 0.21-0.37$, $P < 0.001-0.040$).

253 Tarsus measurements of 59 birds (including 30 unsexed) showed a narrow range of
254 values (17.8-19.4 mm, median = 18.6, mean = 18.5 ± 0.4 s.d.). The median tarsus length of
255 males was longer than that of females (Table 1), but there was no correlation between
256 tarsus and wing length in either sex ($r_s = -0.02$ and 0.25 , $P = 0.942$ and 0.456 for males and
257 females respectively).

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260 **Estimating Marsh Tit population structure**

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262 For adult Marsh Tits, a wing length division of 63 mm or less for females and 64 mm or more
263 for males accurately sexed 96.7% of birds (99.1% of 107 females and 94.4% of 144 males).
264 For first-years a division of ≤ 62 mm for females and ≥ 63 mm for males assigned 93.8% of
265 birds to the correct sex (97.2% of 141 females and 91.0% of 167 males). Combining these
266 statistics for both age classes sexed all 559 Marsh Tits with an overall accuracy of 95.0%,
267 using the different wing length divisions for sexing adults and first-years.

268 The full output of the logistic regression models is given in Appendix 1, showing
269 highly significant relationships between wing length, age and sex in Marsh Tits. Table 2
270 shows the probability of being male for an adult or first-year bird of a given wing length, with
271 associated confidence intervals. Table 2 also gives the wing length frequencies and
272 probability estimates for sexing Marsh Tits where age was unspecified (both age classes
273 combined), which would correspond to unknown birds where age was not determined (e.g. a
274 EURING age code of 2).

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Willow Tit biometrics

The wings and tails of male Willow Tits tended to be longer than those of females, although not significantly so for first-years, and body weights showed no significant difference between the sexes in any age class (Table 1). Wing lengths showed a substantial overlap between the sexes (Table 3, age classes combined due to small sample sizes), and so logistic regression of the wing lengths against sex did not produce good results, with a poor goodness-of-fit (deviance chi-square = 21.8, df = 6, $P = 0.001$) and poor measures of association (73.1% concordant pairs between response variable and predicted probabilities). As such, this approach was not pursued (full model output not shown).

When comparing biometrics of first-year and adult Willow Tits (sexes combined, Table 4) the adults had significantly longer wings ($U = 7183.5$, $P = 0.048$) and tails ($U = 4101.5$, $P = 0.012$) than younger birds, with a near-significant difference in body mass ($U = 5089.5$, $P = 0.051$) following the same trend, but the differences were small.

Differences between Marsh and Willow Tits

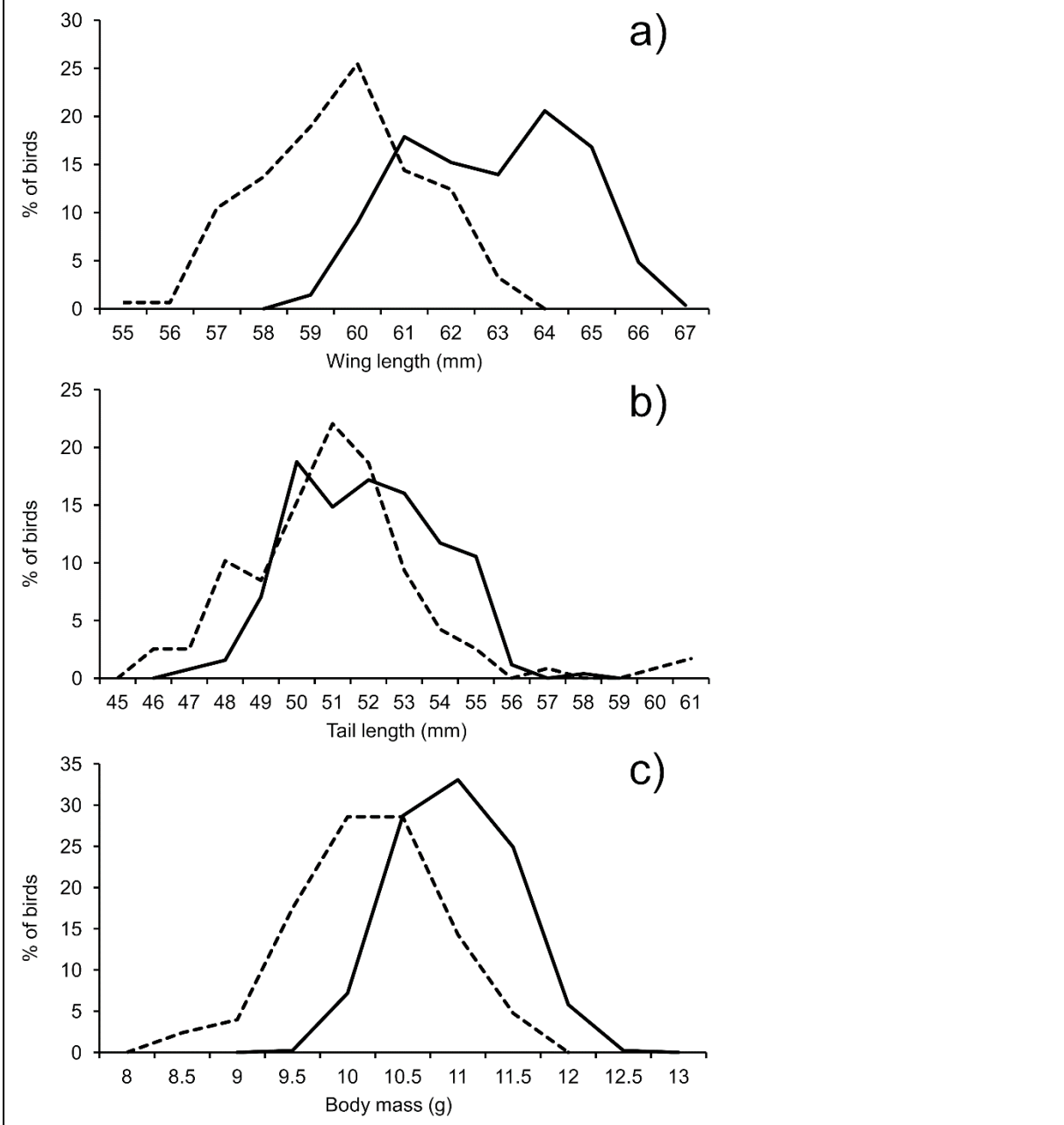
Similar proportions of males and females in samples of both species allowed sexes to be combined for analyses. These showed that Marsh Tits tended to have longer wings than Willow Tits, with proportionately shorter tails (tail/wing index), and were also heavier (Table 4). Marsh Tit wing lengths showed a strongly bimodal distribution, compared to unimodal Willow Tits (Fig. 2a). This reflected the greater overlap in wing lengths between male and female Willow Tits compared to Marsh Tits in both age groups.

Figure 2 illustrates the substantial overlap in wing length, tail length (Fig. 2b) and body mass (Fig. 2c) between species. Nevertheless, the wings of the smallest Willow Tits

303 were 4 mm shorter than the smallest Marsh Tits, and they weighed 1.2 g less, whilst wing
 304 lengths of the largest Marsh Tits were 4 mm longer than Willow Tits and they were 0.7 g
 305 heavier, although the longest tails of Willow Tits were 3.5 mm longer than Marsh Tits (Table
 306 4).

307

308 **Figure 2.** Biometrics of British Willow Tits (dotted lines) and Marsh Tits (solid lines) showing
 309 distributions of: a) wing lengths of 153 Willow Tits and 559 Marsh Tits, b) tail lengths of 118
 310 Willow Tits and 253 Marsh Tits, and c) body mass of 124 Willow Tits and 477 Marsh Tits.



311

312 There were significant differences in tail shape between the two species, with a
313 greater 'tail tip difference' for Willow Tits of all ages, compared to Marsh Tits (Table 4).
314 Despite this, the measurement range of tail tip difference overlapped for 71% of Marsh Tits
315 and 79% of Willow Tits. Nevertheless, for first-year birds, a division of 4 mm or more for
316 Willow Tits and 3.5 mm or less for Marsh Tits correctly identified 96% of both species. For
317 adults, 95% of Willow Tits were correctly identified with a tail tip difference of ≥ 5 mm, but
318 only 72% of Marsh Tits with a measurement of ≤ 4.5 mm. Combining the statistics for both
319 age classes gave an overall accuracy of 89% for the tail tip difference method (44 errors
320 from 389 birds).

321 Willow Tits had significantly higher 'tail tip scores' than Marsh Tits, with generally
322 more tail feathers visible on either side of the closed tail (underside) in all age classes (Table
323 5). Adults of both species had significantly higher scores than first-years (Willow Tit: $U =$
324 264.0 , $P = 0.015$; Marsh Tit, $U = 5043.0$, $P < 0.001$), but the range of scores overlapped
325 completely at 2-5 for each species.

326 Discriminant analysis for separating Marsh and Willow Tits was most successful
327 when using wing length and tail tip difference as predictor variables, assigning almost all
328 birds to the correct species, particularly when adults and first-years were treated separately
329 (Table 5).

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331

332 DISCUSSION

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334

335 **Marsh Tit biometrics**

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337 The results demonstrated that male Marsh Tits were generally larger and heavier than
338 females in both age classes, and that adults were larger than juveniles. Individuals with long
339 wings also had long tails and weighed more than smaller birds. These measurements

340 represent the largest biometric dataset available for live Marsh Tits of the British subspecies,
341 grouped by age and sex. The data come from a very small number of ringers with a high
342 degree of consistency, and with accurate ageing and sexing derived from monitored
343 populations in three English localities.

344 These results offer an alternative to the small sample of measurements from skins in
345 Cramp & Perrins (1993), and also the measurements pooled from a large number of ringers
346 in the BTO database (Robinson 2015), which shows unusual variability (du Feu & du Feu
347 2014). As it has been shown that there is no regional variation in Marsh Tit biometrics across
348 Britain (Broughton et al. 2016), our dataset can be considered as representative of this
349 subspecies.

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352 **Willow Tit biometrics**

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354 Although our sample sizes for Willow Tits were relatively small, the data represent
355 the first material available for the British subspecies that is grouped by age and sex, and the
356 first statistical comparison between these groups. These tests revealed that, like Norwegian
357 Willow Tits (Haftorn 1982, Hogstad 2011) and British Marsh Tits (this study), adult male
358 Willow Tits tended to have longer wings and tails than adult females, but, unexpectedly, this
359 was not the case amongst first-years or for body mass in any age class. More conforming to
360 expectations, adults had longer wings and tails than first-years overall, as in Norway (Haftorn
361 1982, Hogstad 2011) and for British Marsh Tits (this study).

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363

364 **Estimating population structure**

365

366 Based on the large sample of Marsh Tit wing lengths in this study, the sexing probabilities
367 derived from logistic regression could be used to estimate the sex ratio in other ringing data.

368 This could be achieved by simply calculating the percentage of birds of each wing length by
369 the values for the appropriate age class in Table 2 to estimate the percentage of males in a
370 sample. For example, of 125 first-years with a wing length of 61 mm, three birds could be
371 expected to be male (2.4% of 125 = 3), leaving 122 females, whilst all adults of 60 mm
372 would be expected to be female (0.0% probability of being male), and 99.8% of birds of 65
373 mm where age was unknown could be expected to be male.

374 As previous work has shown no variation in Marsh Tit wing length across the British
375 range (Broughton et al. 2016), this probability approach could be applied to samples from
376 any location. The associated confidence intervals indicate the precision of the estimates, and
377 could be used to produce upper and lower estimates. This could test for a gender bias in
378 other populations, which may result from differing survival rates between the sexes and
379 could be a factor in the species' decline in abundance (Broughton & Hinsley 2015).

380 A caveat with using the sexing probabilities from Table 2 is that they are based on a
381 large and relatively balanced sample of birds (56% males, 44% females). Applying these
382 probabilities to very small samples from other populations could be misleading if one sex
383 happens to be grossly over-represented by chance or capture method (e.g. catching at
384 nestboxes, which may be heavily biased towards females). Larger samples, and a random
385 sampling technique, will produce more reliable estimates.

386 For sexing individual Marsh Tits, the probabilities produced by the logistic regression
387 models had very narrow confidence intervals for most wing lengths, suggesting that most
388 individuals could be sexed with a very high degree of reliability (greater than 95%). For
389 example, Table 2 indicates that a first-year Marsh Tit with a wing length of 64 mm would
390 have a 99.7% probability of being male (with a confidence of 99.1-99.9%), whilst a bird of 59
391 mm wing length would be essentially certain to be female. The confidence intervals suggest,
392 however, that greater caution is required when sexing individuals with wing lengths of 62 mm
393 (first-years) or 63 mm (adults), which have much wider confidence intervals.

394 When using the more basic method of sexing Marsh Tits, i.e. the simple cut-off value
395 for wing length, treating adults and first-years separately was an improvement on the method

396 of King & Muddeman (1995) and Broughton et al. (2008). These earlier studies
397 recommended separating females and males of all ages with a wing length division of 62/63
398 mm. Applied to our sample, this method still accurately sexed 93.4% of 559 birds, but by
399 using different divisions for adults (63/64 mm) and first-years (62/63 mm) we improved the
400 accuracy slightly to 95.0%. This compares to 93.5% accuracy for 230 birds in Broughton et
401 al. (2008a), 92% for 89 birds in du Feu & du Feu (2014) and 96% for 50 birds in King &
402 Muddeman (1995). The probabilities given by the logistic regression models offer an
403 advantage by providing confidence intervals as a measure of the reliability of sexing an
404 individual of a given wing length, which is particularly valuable for birds close to the cut-off
405 points.

406 The large degree of overlap between male and female Willow Tits in our relatively
407 small samples meant that biometric sexing was unreliable for this species. Only 74% of birds
408 were assigned to the correct sex if using the best wing length division of ≤ 60 mm for
409 females and ≥ 61 mm for males (94% of females but only 58% of males). These results were
410 surprising, as Markovets (1992) in Russia and Hogstad (2011) in Norway accurately sexed
411 99-100% of nominate race Willow Tits using a simple wing length division (63/64 mm in
412 Russia, 64/65 mm in Norway). Haftorn (1982), also in Norway, sexed 86% of Willow Tits
413 using wing length.

414 Although Willow Tits can be sexed by assessing the brood patch and cloacal
415 protuberance, this method is only valid in spring (April-June, Markovets 1992) and can be
416 unreliable for non-breeding birds (pers. obs.). The ability to sex Willow Tits using biometrics
417 would be a valuable tool throughout the year, as, remarkably, Haftorn (1982) used
418 biometrics to show that a large proportion of skins in the University of Trondheim collection
419 had been wrongly sexed during autopsy. This shows the scientific value of recording
420 biometric measurements to validate other sexing methods. A larger sample of wing lengths
421 from birds of known age and sex could help to confirm whether a greater overlap between
422 the sexes is a feature of British Willow Tits, which may indicate a greater constraint on body
423 size in Britain compared to elsewhere.

424

425

426 **Separating Marsh and Willow Tits**

427

428 Our results show that British Marsh Tits are slightly more robust than Willow Tits, being
429 larger and heavier (by a median 5-7%) with proportionately shorter tails. Tail shape was a
430 useful feature for separating the species, particularly the measurement of ‘tail tip difference’
431 between the shortest and longest tail feathers, as proposed by Amann (1980) for Swiss
432 birds, Abe & Kurosawa (1984) in Japan, and du Feu & du Feu (1996) and Scott (1999) in
433 Britain.

434 Our results supported the findings of these earlier studies by showing that most
435 British Marsh Tits (97%) had a tail tip difference of ≤ 5 mm, with almost all Willow Tits (98%)
436 being ≥ 4 mm, but the overlap of 4-5 mm included a large proportion of birds in our sample.
437 The measured difference was generally greater for adults than first-years, as was also
438 shown by Amann (1980). Therefore, by treating the age classes separately we found that
439 most first-years (96%) could be separated with a sharp division of ≤ 3.5 mm for Marsh Tits
440 and ≥ 4 mm for Willow Tits. Adults showed more overlap than first-years, particularly Marsh
441 Tits, but the division of 4.5/5 mm still identified most birds correctly.

442 Tail tip score, derived from the number of feather tips visible on one or other side of
443 the closed tail, viewed on the underside, was less reliable than tail tip difference due to a
444 significant proportion of adult Marsh Tits and some first-years having a graduated tail that
445 was similar to a typical Willow Tit. Some birds also showed asymmetry in the tail, with one
446 T6 feather noticeably shorter than the other. In these cases measurements taken from the
447 longest T6 feather are recommended as a conservative approach.

448 The discriminant function using tail tip difference and wing length as predictors (Table
449 6) gave the best results for separating Marsh and Willow Tits with two simple
450 measurements, identifying 95-99% of birds of either species (according to age class). The
451 presence/absence of a pale mark at the base of the bill has previously been shown to

452 identify 99% of Marsh Tits and 94% of Willow Tits (Broughton et al. 2008b), and for
453 ambiguous birds the discriminant function based on wing length and tail tip difference now
454 offers an alternative of a similar accuracy. If these two tests are used together then the
455 chances of misidentification seem remote, but where they contradict then other supporting
456 features can be called upon, such as tail tip score and cheek pattern (Broughton 2009).

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458

459 **Using biometrics in analyses**

460

461 The value of biometrics for identifying or sexing birds, or estimating population structure,
462 ultimately relies on the accuracy of the data that are collected, as well as variation among
463 the birds themselves (du Feu & du Feu 2014). For species such as Marsh and Willow Tits,
464 where errors of 0.5-1 mm could make a difference in classification analyses, it is essential
465 that data collection and recording are consistently accurate.

466 When detailed field studies on reasonable samples of Marsh Tits have been carried
467 out in different parts of England, and measurements are consistent within each group of
468 ringers, then the results have been very similar: 92-96% of birds are sexed accurately using
469 a simple wing length division of 62/63 mm (King & Muddeman 1995, Broughton et al. 2008,
470 du Feu & du Feu 2014, and the present study). It was highlighted by du Feu & du Feu
471 (2014), however, that in the BTO ringing database only 71% of sexed Marsh Tits fitted this
472 pattern (Robinson 2015). This means that almost a third of the birds in the national dataset
473 would have been assigned to the wrong sex by the 62/63 mm wing length division,
474 compared to an error rate of just 4-8% in the detailed studies. As there is no regional
475 variation in Marsh Tit biometrics across Britain (Broughton et al. 2016), the most likely
476 explanation for the large discrepancy in the BTO database is variation in data quality. This
477 could result from measurement variability within or between ringers, or incorrect sexing from
478 misinterpretation of brood patch or cloacal protuberance (Redfern & Clark 2001).

479 Significant variability in data quality within the national dataset could undermine the
480 use of Marsh Tit biometrics for the analysis of population structure. Similar issues may also
481 exist for Willow Tit data, but a greater overlap in biometrics between the sexes could make
482 these harder to detect. Although it may be possible to identify the more reliable series of
483 ringing records for these species, perhaps by extracting those from sources of known
484 reliability or by testing the measurement repeatability among recapture records, this case
485 study underlines the need for accurate data collection in ringing schemes.

486

487

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615 **Table 1.** Biometric summary statistics (mean \pm s.d. (median, range), *n*) for British Marsh Tits
 616 and Willow Tits by sex and age (FY = first-years, Ad = adults, All = ages classes combined),
 617 with comparisons of medians by Mann-Whitney *U*-tests.

	Female	Male	<i>U</i>	<i>P</i>
<i>Marsh Tit wing length (mm)</i>				
FY	60.8 \pm 0.8 (61.0, 59.0-63.0), 141	63.7 \pm 0.9 (64.0, 60.0-66.0), 167	10431.0	<0.001
Ad	61.7 \pm 1.0 (62.0, 59.0-65.0), 107	64.8 \pm 0.9 (65.0, 61.0-67.0), 144	5997.0	<0.001
All	61.2 \pm 1.0 (61.0, 59.0-65.0), 248	64.2 \pm 1.1 (64.0, 60.0-67.0), 311	32823.5	<0.001
<i>Marsh Tit tail length (mm)</i>				
FY	49.9 \pm 1.4 (50.0, 46.5-55.5), 63	52.3 \pm 1.4 (52.0, 49.0-57.5), 71	2468.5	<0.001
Ad	50.8 \pm 1.1 (51.0, 49.0-53.5), 53	53.8 \pm 1.0 (54.0, 51.0-56.0), 69	1539.5	<0.001
All	50.3 \pm 1.3 (50.0, 46.5-55.5), 116	53.1 \pm 1.5 (53.0, 49.0-57.5), 140	8188.5	<0.001
<i>Marsh Tit body mass (g)</i>				
FY	10.4 \pm 0.3 (10.4, 9.6-11.3), 125	11.1 \pm 0.4 (11.1, 9.9-12.1), 152	9577.0	<0.001
Ad	10.4 \pm 0.4 (10.5, 9.5-11.2), 96	11.1 \pm 0.4 (11.1, 10.0-11.9), 129	6087.5	<0.001
All	10.4 \pm 0.3 (10.4, 9.5-11.3), 221	11.1 \pm 0.4 (11.1, 9.9-12.1), 281	30878.0	<0.001
<i>Marsh Tit tarsus length (mm)</i>				
All	18.4 \pm 0.4 (18.4, 17.8-18.9), 11	18.8 \pm 0.3 (18.7, 18.4-19.4), 18	102.5	<0.001
<i>Willow Tit wing length (mm)</i>				
FY	59.3 \pm 2.1 (59.5, 56-63), 8	60.0 \pm 1.6 (60.0, 58-62), 10	65.5	0.364
Ad	58.9 \pm 1.4 (59.5, 57-60), 10	61.2 \pm 1.5 (62.0, 58-63), 14	73.0	0.002
All	59.1 \pm 1.7 (59.5, 56-63), 18	60.7 \pm 1.6 (61.0, 58-63), 24	272.0	0.003
<i>Willow Tit tail length (mm)</i>				
FY	50.3 \pm 1.8 (51.0, 48.0-52.5), 6	51.7 \pm 3.9 (50.0, 49.0-60.0), 7	39.0	0.718
Ad	50.0 \pm 2.5 (50.0, 46.0-53.0), 7	52.8 \pm 1.9 (53.0, 49.0-55.0), 11	42.0	0.028
All	50.1 \pm 2.1 (51.0, 48.0-53.0), 13	52.4 \pm 2.8 (52.0, 49.0-60.0), 18	153.0	0.028
<i>Willow Tit body mass (g)</i>				
FY	9.8 \pm 0.6 (9.9, 8.9-10.4), 7	9.9 \pm 0.5 (9.7, 9.2-10.6), 9	56.0	0.750
Ad	10.1 \pm 0.8 (10.2, 8.5-11.0), 8	10.2 \pm 0.6 (10.1, 9.3-11.2), 12	95.5	0.831

All	9.9 ± 0.7 (9.9, 8.5-11.0), 16	10.1 ± 0.6 (9.9, 9.2-11.2), 21	293.5	0.759
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644 **Table 2.** Frequency distribution of Marsh Tit wing lengths for first-years, adults and all birds
 645 (age unspecified), showing the number of females (F) and males (M) of a given wing length
 646 in each category. The probability of being male ($P(M)$) for an individual of a given wing
 647 length is shown as a percentage, with the associated 95% confidence intervals (CI).

Wing (mm)	First-years				Adults				All birds			
	F (n)	M (n)	$P(M)$ (%)	95% CI	F (n)	M (n)	$P(M)$ (%)	95% CI	F (n)	M (n)	$P(M)$ (%)	95% CI
59	6	0	0.0	0.0	2	0	0.0	0.0	8	0	0.0	0.0
60	40	1	0.1	0.0-0.3	9	0	0.0	0.0	49	1	0.1	0.1-0.2
61	70	0	2.4	0.8-7.2	29	1	0.1	0.1-0.2	99	1	1.5	0.9-2.4
62	21	14	37.3	15.7-65.5	50	0	2.4	1.3-4.4	17	14	17.8	11.8-25.9
63	4	51	93.5	82.0-97.9	16	7	37.3	24.2-52.5	20	58	75.7	65.8-83.5
64	0	75	99.7	99.1-99.9	0	40	93.5	88.6-96.4	0	115	97.8	96.5-98.6
65	0	24	100.0	100.0	1	69	99.7	99.5-99.8	1	93	99.8	99.8-99.9
66	0	2	100.0	100.0	0	25	100.0	100.0	0	27	100.0	100.0
67	0	0	100.0	100.0	0	2	100.0	100.0	0	2	100.0	100.0

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657 **Table 3.** Frequency distribution of wing lengths for 18 female and 24 male Willow Tits, with
658 the percentage of birds of each sex for a given wing length.

Wing length (mm)	Female		Male	
	<i>n</i>	%	<i>n</i>	%
56	1	100.0	0	0.0
57	4	100.0	0	0.0
58	0	0.0	4	100.0
59	4	80.0	1	20.0
60	8	62.0	5	38.0
61	0	0.0	4	100.0
62	0	0.0	8	100.0
63	1	33.0	2	67.0

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674 **Table 4.** Comparisons between the biometrics of British Marsh Tits and Willow Tits, with
675 results of Mann-Whitney *U*-tests of medians. Birds are grouped by age class (FY = first-year,
676 Ad = adult, All = age classes combined). Tail tip difference refers to the measurement
677 between the tips of the shortest and longest tail feathers, and tail tip score refers to the
678 number of tail feather tips visible on either side of the underside of the closed tail.

	Marsh Tit	Willow Tit	<i>U</i>	<i>P</i>
<i>Wing length (mm)</i>				
FY	62.4 ± 1.7 (63.0, 59.0-66.0) 308	59.5 ± 1.7 (60.0, 55.0-63.0) 102	75296.0	< 0.001
Ad	63.5 ± 1.8 (64.0, 59.0-67.0) 251	60.1 ± 1.6 (60.0, 57.0-63.0) 51	43282.5	< 0.001
All	62.9 ± 1.8 (63.0, 59.0-67.0) 559	59.7 ± 1.7 (60.0, 55.0-63.0) 153	233048.5	< 0.001
<i>Tail length (mm)</i>				
FY	51.2 ± 1.8 (51.0, 46.5-57.5) 134	50.6 ± 2.6 (50.5, 45.0-61.0) 77	15133.5	0.028
Ad	52.5 ± 1.9 (53.0, 49.0-56.0) 122	51.6 ± 2.5 (52.0, 46.0-61.0) 40	10634.0	0.007
All	51.8 ± 2.0 (52.0, 46.5-57.5) 256	50.9 ± 2.6 (51.0, 45.0-61.0) 117	51768.0	< 0.001
<i>Tail/wing index</i>				
FY	0.82 ± 0.02 (0.82, 0.77-0.91) 134	0.85 ± 0.04 (0.85, 0.77-1.00) 76	10998.0	< 0.001
Ad	0.83 ± 0.01 (0.83, 0.78-0.86) 122	0.86 ± 0.03 (0.85, 0.80-1.00) 39	8200.0	< 0.001
All	0.83 ± 0.02 (0.83, 0.77-0.91) 256	0.85 ± 0.03 (0.85, 0.77-1.00) 115	38354.5	< 0.001
<i>Tail tip difference (mm)</i>				
FY	2.8 ± 0.6 (3.0, 1.5-5.0) 200	5.2 ± 1.0 (5.0, 3.0-8.0) 76	20320.5	< 0.001
Ad	4.2 ± 0.9 (4.0, 2.0-6.0) 71	6.2 ± 1.1 (6.0, 4.0-9.0) 42	2793.5	< 0.001
All	3.2 ± 0.9 (3.0, 1.5-6.0) 271	5.6 ± 1.2 (5.5, 3.0-9.0) 118	38624.0	< 0.001
<i>Tail tip score</i>				
FY	2.1 ± 0.3 (2, 2-3) 107	3.4 ± 0.7 (4, 2-4) 23	5967.5	< 0.001
Ad	2.9 ± 0.7 (3, 2-5) 47	4.3 ± 0.5 (4, 4-5) 8	1156.0	< 0.001
All	2.4 ± 0.6 (2, 2-5) 154	3.6 ± 0.7 (4, 2-5) 29	12460.5	< 0.001
<i>Body mass (g)</i>				
FY	10.8 ± 0.5 (10.7, 9.6-12.1) 277	9.9 ± 0.6 (9.9, 8.3-11.4) 86	58897.5	< 0.001
Ad	10.8 ± 0.5 (10.8, 9.5-11.9) 225	10.2 ± 0.6 (10.2, 8.5-11.4) 40	32468.5	< 0.001

All	10.8 ± 0.5 (10.8, 9.5-12.1) 502	10.0 ± 0.6 (10.0, 8.3-11.4) 126	178884.5	< 0.001
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705 **Table 5.** Linear discriminant function for separating Marsh Tits and Willow Tits of different
706 age classes using measurements of wing length and tail tip difference (TTD, the
707 measurement between the longest and shortest tail feathers on the underside of the tail).
708 Identification is determined by inserting the measurements of wing length and tail tip
709 difference for an individual into the equations for the relevant age class, and then assigning
710 the bird to whichever species gives the highest value from the equation. The functions are
711 based on measurements of 271 Marsh Tits (200 first-years, 71 adults) and 117 Willow Tits
712 (76 first-years, 41 adults), and probabilities of correct identification are expressed as the %
713 of birds in the sample that were correctly assigned to species.

Species	Linear discriminant function	% identified accurately	Overall % accuracy
<i>First-years</i>			
Marsh Tit	$(\text{wing length} \times 24.16) + (\text{TTD} \times 0.31) - 753.35$	99.0	97.8
Willow Tit	$(\text{wing length} \times 22.89) + (\text{TTD} \times 5.04) - 694.57$	94.7	
<i>Adults</i>			
Marsh Tit	$(\text{wing length} \times 23.11) + (\text{TTD} \times -1.05) - 733.36$	95.8	95.5
Willow Tit	$(\text{wing length} \times 21.61) + (\text{TTD} \times 1.49) - 654.21$	95.1	
<i>All ages</i>			
Marsh Tit	$(\text{wing length} \times 22.74) + (\text{TTD} \times -6.88) - 701.68$	96.7	96.4
Willow Tit	$(\text{wing length} \times 21.26) + (\text{TTD} \times -3.77) - 624.76$	95.7	

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723 **Appendix 1**

724 a) Output from Minitab 16 for binomial logistic regression of Marsh Tit wing length versus
 725 sex, with age class (FY = first-year, otherwise adult) as a factor, using a logit link function.

Predictor	Coef	SE Coef	Z	P	Odds Ratio	95% CI	
						Lower	Upper
Constant	-201.53	20.04	-10.06	0.000			
Marsh Tit wing	3.19	0.32	10.06	0.000	24.30	13.05	45.25
Marsh Tit age							
First-year	3.19	0.54	5.95	0.000	24.35	8.50	69.76

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727 Log-Likelihood = -78.08

728 Test that all slopes are zero: $G = 611.67$, $df = 2$, $P < 0.001$

729 Goodness-of-fit test: Deviance chi-square = 23.45, $df = 14$, $P = 0.053$

730

731 b) Output from Minitab 16 for binomial logistic regression of Marsh Tit wing length versus
 732 sex, (age is unspecified), using a logit link function.

Predictor	Coef	SE Coef	Z	P	Odds Ratio	95% CI	
						Lower	Upper
Constant	-607.00	15.41	-10.84	0.000			
Marsh Tit wing	2.67	0.25	10.84	0.000	14.42	8.90	23.27

733

734 Log-Likelihood = -104.28

735 Test that all slopes are zero: $G = 559.26$, $df = 1$, $P < 0.001$

736 Goodness-of-fit test: Deviance chi-square = 11.62, $df = 7$, $P = 0.114$