

Article (refereed) - postprint

Broughton, Richard K.; Day, John C.; Carpenter, Jane E.; Gosler, Andrew G.; Hinsley, Shelley A. 2018. **Offspring sex ratio of a woodland songbird is unrelated to habitat fragmentation.**

© Dt. Ornithologen-Gesellschaft e.V. 2018

This version available <http://nora.nerc.ac.uk/519322/>

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at <http://nora.nerc.ac.uk/policies.html#access>

This is a post-peer-review, pre-copyedit version of an article published in *Journal of Ornithology*, 159 (2). 593-596. The final authenticated version is available online at:

<https://doi.org/10.1007/s10336-018-1546-z>.

There may be differences between this version and the publisher's version. You are advised to consult the publisher's version if you wish to cite from this article.

Contact CEH NORA team at
noraceh@ceh.ac.uk

1 Offspring sex ratio of a woodland songbird is unrelated to habitat fragmentation

2

3 Richard K Broughton¹, John C Day¹, Jane E Carpenter², Andrew G Gosler² and Shelley A
4 Hinsley¹

5 1. Centre for Ecology & Hydrology, Benson Lane, Crowmarsh Gifford, Wallingford,
6 Oxfordshire OX10 8BB, UK

7 2. Edward Grey Institute for Field Ornithology, Department of Zoology, University of Oxford,
8 South Parks Road, Oxford OX1 3PS, UK

9

10 Abstract

11 We assessed whether the sex ratio of nestling Marsh Tits (*Poecile palustris*) varied with
12 habitat fragmentation by sampling broods from two British woodland areas with contrasting
13 patch size, and comparing with published results from an extensive forest in Poland. We
14 found no evidence for manipulation of offspring sex ratios, supporting findings from Poland
15 and suggesting that this trait may be typical of the species. The results are important in
16 helping to understand the origin of biased sex ratios observed among adult Marsh Tits in the
17 declining population in Britain.

18

19

20

21

22

23

24

25

26

27

28

29 Introduction

30 Sex-based differences in life history traits are frequent among birds, resulting in biased
31 selection that skews the sex ratio of populations (Donald 2007). Whether or not birds have
32 facultative control of the sex allocation of their offspring via a genetic or behavioural
33 mechanism, as a means of addressing or exploiting this bias, is a controversial topic that has
34 produced mixed empirical results (reviewed in Hasselquist and Kampenaeers 2002; Ewen et
35 al. 2004).

36 At the population level, a biased adult sex ratio may be expected to result in parents
37 producing more offspring of the rarer or more competitively advantaged sex, in order to
38 improve the settling and breeding potential of their progeny (Bensch et al. 1999; Stauss et al.
39 2005). Under environmental stress, however, such as deteriorating habitat quality, parents
40 may adjust the brood sex ratio to favour the sex that is least costly to produce (Hasselquist
41 and Kampenaeers 2002).

42 Habitat fragmentation is a major cause of environmental degradation for woodland birds, and
43 Suorsa et al. (2003) found that fragmentation favoured production of less-costly female
44 offspring by Eurasian Treecreepers (*Certhia familiaris*). In fragmented British woodland,
45 adult Marsh Tits (*Poecile palustris*) show a male-biased sex ratio, exhibited by frequent
46 bachelor males holding territory throughout the spring (Broughton and Hinsley 2015).
47 However, in continuous habitat in Poland's Białowieża Forest unpaired males regularly
48 appear early in the spring (Czyż et al. 2012) but never hold territory throughout the breeding
49 season (T. Wesółowski pers. comm., Broughton and Hinsley 2015), suggesting that these
50 may be dispersing birds that eventually find a mate in the forest.

51 Czyż et al. (2012) showed that the Marsh Tit population in Białowieża produced offspring
52 with an unbiased sex ratio, reflecting the adult population in the breeding period. However, it
53 is unknown whether the male bias in British populations of adult Marsh Tits results from a
54 male-biased sex ratio among offspring or from differential mortality between full-grown birds,
55 perhaps related to costs associated with female-biased dispersal in fragmented habitat
56 (Broughton et al. 2010; Wesółowski 2015). Under the environmental stress of a declining

57 population in fragmented habitat (Broughton and Hinsley 2015) British females could be
58 expected to manipulate their offspring towards daughters, which are rarer, more dispersive
59 and less costly to produce, although manipulation towards males may produce offspring that
60 are more competitively advantaged and able to settle successfully.

61 We examined the secondary sex ratio of Marsh Tits nestlings in two areas of Britain with
62 contrasting habitat fragmentation, and compared results with those previously reported from
63 the continuous forest of Białowieża (Czyż et al. 2012). A male-biased sex ratio among
64 nestlings in the British populations, increasing with habitat fragmentation, would indicate the
65 origin of the male skew among adults. Conversely, an unbiased or female-skewed sex ratio
66 would suggest sex-biased mortality of full-grown birds, aiding the understanding the Marsh
67 Tit's long-term decline in Britain (Broughton and Hinsley 2015).

68

69 Methods

70 The study compared a single 312 ha patch of deciduous woodland at Wytham Woods,
71 south-central England (51°46' N, 1°20' W), and 201 ha of fragmented woodland patches
72 located 101 km to the north-east, comprising the 160 ha Monks Wood (52°24' N, 0°14' W)
73 and two neighbouring woods of 13 ha and 28 ha (hereafter 'Monks Wood'). Inter-patch
74 distances at Monks Wood were 301-719 m across an arable landscape matrix.

75 Sex was determined for 190 nestlings from Wytham, comprising 19 complete first broods in
76 2007 (n = 113 nestlings) and 13 in 2008 (n = 77). At Monks Wood 195 nestlings were
77 sampled from 16 complete first broods in the three woodland patches in 2007 (n = 106) and
78 13 in 2008 (n = 89). Total genomic DNA was isolated from whole blood taken from the
79 brachial vein of nestlings when aged 11-15 days post-hatching, which was approximately 5-9
80 days prior to fledging. Aside from four cases of later predation causing total brood loss, no
81 mortality was detected after sampling and so the recorded sex ratio was considered to
82 represent that of fledged juveniles entering the populations.

83 Blood samples were archived on Whatman FTA Classic Cards (GE Healthcare Life
84 Sciences, Maidstone, UK). A 1.25 mm disc was cut from the cards using a Uni-Core punch

85 (Whatman) for DNA extraction using FTA purification reagent (Whatman) and the ZR DNA-
86 Card Extraction Kit (Zymo Research, Irvine, California, USA) according to the manufacturer
87 instructions. The sex identification test employed the P8 (5'-CTCCCAAGGATGAGRAAYTG-
88 3') and P2 (5'-TCTGCATCGCTAAATCCTTT-3') primers (Griffiths et al. 1998) and PCR
89 amplification was conducted in a total volume of 10 μ L using the PCR conditions reported by
90 Griffiths et al. (1998).

91 At least five adult females, identified from leg rings, contributed broods to both years at
92 Monks Wood, with an unknown number at Wytham. However, because Czyż et al. (2012)
93 reported no effect of female age or identity on Marsh Tit brood sex ratios, and also no effect
94 of habitat, year, brood size or breeding phenology, we included all broods in analyses and
95 limited investigation to the population level sex ratio. We hypothesised that the offspring sex
96 ratio would be more male-biased at the fragmented Monks Wood site than at the single,
97 larger woodland patch at Wytham, and both sites would deviate from the even sex ratio
98 reported from the extensive forest at Białowieża (Czyż et al. 2012).

99 We employed a binomial generalised linear model (GLM) with a logit link function in R
100 version 3.0.2 (R Core Team 2013) to test for main effects and an interaction of site and year
101 on sex ratio, with the response variable being the number of males and females in each
102 brood. A power analysis assessed the ability of a binomial test to detect up to a 60% skew
103 towards males among nestlings, using the pwr package (Champely 2015), to replicate the
104 approach of Czyż et al. (2012).

105

106 Results

107 The overall number of male nestlings at Monks Wood totalled 100, with 95 females, and
108 respective totals at Wytham were 88 and 102. The proportion of male nestlings in broods
109 ranged from zero at both sites to 0.80 at Wytham and 0.86 at Monks Wood (Fig. 1), with
110 medians of 0.50 and respective brood sizes of 2-8 (mean = 5.9) and 1-10 (mean = 6.6). The
111 GLM analysis indicated a good fit of the full model, which included site and year effects, with
112 the ratio of residual deviance to 57 degrees of freedom being 0.88. However, none of the

113 explanatory terms were significant (Table 1) and the most parsimonious was the null model
114 (deviance ratio = 0.86) in returning a proportion of 0.48 males with 95% CI of 0.43-0.53. The
115 power analysis produced a curve estimating a 0.98 probability of detecting a proportion of
116 0.6 males in a binomial test of the population-level data (Fig. 2).

117

118 Discussion

119 Our results from two British sites replicate those previously reported from the Białowieża
120 Forest (Czyż et al. 2012) in finding no evidence of significant manipulation of the sex ratio of
121 Marsh Tit broods. Regardless of the degree of habitat fragmentation, the Marsh Tit
122 populations studied appeared to produce male and female offspring in unbiased proportions.
123 These results have two significant implications. Firstly, they indicate that an unbiased sex
124 ratio among offspring appears to be a general feature of Marsh Tit populations under the
125 conditions and spatial scales studied to date. Ramsay et al. (2003) obtained similar results
126 for the closely-related Black-capped Chickadee (*P. atricapilla*), suggesting a more
127 widespread inability to manipulate offspring sex ratios among the *Poecile* tits. These results
128 add to a growing literature of unbiased brood sex ratios among some groups of birds
129 (Hasselquist and Kampenaeers 2002).

130 The second important implication of the results is the indication that the male-biased sex
131 ratio observed among British populations of adult Marsh Tits (Broughton and Hinsley 2015)
132 is unlikely to originate in the nest. Instead, differences in mortality or settling success
133 between males and females must be occurring during the juvenile (post-fledging) or adult
134 stages. A possible mechanism is the greater costs associated with female-biased dispersal
135 of juvenile Marsh Tits. When habitat is fragmented and scattered, the tendency of females to
136 disperse further than males before settling will often necessitate leaving the natal patch of
137 woodland. There are likely to be significant risks of starvation and/or predation associated
138 with crossing the more open landscape matrix between woodland habitat patches. There is
139 also no guarantee that even favourable routes such as hedgerows will lead to a vacant patch
140 of suitable woodland in which to settle (Broughton et al. 2010; Wesolowski 2015).

141 Consequently, males with shorter dispersal distances, which may reduce or avoid inter-patch
142 movements, are likely to have a greater chance of successful dispersal and settlement.

143 In extensive forest, such as Białowieża, the risks associated with inter-patch dispersal are
144 avoided by both sexes, which can travel for many kilometres in any direction through
145 continuous, high quality habitat (Wesołowski 2015). As such, exchange of juveniles across
146 the habitat should be broadly equal for both sexes, even where dispersal distances are
147 greater for females. This would result in population-level recruitment with an unbiased sex
148 ratio during the breeding period, reflecting that of the nestlings.

149 The insights provided by comparison of offspring sex ratios between Marsh Tit populations in
150 Britain and Białowieża are essential in understanding the potential drivers behind the
151 substantial decline in abundance and range of British Marsh Tits (Broughton and Hinsley
152 2015). The results provide support for the theory of habitat fragmentation hindering the
153 recruitment of juvenile females, resulting in unpaired males that are unable to breed and,
154 therefore, reduced population productivity (Broughton et al. 2010). As such, the results
155 provide important evidence supporting the implementation of habitat management that could
156 facilitate inter-patch movement of Marsh Tits, such as creation and conservation of dispersal
157 corridors between fragmented breeding habitat (Broughton and Hinsley 2015).

158

159 Acknowledgements

160 We thank Natural England and the Conservator of Wytham Woods for access to study sites.

161 This work was funded by the Natural Environment Research Council.

162

163 References

164

165 Bensch S, Westerdahl H, Hansson B, Hasselquist D (1999) Do females adjust the sex of
166 their offspring in relation to the breeding sex ratio? *J. Evol. Biol.* 12: 1104-1109.

167

168 Broughton RK, Hill RA, Bellamy PE, Hinsley SA (2010) Dispersal, ranging and settling
169 behaviour of Marsh Tits *Poecile palustris* in a fragmented landscape in lowland England.
170 Bird Study 57: 458-472.
171

172 Broughton RK, Hinsley SA (2015) The ecology and conservation of the Marsh Tit in Britain.
173 Br. Birds 108: 12-29.
174

175 Champely S (2015) pwr: Basic Functions for Power Analysis. R package version 1.1-2.
176 <http://CRAN.R-project.org/package=pwr>
177

178 Czyż B, Rowiński P, Wesolowski T (2012) No evidence for offspring sex ratio adjustment in
179 Marsh Tits *Poecile palustris* breeding in a primeval forest. Acta Orn. 47: 111-118.
180

181 Donald P (2007) Adult sex ratios in wild bird populations. Ibis 149: 671-692.
182

183 Ewen JG, Cassey P, Møller AP (2004) Faculative primary sex ratio variation: a lack of
184 evidence in birds? Proc. R. Soc. Lond. B 271: 1277-1282.
185

186 Griffiths R, Double MC, Orr K, Dawson RJG. (1998) A DNA test to sex most birds. Mol. Ecol.
187 7: 1071-1076.
188

189 Hasselquist D, Kampenars B (2002) Parental care and adaptive brood sex ratio
190 manipulation in birds. Phil. Trans. R. Soc. B 357: 363-372.
191

192 R Core Team (2013) R: A language and environment for statistical computing. R Foundation
193 for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
194

195 Ramsay SM, Mennill DJ, Otter KA, Ratcliffe LM, Boag PT (2003) Sex allocation in black-
196 capped chickadees *Poecile atricapilla*. J. Avian Biol. 34: 134-139.

197

198 Stauss M, Segelbacher G, Tomiuk J, Bachmann L (2005) Sex ratio of *Parus major* and *P.*
199 *caeruleus* broods depends on parental condition and habitat quality. Oikos 109: 367-373.

200

201 Suorsa P, Helle H, Huhta E, Jännti A, Nikula A, Hakkarainen H (2003) Forest fragmentation
202 is associated with primary brood sex ratio in the treecreeper (*Certhia familiaris*). Proc. R.
203 Soc. Lond. B 270: 2215-2222.

204

205 Wesolowski T (2015) Dispersal in an extensive continuous forest habitat: Marsh Tit *Poecile*
206 *palustris* in the Białowieża National Park. J. Orn. 156: 349-361.

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223 Table 1. Significance of deleted terms from a binomial GLM during stepwise deletion using
 224 analysis of deviance to compare the larger and reduced model in each case.

225

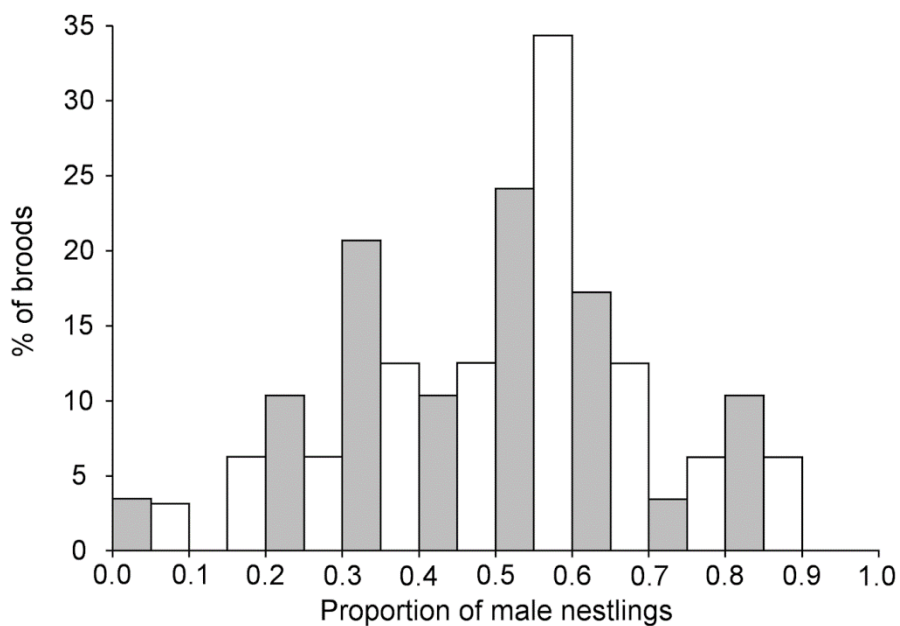
Model	χ^2	df	<i>P</i>
Site * Year (saturated)	0.27	1	0.61
Site	0.51	1	0.48
Year	0.66	1	0.42

226

227

228

229



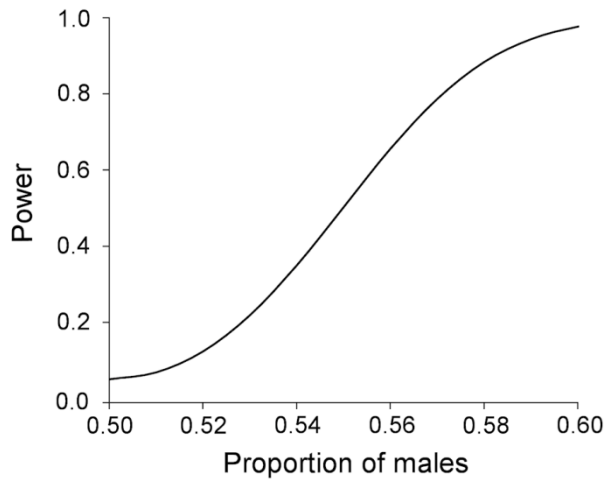
230

231 Figure 1. Distribution of the proportion of males in 29 Marsh Tit broods (195 nestlings) at
 232 Monks Wood (grey bars) and 32 broods (190 nestlings) at Wytham (white bars), both in
 233 southern Britain, pooled over two breeding seasons.

234

235

236



237

238 Figure 2. Curve produced by power analysis indicating the probability (power) of detecting a

239 skew of up to 0.6 in favour of males in a sample of 385 Marsh Tit nestlings.