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1 **PROVENANCE DOES MATTER: LINKS BETWEEN WINTER TROPHIC**

2 **SEGREGATION AND THE MIGRATORY ORIGINS OF EUROPEAN ROBINS**

3   Paulo Catry[1], Ana R. Campos[2], José Pedro Granadeiro[3], Júlio M. Neto[4,5], Jaime Ramos[2], Jason

4 Newton[6,7], Stuart Bearhop[7]

5

6 [1] MARE – Marine and Environmental Sciences Center, ISPA - Instituto Universitário, Rua

7 Jardim do Tabaco 34, 1149-041 Lisboa, Portugal. Email: paulo.catry@gmail.com

8 [2] MARE – Marine and Environmental Sciences Centre, Department of Life Sciences, University

9 of Coimbra, 3004-517 Coimbra, Portugal.

10 [3] CESAM and Departamento de Biologia Animal, Faculdade de Ciências da Universidade de

11 Lisboa, Lisboa, Portugal.

12 [4] CIBIO/UP — Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade

13 do Porto, Portugal

14 [5] Molecular Ecology and Evolution Lab, MEMEG, Department of Biology, University of Lund,

15 Sweden

16 [6] NERC Life Sciences Mass Spectrometry Facility, SUERC, Rankine Avenue, East Kilbride,

17 G75 0QF, UK.

18 [7] Centre for Ecology and Conservation, University of Exeter, Cornwall Campus, TR10 9FE, UK.

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23

24   *Abstract.*

25   Amongst migratory species, it is common to find individuals from different populations or

26   geographical origins sharing staging or wintering areas. Given their differing life histories,

27   ecological theory would predict that the different groups of individuals should exhibit some level

28   of niche segregation. This has rarely been investigated because of the difficulty in assigning

29   migrating individuals to breeding areas. Here, we start by documenting a broad geographical

30   gradient of hydrogen isotopes (δ^2H) in robin *Erithacus rubecula* feathers across Europe. We then

31   use δ^2H, as well as wing-tip shape, as surrogates for broad migratory origin of birds wintering in

32   Iberia, to investigate the ecological segregation of populations. Wintering robins of different

33   sexes, ages and body sizes are known to segregate between habitats in Iberia. This has been

34   attributed to the despotic exclusion of inferior competitors from the best patches by dominant

35   individuals. We find no segregation between habitats in relation to δ^2H in feathers, or to wing-tip

36   shape, which suggests that no major asymmetries in competitive ability exist between migrant

37   robins of different origins. Trophic level (inferred from nitrogen isotopes in blood) correlated

38   both with δ^2H in feathers and with wing-tip shape, showing that individuals from different

39   geographic origins display a degree of ecological segregation in shared winter quarters. Isotopic

40   mixing models indicate that wintering birds originating from more northerly populations

41   consume more invertebrates. Our multi-scale study suggests that trophic-niche segregation may

42   result from specializations (arising in the population-specific breeding areas) that are transported

43   by the migrants into the shared wintering grounds.

44

45   Keywords: Seasonal matching, ecological segregation, *Erithacus rubecula*, migration, stable

46   isotopes

INTRODUCTION

48   Billions of migratory birds, as well as countless other animals, seasonally shift positions across

49   vast geographical areas. At the end of the migratory journey, they often face numerous

50   conspecific competitors from diverse geographical origins that share common non-breeding

51   quarters (Block et al 2005, Newton 2008, Blumenthal et al 2009). The degree of population

52   overlap and competition in the non-breeding season may have varied implications, from

53   demographic regulation to the evolution and shaping of migratory systems (Rickleffs 1992, Bell

54   2005), but because of difficulties in assigning individuals to different origins, thus far virtually

55   nothing is known of the ways individuals from different populations of the same species coexist.

56   There are theoretical reasons to expect segregation and ecological differentiation when

57   multiple populations locally coexist. For example, local adaptations or specializations associated

58   with the breeding grounds in different latitudes and habitats (Peterson 1993) may well be

59   transported to wintering sites by migrating individuals (Price & Gross 2005, Rayner et al. 2011).

60   Alternatively, competitive asymmetries on the wintering grounds may force habitat/trophic

61   segregation of different populations where resources are limited (Fretwell & Lucas 1970,

62   Greenberg 1986). For example, if some migrants arrive earlier, they may gain advantage from

63   earlier ownership of non-breeding territories and win more territorial contests against later

64   arrivals (Tobias 1997). In this scenario, populations at disadvantage may have no alternative but

65   occupying different niches (Greenberg et al. 2001), perhaps with time adapting to them.

66   Asymmetries may also occur due to carry-over effects (Catry et al. 2013) and matching of habitat

67   quality between breeding and wintering sites has also been reported for non-territorial migrants

68   (Gunnarsson et al. 2005).

69   In species such as European robins *Erithacus rubecula* (hereafter "robins") and in

70   blackcaps *Sylvia atricapilla*, sedentary Iberian populations mostly occupy woodland habitats

71   where they also nest, whereas migrants predominate at shrubland sites where no breeding takes

72   place (Pérez-Tris & Tellería 2002, Tellería & Pérez-Tris 2004). It is unclear whether such

73   segregation results from the predominance of sedentary individuals at sites where they hold year-

74   round territories (Campos et al. 2011a), or whether there is a difference in winter habitat choice

75   irrespective of its potential value for future reproduction. A recent study suggests that amongst

76   migratory blackcaps there is no difference in the habitat distribution of birds of different origins

77   (de la Hera et al. 2012). However, segregation may also occur at finer ecological scales, such as

78   among micro-habitats or dietary preferences.

79   In this paper, we use robins as a model species to determine whether there is an

80   ecological segregation of wintering migratory individuals of different geographical origins at

81   multiple ecological scales. We used stable hydrogen isotope ratios (δ^2H) in robin feathers to give

82   an indication of geographic origin, as these ratios in amount-weighted mean annual precipitation

83   occur in a pronounced gradient across Europe along a southwest-northeast axis (Hobson et al.

84   2004). This is the main direction of migration of robins occurring in western Iberia (Bueno

85   1998). We also use stable carbon and nitrogen isotope ratios and multiple source mixing models

86   to assess differences in diet (Inger & Bearhop 2007, Parnell *et al* 2010). Specifically we

87   investigate the question at three hierarchical spatial scales plus at one further ecological

88   dimension: (1) regional, by comparing different sectors of Iberia; (2) macro-habitat, by

89   comparing two contrasting habitats in the same region; (3) micro-habitat, by making detailed

90   habitat measurement within two study sites; and (4) diet, as assessed through stable-nitrogen

91   isotope ratios in blood, a proxy for trophic level.

92

93   METHODS

94   *Study species*

95   European robins are small passerines common across almost the entire continent. Northern and

96   eastern populations are fully migratory, while at the western and southern edge of the range

97   (including much of Iberia) they are mostly sedentary. At mid-latitudes, robins are partial

98   migrants (Cramp 1988). In western Iberia, robins are extremely common winter visitors,

99   occupying virtually every type of habitat with some tree or shrub cover, feeding on invertebrates,

100   berries, olives and acorns (Herrera 1977, 1998). Some individuals defend winter territories while

101   others behave as floaters (Cuadrado 1997).

102

103   *Feather sampling across Europe*

104   Because feathers, once fully developed, are metabolically inert, they reflect the environment in

105   which they were grown. Flight feathers of robins <1 year old are grown in the nest. Adult

106   feathers are moulted after reproduction, while birds are still on the breeding grounds (Cramp

107   1988). Note that robins are highly faithful to nesting areas and breeding dispersal, when it

108   occurs, is mostly of less than 10km (Paradis et al. 1998). We sampled robin feathers grown in

109   2007 across multiple European locations in Portugal, France, Sweden and Russia. Stable isotope

110   ratios in terrestrial ecosystems are known to show site-specific variations that are superimposed

111   on broad geographical trends (Wunder et al. 2005). Hence, to avoid obtaining spurious results

112   caused by local variation, within each region/country we tried, as much as possible, to sample

113   individuals from as many different locations (covering a broad range of latitudes) as possible.

114   Adult robins were sampled during the 2008 breeding season in Portugal (16 sites from north to

115 south; late April to mid July), France (10 sites, from north to centre, late May to early July) and

116 Russia (2 sites, Kaliningrad enclave, Baltic region, late May and June). Scandinavian robins

117 were sampled during migration at the southern tip of Sweden, at Falsterbo (55º 23' N, 12º 50' E),

118 where migrant robins originating from all over Sweden and (less so) from Finland occur during

119 autumn (Karlsson et al. 1988). Trapping took place on 5 different dates, from mid September to

120 mid-October 2007, covering the main migratory period. It is therefore likely that each of the 36

121 individuals sampled here came from a different breeding site. Note that individuals sampled in

122 autumn 2007 and adults sampled in spring 2008 would have all grown their feathers in the same

123 breeding season.

124   Each sampled robin was ringed. The innermost secondary was cut and preserved for

125 isotope analysis. This feather was chosen as it is rarely lost by accident, which prevents the

126 unintended sampling of replacement feathers grown away from the breeding site.

127

128 *Winter sampling in Iberia*

129 Robins were captured using mist-nets and baited traps during the winter (from mid-November

130 2008 to mid-February 2009) at 5 locations, covering the full wintering range along a NE-SW

131 axis on the western part of Iberia: (1) Irun, Northern Spain (43º20'N, 1º 47'W) in mostly

132 woodland habitats (10m altitude; N = 22 robins); (2) Salvaterra do Extremo, Central Portugal

133 (39º 52'N, 06º 54' W) in open cork oak woodland (350m altitude; N = 12 robins); (3) Charneca

134 (near Alcochete), Central/South Portugal (38º 49'N, 08º 49' W) in open cork oak woodland with

135 virtually no undergrowth (25m altitude; N = 45 robins); (4) Arrábida, Central/South Portugal

136 (38º 27'N, 09º 01' W) in Mediterranean shrubland (200m altitude; N = 77 robins); (5) Charito,

137 Algarve, South Portugal (37º10'N, 08º 27'W), in an open mixture of woodland/shrubland with

138   carob trees, mastic and olive trees (50m altitude; N = 17 robins). Robins do not nest at any of the

139   sites where traps or nets were placed.

140   Sites 3 and 4 are separated by just 45km and have a similar climate. They were selected

141   to represent the greatest possible contrast in terms of shrub and tree cover, as these variables are

142   known to influence the distribution of robin of different age, sex and size classes (Tellería *et al*.

143   2001, Catry *et al*. 2004, own unpubl. data). Within these main study sites (sites 3 & 4), robins

144   were trapped with spring traps baited with mealworms only, to ensure they were foraging at the

145   site of capture, and not just commuting to another place. At each exact capture location (and

146   following Catry *et al*. 2004) we visually estimated the following micro-habitat variables, in a

147   radius of 20m around the trap: a) mean canopy height, b) percentage canopy cover, c) mean

148   height of the shrub layer (shrubs were defined as woody plants less than 3 m tall), d) percentage

149   shrub cover, e) percentage bare ground or short-grass (< 10 cm tall) cover and f) percentage

150   long-grass cover.

151   Robins captured in winter were aged according to Svensson (1992) and processed in the

152   same way as described above for spring. Additionally, for birds captured at Charneca and

153   Arrábida, we measured each of the 9 primaries using a ruler with a pin. Pectoral muscle profiles

154   were scored with reference to the prominence of the sternal keel and muscle shape, on a 4-point

155   scale following Bairlein (1995; see also Gosler, 1991).

156   Approximately 60 μl of blood were obtained by puncturing the *vena ulnaris*. A drop was

157   preserved in ethanol for molecular sexing and the remaining was frozen until further processing.

158   Sexing was done through the amplification by PCR (polymerase chain reaction) of a fragment of

159   the CHD gene, using the primers P2 and P8 (Griffiths *et al*. 1998).

160   At Charneca and Arrábida, samples of the most common invertebrates, berries and acorns

161   likely to be part of robin diet (Herrera 1977, 1998, Debusse & Isenmann 1985) were collected

162   during winter and frozen for subsequent laboratory analysis.

163

164   *Stable isotopes*

165   i) Hydrogen isotopes

166   The sampled feathers from our study birds were carefully washed in distilled water and oven

167   dried and left for a week equilibrating. Feathers were then cut very finely and weighed into silver

168   cups and loaded into a zero-blank autosampler ready for analysis by continuous flow isotope

169   ratio mass spectrometry (CF-IRMS), using a high temperature reduction system (TC/EA)

170   interfaced with a Thermo-Fisher-Scientific Delta V Plus IRMS. We used the "comparative

171   equilibration" (Wassenaar and Hobson, 2003) approach to correct the δ^2H isotope data, Briefly,

172   the steam equilibration takes place in a modified Costech zero-blank autosampler containing

173   keratin standards. Water of known δ^2H_{VSMOW} (W64444, d2H=-399.1‰, provided by USGS-

174   RSIL; or alternatively S9, an internal water standard, $\delta^2H_{VSMOW} = +99.09‰$) is injected into the

175   evacuated hot autosampler in a 110°C oven. Following equilibration, evacuation and cooling to

176   room temperature the autosampler is reattached to the TC/EA, purged with helium gas and run as

177   normal. Non-exchangeable standards are used to correct raw hydrogen isotope data; these were

178   IAEA-CH7 (polyethylene foil, $\delta^2H_{VSMOW} = -100.3‰$), C36 (hexatriacontane provided by A.

179   Schimmelmann, $\delta^2H_{VSMOW} =246.7‰$) and an internal polythene foil standard "BOS" (δ^2H_{VSMOW}

180   = -82.1‰). Two runs using the two widely disparate water standards above, allows %H

181   exchangeability and unexchangeable δ^2H_{VSMOW} to be calculated. As such that the δ^2H data here

182   represent the unexchangeable hydrogen and fixed to the VSMOW-VSLAP scale.

183   The standards used for this approach are CFS (chicken feathers; δ^2H_{VSMOW} = -147 ± 5 ‰,

184   Hobson and Wassenaar 2008), BWB-II (bowhead whale baleen; δ^2H_{VSMOW} = -108 ± 4 ‰,

185   Hobson and Wassenaar 2008) and ISB (Icelandic black-legged kittiwake, *Rissa tridactyla*

186   feathers δ^2H_{VSMOW} = -72 ± 3 ‰, Fox et al. 2007). A fourth keratin standard WG (Willow grouse

187   *Lagopus lagopus* feathers, δ^2H_{VSMOW} = -135 ± 1 ‰, unpublished data) is run independently of

188   the calibration (see also Evans et al. 2012). All four standards were run in triplicate in five

189   measurement runs; collectively the fifteen WG standards had a δ^2H_{VSMOW} of -135.38 ± 2.89‰.

190   All δ^2H values quoted are non-exchangeable hydrogen derived from the comparative steam

191   equilibration technique.

192   ii) Carbon and nitrogen isotopes

193   Whole blood, whole insects, berries and acorns were oven dried, reduced to a homogeneous

194   powder, and weighed into tin cups then also analysed for nitrogen ($\delta^{15}N_{AIR}$) and carbon ($\delta^{13}C_{V-}$

195    $_{PDB}$) isotope ratios by CF-IRMS, using a Costech ECS 4010 elemental analyser connected to a

196   Thermo-Fisher-Scientific Delta XP Plus IRMS. Internal laboratory standards included gelatin,

197   and two alanine standards spiked with ^{13}C- and ^{15}N-enriched alanine respectively. These internal

198   standards are checked monthly against international reference materials USGS 40 and USGS 41

199   (glutamic acid), IAEA N1, N2 and USGS25 (ammonium sulphate) and IAEA CH6 (sucrose).

200   Repeated (>20) $\delta^{15}N$ and $\delta^{13}C$ measurements in each run gave standard deviations of ~0.2 and

201   0.1‰ respectively.

202   All the analyses were performed at the same laboratory of the NERC Life Sciences Mass

203   Spectrometry Facility at East Kilbride, Scotland.

204

205   *Statistical analysis*

206   An index of shrub volume at each trapping location was obtained by multiplying shrub cover by

207   its mean height. This micro-habitat variable has been shown to contribute to predict size, age and

208   sex-ratios of captured robins in winter (Catry et al. 2004).

209   Bird populations with a greater migratory tendency tend to have longer and more pointed

210   wings, and this general pattern applies to robins, with more northerly populations differing from

211   southern and predominantly sedentary ones (Pérez-Tris *et al*. 2000, Tellería *et al*. 2001). Hence,

212   using the measurements of individual primary feathers, we calculated a wingtip pointedness

213   index (C_2) derived from Size-Corrected Components Analysis - SCCA (Lockwood *et al.* 1998).

214   This was only done for robins wintering at Charneca and Arrábida, as most other samples had

215   been collected by collaborators who were unable to provide measurements of primary feathers.

216   In order to estimate the contribution of each prey type in the diet of individual robins, we

217   developed an isotopic mixing model in the "R" package SIAR (Parnell et al. 2010, R

218   Development Core Team 2012). The procedure implements a Markov Chain Monte Carlo

219   algorithm to seek likely combination of prey contributions that best match the isotopic signature

220   of the consumer, taking into account tissue- and species-specific discrimination factors of dietary

221   items. Natural variability in isotopic signature of food sources and in trophic discrimination is

222   dealt with within the Bayesian approach implemented by SIAR (Parnell *et al*. 2010). Prey items

223   were aggregated according to prior knowledge of their feeding habits in the regions and included

224   four major types: Ants, other insects, *Quercus sp.* acorns and berries, the latter only present in

225   one of the sites (Arrábida). Trophic enrichment factors were set at 1.7±0.17‰ and 2.4±0.24‰

226   and for carbon and nitrogen respectively (Hobson and Bairlein 2003).

227   We used general linear models to investigate the effects of single factors/variables on

228   stable hydrogen isotope ratios or C_2, and checked their significance using likelihood ratio tests

229 against the corresponding null models (i.e. with the variable of interest removed). We also

230 examined the extent to which $\delta^{15}N$ in blood was influenced by age, sex, month, habitats and

231 either δ^2H or C_2 (as indicators of origin), for which general linear models within a Bayesian

232 model averaging (BMA) framework were used. Variables were checked for normality and

233 homogeneity of variance. To avoid over-fitting we tested all combinations of variables, but only

234 included first-order interactions between month, age, sex and study site. Computations were

235 carried out using function bic.glm of BMA package (Raftery et al. 2013), assuming uniform

236 priors for the model parameters and using the Bayesian Information Criterion (BIC)

237 approximation to estimate the integrated model likelihood. We only averaged a subset of all

238 possible models, by constraining the ratio of the posterior probabilities of the candidate model to

239 the best model to be higher than 0.05 (Occam's window criteria Madigan and Raftery 1994). For

240 models including either δ^2H or C_2 we calculated the posterior probability that each variable is in

241 the model (in %), as well as the posterior mean and standard deviation for all estimated

242 coefficients.

243

244   RESULTS

245 *Hydrogen isotope trends in Europe*

246 There were highly significant differences in δ^2H values in feathers sampled across a range of

247 different European countries (GLM log-likelihood ratio $\chi^2 = 16499$, $P < 0.001$), with a clear

248 trend for more negative values with increasing distance from Portugal towards north-eastern

249 Europe (Fig. 1). The patterns for the more extensive Portuguese data set are similar, illustrating

250 that even within a smaller region, closer to our winter study sites, latitude of a site and mean δ^2H

251 values of robins from that same location correlate significantly (r = -0.67, N = 16 sites, P =

252 0.005).

253

254 *Segregation in winter quarters*

255 As expected there was a correlation between wing pointedness (C_2) and δ^2H in feathers (r = 0.38,

256 N = 121, P < 0.001) for birds captured in central Portugal during winter, indicating that birds

257 with more pointed wings had bred (or were hatched) further northeast. Using the larger data set

258 of birds captured across Iberia, wing length (no data for C_2) also correlated with δ^2H (r = -0.27,

259 N = 170, P < 0.001), again indicating that birds with longer wings originated further northeast.

260   The δ^2H in feathers did not correlate with latitude of the Iberian wintering site (r = 0.03,

261 N = 174, P = 0.69). Although this analysis is pseudoreplicated (as many individuals came from

262 the same wintering site), we choose to present it as a conservative example of there being no

263 relationship between breeding latitude and wintering latitude. Furthermore, as shown below,

264 there are no reasons to expect important site-related differences in δ^2H composition of feathers of

265 *wintering* robins. The correlation between mean δ^2H in robin feathers from a given wintering site

266 and latitude is also not significant (r = 0.33, N = 5, P = 0.59).

267   The feathers of robins wintering in open woodland and in shrubland in nearly adjacent

268 sites in central Portugal did not differ significantly in δ^2H (respectively, -87.4 ± 9.6‰, N = 45

269 and -86.1 ± 12.6‰, N = 77, GLM log-likelihood test χ^2 = 0.002, P=0.997). None of the

270 microhabitat variables measured at Arrábida and at Charneca correlated with stable hydrogen

271 isotope ratios or with the wing pointedness index (all correlations with P > 0.05, N = 45 at

272 Charneca and N = 77 at Arrábida).

273   Model averaging revealed that values of $\delta^{15}N$ in whole blood differed between habitats,

274   among sampling months and were negatively correlated with δ^2H (Table 1, Fig. 2), while age

275   and sex effects and interactions were non-significant. The same highly significant relationship

276   holds when δ^2H replaces the wing pointedness index C_2 (Table 1, Fig. 2). The percentage

277   contribution of invertebrates in the diet of individual birds (estimated with isotopic mixing

278   models; see Electronic Supplementary Materials for data on Carbon and Nitrogen isotope ratios

279   in prey and robins) showed a negative relationship with δ^2H levels (GLM, t= -2.16 , P=0.034),

280   and also a significant effect of site (GLM, t=14.6, P<0.001).

281

282   DISCUSSION

283   For the first time we have systematically measured variation across multiple niche dimensions

284   amongst sympatrically wintering conspecific migrants from allopatric breeding areas. Perhaps

285   surprisingly we found that robins from a wide range of breeding latitudes within Europe show no

286   evidence of geographic structuring or migratory connectivity within Iberia. Likewise we could

287   find no consistent differences in habitat and microhabitat selection. However, individuals from

288   different origins differed in their trophic position during the winter months, suggesting a form of

289   seasonal matching (Gunnarsson et al. 2005) between the region of birth or reproduction and the

290   wintering ecology of migrants. Trophic specialisation of this nature may play a key role in

291   allowing these populations with different migratory tendencies to co-exist during the non-

292   breeding season.

293   We began by documenting a clear geographical cline of hydrogen isotope ratios in robin

294   feathers across Europe. The relationship between wing length and shape and δ^2H in wintering

295   birds is also in line with the initial expectation of more negative ratios for birds with longer and

13

296 more pointed wings, which likely belong to more migratory and northerly distributed populations

297 (Pérez-Tris et al. 2000).

298   The distribution of δ^2H values evidenced in Fig. 1 clearly indicates that the robin

299 communities wintering in our study sites are a mixture of individuals originating from a wide

300 range of latitudes in Europe (a condition necessary for our study) and is in agreement with

301 previous research (Bueno 1998, Catry et al. 2010, Campos et al. 2011a). This panmitic

302 overwintering model at the level of the Iberian Peninsula is similar to that found on

303 overwintering monarch butterflies (Wassenar & Hobson 1998).

304   Many conspecific migrant populations show some segregation in winter quarters, a

305 phenomenon known as migratory connectivity (Newton 2008). Segregation by habitat has more

306 rarely been documented (Marion 1995, Duijns et al. 2009, Alves et al. 2010). In robins, however,

307 we found no habitat influence on the geographical composition of wintering birds, despite the

308 fact that we compared contrasting habitats which, for example, harbour robin communities with

309 markedly different sex-ratios (Catry et al. 2004 and own unpubl. data). A similar result has been

310 obtained for blackcaps, a mostly non-territorial passerine wintering in southern Iberia (de la Hera

311 et al. 2012).

312   Amongst migrants that are territorial during winter, asymmetries in resource-holding

313 potential may result in a despotic distribution, where dominant individuals relegate weaker

314 competitors to less suitable habitats (e.g. Marra 2000). Detailed studies of wintering robins in

315 Iberia have indicated that local birds may have a competitive advantage over visiting migrants,

316 given that they occupy the putative best habitats where they nest throughout the year (Tellería et

317 al. 2001, Tellería & Pérez-Tris 2004). However, the same patterns of population segregation

318 might not necessarily arise from the competitive superiority of southern individuals, but rather

14

319 from them opting to invest in territorial defence over winter at appropriate sites, in preparation

320 for future breeding attempts. The present study was conducted only at sites where no robins nest.

321 Under these conditions, there was no evidence for habitat separation between birds from

322 different origins, which would be expected if robins from different latitudes had dissimilar

323 abilities to compete for the best wintering sites. Experimental work with artificial feeders also

324 suggested no differences in the resource holding potential linked to geographical origin of robins

325 (Campos et al. 2011b).

326   When potentially competing groups do not segregate in one niche dimension, they are

327 more likely to differ in another one, and this is exactly what we found, with robins from more

328 northerly origins feeding at a higher trophic level (consuming more invertebrates). Wintering

329 robins are broadly omnivorous (Herrera 1977, 1978, 1998, Debusse & Isenmann 1985), which

330 means that any dietary specialization or differentiation is likely to be picked up by an analysis of

331 trophic level given by an indicator such as $\delta^{15}N$ in blood. Most robins arrive in October (Bueno

332 1998, Catry et al. 2010), and given the fact that turnover half-life of whole blood for birds the

333 size of a robin is ca. 5 days (Hobson 2008), it is almost impossible that any isotopic

334 measurements made during late-November to February retain any signature of the migratory

335 habitats or of the migratory journey itself.

336   Virtually no studies have documented dietary segregation of coexisting migratory

337 populations in common wintering grounds, and the few exceptions reported very small

338 differences between distinct subspecies (Duijns et al. 2009, Fonteneau et al. 2009). An exception

339 is a study of black-tailed godwits *Limosa limosa* that documented important dietary differences

340 between subspecies, but those were linked to a clear segregation by habitat (Alves et al. 2010). A

341 study of collared flycatchers *Ficedula albicollis* using isotopes also suggested there may be

15

342   consistent interpopulation differentiation in the feeding ecology at the wintering sites, but

343   alternative explanations for the patterns found could not be rejected (Hjernquist et al. 2009).

344   Individual specialization in diet has been suggested for robins of multiple populations wintering

345   in southern Iberia, which was attributed to differences in morphology, but those were not linked

346   to geographical origin of migrants (Herrera 1978). Our study provides the first strong evidence

347   that different migratory populations may have different diets, even when sharing wintering

348   habitats.

349   Robins from different breeding origins may have slightly divergent adaptations, allowing

350   them to better exploit the resources of their nesting habitats, resulting in an intraspecific niche

351   differentiation (Peterson & Holt 2003). Feeding specializations have been documented in

352   passerine birds even at small spatial scales (10s of kilometres, along an altitudinal gradient) and

353   despite gene flow (e.g. McCormack & Smith 2008). Hence, it seems plausible to speculate that

354   robins originating from different parts of the wide European range could carry different

355   morphological, physiological or behavioural specializations, resulting from local adaptations,

356   into the winter quarters (see Price & Gross 2005, Rayner et al. 2011 for this type of phenomenon

357   amongst closely related migratory species and subspecies). This would then lead them to adopt

358   different diets when sharing a common environment. Such specializations could even be

359   reinforced by selection during winter-time, if they were to reduce competition for resources. An

360   alternative explanation would be that longer-distance migrants have different nutritional

361   requirements than shorter-distance migrant conspecifics (Tellería et al. 2013), but with the

362   present knowledge it is hard to explain why would migrants need a diet richer in protein (from

363   animal prey) and presumably poorer in lipids (Bairlein 2002). It should be noted that muscle-

364   score was not a significant predictor of trophic level, suggesting that body condition or

365 composition were not strongly correlated with diet. Furthermore, given that differences were

366 present throughout winter, well before spring migratory preparation (fattening) occurs, this

367 alternative explanation seems less likely.

368   Our findings have implications for models tackling the conditions for the evolution and

369 maintenance of migratory systems. For example, in a theoretical modelling exercise, Taylor &

370 Norris (2007), based on empirical evidence from previous studies (e.g. Adriaensen & Dhondt

371 1990, Pérez-Tris & Tellería 2002) assumed that migrants experience reduced competitive ability

372 during winter. The results of the present study suggest that (longer-distance) migrants may not

373 necessarily be competitively inferior. Northern robins were not only able to occupy the same

374 habitats as their southern conspecifics despite arriving later to Iberia (Bueno 1998) but they were

375 at a higher trophic level due to a greater consumption of invertebrates. Robins are known to

376 prefer animal to plant foods (Berthold 1976), and so northern individuals may not be faring

377 worse than southern counterparts.

378

379 *Conclusions*

380 Wintering robins in Iberia have been conclusively shown to clearly segregate between habitats

381 by age, sex and size classes, which has been mostly attributed to the competitive exclusion of

382 weaker individuals from preferred sites by conspecific dominants (Figuerola et al. 2001, Tellería

383 et al. 2001, Catry et al. 2004). The evidence from our work shows that such segregation does not

384 apply to migrants from different regions, suggesting no important asymmetries in the resource

385 holding potential of birds originating from different populations. However, although there are no

386 reported differences in diet between sex and age classes, there is clear evidence for dietary

387 differences between northern and southern individuals. These are perhaps more readily explained

388   by the transportation to the wintering grounds of specializations originating in the breeding areas,

389   but more research is needed to better evaluate the factors underlying these fascinating patterns,

390   and to understand whether this particular type of ecological matching is a frequent trait in

391   migratory species.

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407

408   LITERATURE CITED

409   Adriaensen F, Dhondt AA (1990) Population dynamics and partial migration of the European

410   robin *Erithacus rubecula* in different habitats. J Anim Ecol 59:1077-1090.

411 Alves JA, Lourenço PM, Piersma P, Sutherland WJ, Gill JA (2010) Population overlap and
412   habitat segregation in wintering black-tailed godwits *Limosa limosa*. Bird Study 57:381-
413   391.

414 Bairlein F (comp.) (1995) Manual of field-methods. European-African songbird migration
415   network. Wilhelmhaven, Germany.

416 Bairlein F (2002) How to get fat: nutritional mechanisms of seasonal fat accumulation in
417   migratory songbirds. Naturwissenschaften 89:1–10

418 Bell CP (2005) Inter- and intrapopulation migration patterns: ideas, evidence and research
419   priorities. In: Greenberg R, Marra PP (eds.). Birds of two worlds. The Ecology and
420   Evolution of Migration. John Hopkins University Press, Baltimore.

421 Berthold P (1976) The control and significance of animal and vegetable nutrition in omnivorous
422   songbirds. Ardea 64:140-154.

423 Block BA, Teo SLH, Walli A, et al. (2005) Electronic tagging and population structure of
424   Atlantic bluefin tuna. Nature 434:1121-1127

425 Blumenthal JM, Abreu-Grobois FA, Austin TJ, et al. (2009) Turtle groups or turtle soup:
426   dispersal patterns of hawksbill turtles in the Caribbean. Mol Ecol 18:4841-4853.

427 Bueno JM (1998) Migracion e invernada de pequeños turdinos en la Peninsula Ibérica V.
428   Petirrojo (*Erithacus rubecula*). Ardeola 45:193-200.

429 Campos AR, Catry P, Tenreiro P, Neto JM, Pereira AC, Brito R, Cardoso H, Ramos JA, Bearhop
430   S, Newton J (2011a) How do Iberian robins *Erithacus rubecula* respond to seasonal
431   flooding by conspecific migrants? Bird Study 58:435–442.

432   Campos AR, Catry P, Ramos J, Robalo J (2011b) Competition among European robins *Erithacus*

433   *rubecula* in the winter quarters: sex is the best predictor of priority of access to

434   experimental food resources. Ornis Fenn 88:226-233.

435   Catry P, Campos A, Almada V, Cresswell W (2004) Winter segregation of migrant European

436   robins *Erithacus rubecula* in relation to sex, age and size. J Avian Biol 35:204-209.

437   Catry P, Costa H, Elias G, Matias R (2010) Aves de Portugal. Ornitologia do Território

438   Continental. Assírio & Alvim, Lisboa.

439   Catry P, Dias MP, Phillips RA, Granadeiro JP (2013) Carry-over effects from breeding modulate

440   the annual cycle of a long-distance migrant: an experimental demonstration. Ecology

441   94:1230-1235

442   Cramp S (Ed.) (1988) The Birds of the Western Palearctic, Vol V. Oxford University Press,

443   Oxford.

444   Cuadrado M (1997) Why are migrant robins *Erithacus rubecula* territorial in winter?: the

445   importance of the anti-predation behaviour. Ethol Ecol Evol 9:77-88.

446   de la Hera I, Pérez-Tris J, Tellería JL (2012) Habitat distribution of migratory and sedentary

447   blackcaps *Sylvia atricapilla* wintering in southern Iberia: a morphological and

448   biogeochemical approach J Avian Biol 43:333–340.

449   Debusse M, Isenmann P 1985. Frugivory of transient and wintering European robins *Erithacus*

450   *rubecula* in a Mediterranean region and its relationship with ornithochory. Holarctic Ecol

451   8:157-163.

452   Duijns S, van Dijk JGB, Spaans B, Jukema J, de Boer WF, Piersma T (2009) Foraging site

453   selection of two subspecies of bar-tailed godwit *Limosa lapponica*: time minimizers

454   accept greater predation risk than energy minimizers. Ardea 97:51-59.

455 Evans KL, Newton J, Gaston KJ, Sharp SP, McGowan A, Hatchwell BJ (2012) Colonisation of

456   urban environments is associated with reduced migratory behaviour, facilitating

457   divergence from ancestral populations. Oikos 121:634-640.

458 Figuerola J, Jovani R, Sol D (2001) Age-related habitat segregation by robins *Erithacus rubecula*

459   during the winter. Bird Study 48:252-255.

460 Fonteneau F, Paillisson J-M, Marion L 2009 Relationships between bird morphology and prey

461   selection in two sympatric great cormorant *Phalacrocorax carbo* subspecies during

462   winter. Ibis 151:286-298

463 Fretwell SD, Lucas HL Jr. (1970) On territorial behavior and other factors influencing habitat

464   distributions in birds.. Acta Biotheoret 19:16-36.

465 Fox AD, Christensen TK, Bearhop S, Newton J (2007) Using stable isotope analysis of multiple

466   feather tracts to identify moulting provenance of vagrant birds: a case study of Baikal teal

467   *Anas formosa* in Denmark. Ibis 142:622-625.

468 Gosler AG (1991) On the use of greater covert moult and pectoral muscle as measures of

469   condition in passerines with data for the great tit *Parus major*. Bird Study 31:1-9.

470 Greenberg R (1986) Competition in migrant birds in the nonbreeding season. In: Johnston RF

471   (ed) Current Ornithology 3. Plenum Press, New York and London, pp 281-307.

472 Greenberg R, Gonzalez CE, Bichier P, Reitsma R (2001) Nonbreeding habitat selection and

473   foraging behavior of the black-throated green warbler complex in Southeastern Mexico.

474   Condor 103:31-37.

475 Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. Mol Ecol

476   7:1071- 1075.

477   Gunnarsson TG, Gill JA, Newton J, Potts PM, Sutherland WJ (2005) Seasonal matching of

478   habitat quality and fitness in a migratory bird. Proc R Soc B 272:2319-2323.

479   Herrera CM (1977) Ecología alimenticia del petirrojo (*Erithacus rubecula*) durante su invernada

480   en encinares del sur de España. Doñana, Acta Vert 4:35-59.

481   Herrera CM (1978) Individual dietary differences associated with morphological variation in

482   robins *Erithacus rubecula*. Ibis 120:542-545.

483   Herrera CM (1998) Long-term dynamics of Mediterranean frugivorous birds and fleshy fruits: a

484   12-year study. Ecol Monogr 68:511-538.

485   Hjernquist MB, Veen T, Font L, Klaassen (2009) High individual repeatability and population

486   differentiation in stable isotope ratios in winter-grown collared flycatcher *Ficedula*

487   *albicollis* feathers. J Avian Biol 40:102-107.

488   Hobson KA and Bairlein F (2003) Isotopic fractionation and turnover in captive garden warblers

489   (*Sylvia borin*): implications for delineating dietary and migratory associations in wild

490   passerines. Can J Zool 81:1630–1635

491   Hobson KA (2008) Applying isotopic methods to tracking animal movements. In: Hobson KA,

492   Wassenaar LI (eds) Tracking Animal Migration with Stable Isotopes. Academic Press,

493   London.

494   Hobson KA, Wassenaar LI (2008) Tracking Animal Migration with Stable Isotopes. Academic

495   Press, London.

496   Hobson KA, Bowen G, Wassenaar LI, Ferrand Y, Lormee H (2004) Using stable hydrogen

497   isotope measurements of feathers to infer geographical origins of migrating European

498   birds. Oecologia 141:477-488.

499   Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. Ibis

500   150:447-461.

501   Karlsson L, Persson K, Pettersson J, Walinder G (1988) Fat-weight relationships and migratory

502   strategies in the robin *Erithacus rubecula* at two stop-over sites in South Sweden. Ring

503   Migr 9:160-168.

504   Lockwood R., Swaddle JP, Rayner JMV (1998) Avian wing tip shape reconsidered: wingtip

505   shape indices and morphological adaptations to migration. J Avian Biol 29:273-292.

506   Madigan D, Raftery AE (1994) Model selection and accounting for model uncertainty in

507   graphical models using Occam's window. J Am Stat Assoc 89:1535–1546

508   Marion L (1995) Where two subspecies meet: origin, habitat choice and niche segregation of

509   cormorant *Phalacrocorax c. carbo* and *P. c. sinensis* in the common wintering area

510   (France), in relation to breeding isolation in Europe. Ardea 83:103–114.

511   Marra PP (2000) The role of behavioral dominance in structuring patterns of habitat occupancy

512   in a migrant bird during the nonbreeding season. Behav Ecol 11:299-308.

513   McCormack JE, Smith TB (2008) Niche expansion leads to small-scale adaptive divergence

514   along an elevation gradient in a medium-sized passerine bird. Proc R Soc B 275:2155-

515   2164.

516   Newton I (2008) The migration ecology of birds. Academic Press, Amsterdam.

517   Paradis E, Baillie SR, Sutherland WJ, Gregory RD 1998. Patterns of natal and breeding dispersal

518   in birds. J Anim Ecol 67:518-536.

519   Parnell AC, Inger R, Bearhop S & Jackson AL (2010) Source partitioning using stable isotopes:

520   coping with too much variation. PLoS ONE 5:e9672.

521   Pérez-Tris J, Carbonell R, Tellería JL (2000) Identificación e importancia poblacional de los

522   petirrojos *Erithacus rubecula* locales durante la invernada en el sur de España. Ardeola

523   47:9-18.

524   Pérez-Tris J, Tellería JL (2002) Migratory and sedentary blackcaps in sympatric non-breeding

525   grounds: implications for the evolution of avian migration. J Anim Ecol 71:211-224.

526   Peterson AT (1993) Adaptive geographical variation in bill shape of scrub jays (*Aphelocoma*

527   *coerulescens*). Am Nat 142:508-527.

528   Peterson AT & Holt RD (2003) Niche differentiation in Mexican birds: using point occurrences

529   to detect ecological innovation. Ecol Let 6:774-782.

530   Price T, Gross S (2005) Correlated evolution of ecological differences among the Old World leaf

531   warblers in the breeding and nonbreeding seasons. In: Greenberg R, Marra PP (eds.)

532   Birds of two worlds. The Ecology and Evolution of Migration. John Hopkins University

533   Press, Baltimore.

534   R Development Core Team (2012) R: a language and environment for statistical computing.

535   Vienna: R Foundation for Statistical Computing. http://www.R-project.org/

536   Raftery A, Hoeting J, Volinsky C, Painter I, Yeung KY (2013) BMA: Bayesian Model

537   Averaging, R package version 3.16.2.3, http://CRAN.R-project.org/package=BMA

538   Rayner MJ, Hauber ME, Steeves TE, Lawrence HA, Thompson DR, Sagar PM, Bury SJ,

539   Landers TJ, Phillips RA, Ranjard L, Shaffer SA (2011) Contemporary and historical

540   separation of transequatorial migration between genetically distinct seabird populations.

541   Nature Com 2: article number 332.

542   Rickleffs RE (1992) The megapopulation: a model of demographic coupling between migrant

543   and resident landbird populations. In: Hagan III JM & Johnston DW (eds) Ecology and

544   Conservation of Neotropical Migrant Landbirds. Smithsonian Institution Press,

545   Washington.

546   Svensson L (1992) Identification Guide to European Passerines. Svensson, Stockholm.

547   Taylor CM, Norris DR (2007) Predicting conditions for migration: effects of density dependence

548   and habitat quality. Biol Let 3:280-283.

549   Tellería JL, Pérez-Tris J (2004) Consequences of the settlement of migrant European robins

550   *Erithacus rubecula* in wintering habitats occupied by conspecific residents. Ibis 146:258-

551   268.

552   Tellería JL, Blázquez M, de la Hera I, Pérez-Tris J (2013) Migratory and resident blackcaps

553   *Sylvia atricapilla* wintering in southern Spain show no resource partitioning. Ibis

554   155:750-761.

555   Tellería JL, Pérez-Tris J, Ramírez A, Fernández-Juridic E, Carbonell R (2001) Distribution of

556   robins *Erithacus rubecula* in wintering grounds: effects of conspecific density, migratory

557   status and age. Ardea 89:363-373.

558   Tobias J (1997) Asymmetric territorial contests in the European robin: the role of settlement

559   costs. Anim Behav 54:9-21.

560   Wassenaar LI, Hobson KA (1998) Natal origins of migratory monarch butterflies at wintering

561   colonies in Mexico: new isotopic evidence. Proc Natl Acad Sci 95:15436-15439.

562   Wassenaar LI, Hobson KA (2003) Comparative equilibration and online technique for

563   determination of non-exchangeable hydrogen of keratins for use in animal migration

564   studies. Isotopes in Environmental and Health Studies 39:211-217.

565   Wunder MB, Kester CL, Knopf FL, Rye RO (2005) A test of geographic assignment using

566   isotope tracers in feathers of known origin. Oecologia 144:607-617.

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Table 1. Effects of several predictors on $\delta^{15}N$ levels in blood of robins, using δ^2H (a) and C2 (b) as proxy for geographic origin, estimated by Bayesian model averaging. Values represent posterior probability of each variable has a non-zero coefficient in the model (PP), averaged coefficients (conditional on the variable being in the model) and corresponding standard deviation. Variables with high relevance are represented in bold.

(a) Seventeen models averaged, the best 5 of which account for a cumulative probability of 0.67	Posterior Probability (PP)	Averaged Coefficients	SD of averaged coefficients
Intercept	**100**	**2.115**	**1.049**
Site (habitat)	**100**	**1.532**	**0.209**
Age	11.5	-0.215	0.235
Sex	7.5	-0.161	0.224
δ^2H	**83.2**	**-0.023**	**0.009**
Month (February)	**100**	**-0.648**	**0.866**
Month (After February)	**100**	**0.654**	**1.043**
Muscle	8.1	-0.136	0.201
Month*Site	0	0.000	0.000
Month*Age	0	0.000	0.000
Month*Sex	0	0.000	0.000
Site*Age	5.7	-0.088	0.333
Site*Sex	7.6	-0.319	0.435

Age*Sex	31.4	0.438	0.259

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(b) Seven models averaged, the best 5 of which account for a cumulative probability of 0.67	Posterior Probability	Averaged Coefficients	SD of averaged coefficients
Intercept	**100**	**8.310**	**1.274**
Site (habitat)	**100**	**1.371**	**0.201**
Age	7.1	-0.106	0.201
Sex	7	-0.101	0.201
C2	**100**	**-2.495**	**0.690**
Month (February)	**100**	**-0.854**	**0.712**
Month (After February)	**100**	**0.490**	**0.714**
Muscle	6.6	0.074	0.208
Month*Site	0	0.000	0.000
Month*Age	0	0.000	0.000
Month*Sex	0	0.000	0.000
Site*Age	6.3	-0.065	0.314
Site*Sex	6.3	-0.050	0.349
Age*Sex	11.7	0.278	0.247

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579   **Fig. 1. Box-plot of δ^2H values of robins sampled during the breeding season in Portugal (N=69),**

580   **France (N = 20), Scandinavia (N = 36) and Kaliningrad enclave, Russia (N = 7) and in winter in**
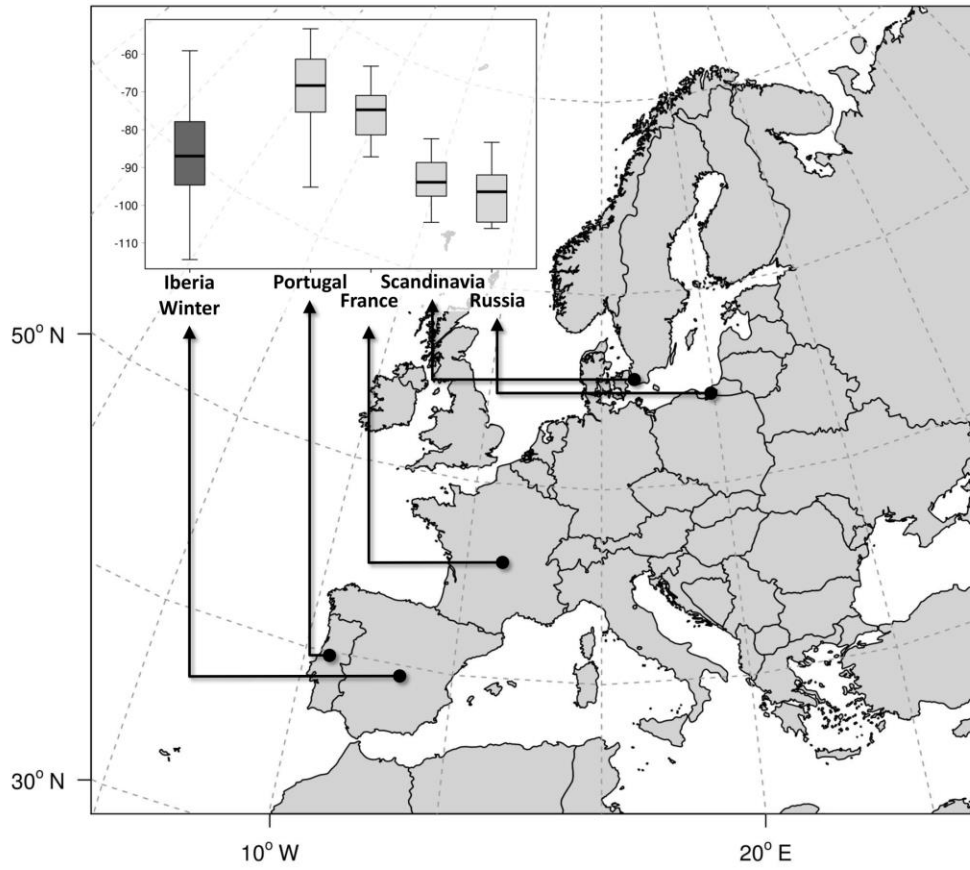
581   **Iberia (n=170).**

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583   **Fig 2. Relationship between (A) δ^2H in feathers (grown in spring/summer) or (B) wing-pointedness**

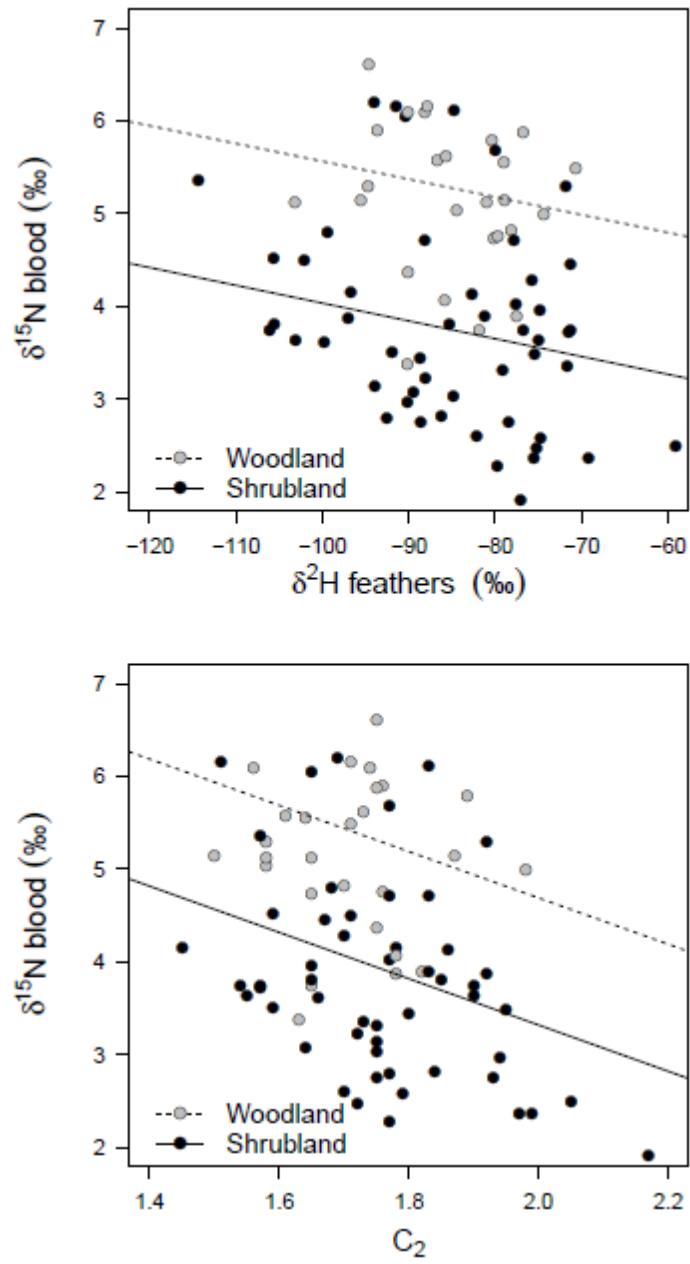584   **index (C_2), and δ^{15}N in whole blood of wintering robins. See Table 1 for statistics.**

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587   **Fig. 1**

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590   **Fig. 2**

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