1   **Faecal metabarcoding reveals pervasive long-distance impacts of garden bird feeding**

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14   **Abstract**

15

16   Supplementary feeding of wildlife is widespread, being undertaken by more than half of households

17   in many countries. However, the impact that these additional artificial resources have is unclear, and

18   impacts are assumed to be restricted to urban ecosystems. Using faecal metabarcoding of blue tit

19   (*Cyanistes caeruleus*) faeces collected in early spring from a 220km transect in Scotland with a large

20   urbanisation gradient we reveal the pervasiveness of supplementary foodstuffs in the diet of a wild

21   bird, being present in the majority of samples, with peanut (*Arachis hypogaea*) the single

22   commonest (either natural or artificial) dietary item. Consumption rates exhibited a distance decay

23   but remained high at several hundred metres from the nearest household and continued to our

24   study limit of 1.4km distant. Supplementary food consumption was associated with a near

25   quadrupling of blue tit breeding density and five days earlier breeding phenology. We show

26   woodland bird species using supplementary food to have increasing UK population trends on

27   average, while species that don't, and/or are outcompeted by blue tits, are likely to be declining. We

28   suggest that supplementary feeding impacts are larger and extend further than previously

29   appreciated and could be disrupting ecosystem dynamics and contributing to population-level

30   effects.

31

32   **Introduction**

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34   Supplementary feeding of garden wildlife is the most common active form of human-wildlife

35   interaction and occurs globally [1,2]. It is particularly widespread in the Western world, with over

36   half of all households participating in many Northern European and North American countries,

37   providing an ever-increasing variety of foodstuffs and feeder designs targeting more diverse species

38   each year [3,4]. Many mammal and insect species are intentionally provided with supplementary

39   food, but bird feeding is the commonest activity [2,5]. In the UK, for example, the wild birdfood

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40 market is estimated to be worth £241 million and supply around 150,000 tonnes of supplementary

41 food annually [6] while in the USA over 500,000 tonnes are supplied annually [2,7]. In the UK there is

42 estimated to be one supplementary bird feeder per 9 feeder-using birds [5], providing enough

43 resources nationally to feed three times the entire breeding populations of the ten commonest

44 feeder-using species year-round if they consumed nothing else [8]. Many mammal species such as

45 squirrels and rats also use these resources incidentally but at high frequencies [2,9,10]. While garden

46 wildlife feeding is actively encouraged by conservation organisations and official policy in a majority

47 of countries, including the UK and USA [11,12], such an enormous resource addition is likely to have

48 profound effects on both the organisms benefitting from it and their natural competitors and prey,

49 and these effects are far from well understood [1,13,14].

50

51 Research into the direct effects of supplementary garden wildlife feeding on the species utilising it to

52 date has developed a rather contradictory and mixed evidence base. While some studies have found

53 that supplementary feeding advances breeding phenology and improves reproductive success due to

54 increased resources [15–17], others have found the opposite, possibly due to poor nutrition [18,19].

55 Similarly, some studies have found benefits to individual user health [20] while others have found

56 detrimental effects [21]. Group health is also at risk as promoting artificial long-term aggregations of

57 novel individual and species interactions has facilitated disease spread and crossover, causing large

58 declines in some susceptible species [22,23]. There is consensus as to overwinter survival benefits,

59 to such an extent that migration patterns can be altered due to novel year-round resources [24,25].

60 Such a large-scale change in diet and feeding behaviour is also likely to have further effects that are

61 just being realised, such as changes to blood chemistry [26] and evolutionary traits [27]. Elucidating

62 any effects on the breeding ecology of feeder-using species is particularly important due to the

63 immediate fitness and population impacts.

64

65   One reason why the evidence to date is conflicting may lie in many studies not being able to account

66   for supplementary food uptake rates in their study organisms due to difficulties in diet detection,

67   and without this critical information it is impossible to assess large-scale impacts and background

68   consumption rates [1,13]. The advent of faecal metabarcoding provides a mechanism whereby this

69   can be overcome [28]. This method detects fragments of prey DNA contained within faeces non-

70   invasively, and while the technique is in its infancy and primarily applied to insect prey DNA [29,30],

71   many food types can be distinguished, including plant DNA [31] which are traditionally the

72   commonest supplementary foods provisioned for garden wildlife [1].

73

74   Most studies to date have provided additional experimental supplementary food and assumed a

75   distance decay in uptake [18,19], however this has two major caveats. Firstly, it does not account for

76   background supplementary feeding rates from resources provided by the local human population

77   unconnected to the study itself, with such cross-contamination rates probably high due to the

78   ubiquity of supplementary provisioning [1,5]. Secondly, the distance decay is unknown in most

79   species and therefore supplementary feeding may be occurring over a wider scale than imagined

80   [17,32]. Assessing diet composition directly through faecal metabarcoding without providing

81   additional experimental resources overcomes these caveats.

82

83   The largely unknown distance over which the effects of supplementary feeding occur is also

84   evidenced by the literature focus that impacts are only, or overwhelmingly, encountered in the

85   urban environment, even altering community structure there [33–35]. This ignores that many

86   provisioned bird, mammal and insect species are able to move long distances in search of reliable

87   feeding opportunities [32], and that rural human dwellings are likely to provide more supplementary

88   food per household than urban dwellings [4]. Additionally, research to date has focussed solely on

89   the species that utilise supplementary feeding without considering those that do not. It is likely that

90   increases in populations of supplementary food-using species [33] and individual competitiveness

91   will have a negative effect on their competitor and prey species that do not benefit from

92   supplementary feeding, as background habitat availability is unchanged. Furthermore, if the effects

93   of supplementary feeding are felt over a wider area than solely urban environments, the impacts on

94   community composition and conservation could be far-reaching [16]. Therefore, it is crucial to

95   understand over what distances feeder-using species are travelling to make use of supplementary

96   food resources and what impact this is having upon their ecology, fitness and populations.

97

98   In this study, we analyse data from a widespread and common avian supplementary food user, the

99   blue tit (*Cyanistes caeruleus*), across a 220km transect of Scotland [36] with a large gradient in

100   distance to human habitation and therefore supplementary feeding. We use faecal metabarcoding

101   to uncover what proportion of faeces contain supplementary garden bird food immediately prior to

102   breeding and over what distance supplementary food use is occurring, predicting that use will

103   decline with increasing distance. We then use site average supplementary food use to determine

104   effects on breeding ecology. Finally, we assess whether the utilisation of supplementary food is

105   affecting recent population trends in blue tits and their competitors (insectivorous forest bird

106   species) across the UK, hypothesising that if supplementary feeding is supporting higher populations

107   of those species using it, these inflated populations may be having detrimental effects on the

108   populations of competitor species that do not, contributing to human-mediated homogenising

109   impacts on biodiversity [37,38]. We believe that this focal study system is highly representative of

110   many supplementary feeding systems and that insights garnered should extrapolate across many

111   systems.

112

113   **Methods**

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115   **Field data collection**

116

117   Field data were collected from a transect of 39 predominantly deciduous woodlands during the

118   springs of 2014-2016 [36]. At each site there were six Schwegler 1B 26 mm hole nestboxes

119   distributed at approximately 40 m intervals. From mid-March in 2014 and 2015 the base of each

120   nestbox was lined with greaseproof paper which was replaced when damaged or heavily soiled and

121   removed at the onset of nest building or once a bird had attempted removal by pulling it through

122   the hole [29]. Each nestbox was visited on alternate days and all faeces on the greaseproof paper

123   were removed with sterilised tweezers (after use they were wiped with laboratory tissue and

124   flamed), with up to a maximum of three faeces collected in a 2 ml Eppendorf tube containing pure

125   ethanol, and the number of faeces collected recorded (with the exception of samples in early 2014).

126   Faecal samples were stored at −18°C within a day of collection and transferred to a −20°C freezer at

127   the end of each field season. Samples were collected from 19 March in 2014 and 18 March in 2015

128   until nest building, giving a median sampling range of 20 days per site in 2014 and 24 days in 2015,

129   and a maximum sampling range at a site of 34 days. Faecal samples were not collected in 2016.

130

131   The date of first egg laying was recorded for each nestbox (taken as the previous day if two eggs

132   found, as blue tits lay one egg daily [39]) and nestboxes were designated as occupied in a particular

133   year if at least one egg was laid in a nest by a blue tit. Clutch size was counted once all eggs were laid

134   and incubation had begun. All nestlings were fitted with a metal identification ring under license and

135   productivity was defined as the number of nestlings successfully fledged (number of nestlings alive

136   at day 12 – number of dead nestlings found in nestbox subsequently). Parent birds of both sexes

137   were also captured and fitted with metal identification rings under license and their mass, sex and

138   age (first year breeder or second year plus) recorded. Latitude (site range 55.98 - 57.88°N) and

139   elevation (10 - 433 m) were obtained for each nestbox [36] and the Euclidian distance to nearest

140   human habitation (33 – 1384m) was calculated for each nestbox after finding the coordinates of the

141   nearest human dwelling via Google maps [40]. Due to the high incidence of supplementary bird

142   feeding in the UK [2,4] this should be a good predictor of feeder availability.

143

144 **Molecular protocol and bioinformatics**

145

146 A total of 959 faecal samples were collected and 793 fully processed, with these selected by

147 balancing subsampling across nestboxes and dates and an upper limit of ten samples per nestbox

148 per year enforced [29]. If multiple faeces were present in the sample tube, part of each was used for

149 DNA extraction. Thirty samples were processed in duplicate by dividing the faecal sample into two to

150 assess repeatability. Twenty-four controls were also included (six extraction negatives, nine PCR

151 negatives and nine PCR positives using *Inga pezizifera* as a non-native plant PCR positive).

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153 DNA extraction was performed using the QIAamp DNA Stool Mini kit, following the protocol for

154 pathogen detection with a few customisations to improve yields [29]. PCR amplification was

155 targeted on three loci (of importance to this manuscript including the standard plant barcoding gene

156 rbcL, also COI and 16S) and amplified a 'minibarcode' of 184-220 base pairs. The second stage of PCR

157 added indexed Illumina adaptors to the amplicons from each sample with amplicons multiplexed in

158 three pools; each pool was then sequenced on an Illumina MiSeq using 150 base pair paired-end

159 reads.

160

161 Sequencing reads were demultiplexed and processed as per the bioinformatics protocol detailed in

162 Shutt *et al* 2020. Output sequences were clustered into molecular operational taxonomic units

163 (MOTUs) and the taxonomic identity of MOTUs determined using a BLAST search of the reference

164 set of MOTUs against GenBank and BOLD public databases.

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166 Samples were tested for the presence of blue tit at the 16S locus and those with fewer than 100

167 reads of blue tit were excluded from further analyses (n=9) following [29]. No non-blue tit avian DNA

168 was found in any sample. All nine PCR positive control samples contained MOTUs attributable to

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169   *Inga pezizifera* (range of reads = 4,007 – 12,697) and no more than 19 reads of another MOTU. All

170   nine PCR negative control samples and three of the six extraction negative control samples

171   contained no more than 22 reads of any MOTU. The remaining three extraction negative control

172   samples showed high numbers of reads (n = 991 – 6,302) from contaminating tomato but nothing

173   else. Systematic contamination at the rbcL locus was investigated by assessing row and column

174   content correlation [29] and no systematic contamination found. As there were few cases where a

175   control had >20 reads for any nontarget MOTU, we adopted 20 reads as the cut-off for identifying

176   MOTU presence.

177

178   Present MOTUs with a less than 90% match to their best hit were then discarded as inconclusively

179   identified and remaining MOTUs amalgamated based on their genus identification, as species

180   identification was seen to be very slight and not reliable at the rbcL locus. 185 plant genera were

181   identified and compared with common artificial garden bird foods to extract relevant genera. All

182   further analyses were carried out only on these identified artificial food taxa within focal samples

183   (excluding duplicate and control samples, and those not confirmed to be from blue tit).

184

185   **Statistical analyses**

186

187   The first model examined how artificial food consumption varied with regards to environmental

188   factors. A binary value of whether artificial food was present in a faecal sample or not was used as

189   the response variable of a Bayesian generalised linear mixed model (GLMM) [41], with distance to

190   nearest human habitation, date, elevation, latitude, year as a factor and number of faeces in sample

191   (1-3 and U for unknown, factor) as fixed predictor variables. All numeric predictor variables were

192   mean centred for ease of interpretation [42] and to facilitate model convergence. Distance to

193   human habitation was analysed on the logarithmic scale due to right skewed data and from the

194   perspective of a distance decay. Date was analysed as a deviation from the respective sample site

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195   mean per year, as different sites and years have different blue tit breeding phenology. Site and

196   nestbox were included as random effects and the model was run for ten million iterations, removing

197   the first 100,000 as burn-in and thinning every 100. A binomial error structure was used along with

198   parameter expanded priors for the variance terms with residual variance fixed at 0.5. Repeatability

199   in the detection of artificial food consumption was analysed on 29 repeat samples (one was

200   removed during quality control steps above) and their corresponding focal samples by calculating

201   Jaccard similarity.

202

203   The second set of models aimed to infer whether artificial food consumption affected the breeding

204   parameters and fitness of the birds and to do so the mean artificial food consumption at each site

205   was calculated. Site mean consumption was used rather than nestbox level consumption for two

206   reasons: i) blue tits often do not nest in a nestbox they are roosting in prior to breeding, but rather

207   nearby, precluding direct attribution, ii) faeces were only produced in certain nestboxes so most

208   nesting attempts are not in a nestbox from which faeces were collected. In addition, if we assume

209   that the estimated mean artificial food consumption at a site is representative of the nests then

210   framing the analysis at the site level benefits the sample size and power. Firstly, nestbox occupancy

211   was treated as the response variable in a Bayesian GLMM [41] containing mean artificial food

212   consumption (varying 0-1), elevation, latitude and year as a factor as fixed predictor variables, and

213   site and nestbox as random effects, with all numeric predictor variables mean-centred. A binomial

214   error structure was used with similar priors to the first model. Similar models were then run with

215   first egg laying date, clutch size, productivity and adult blue tit mass as response variables with

216   Gaussian error structures and no fixed residual variance. In addition to the standard fixed predictor

217   variables mentioned above, the mass model also contained the age and sex of the bird.

218

219   To gain an indication of whether supplementary feeder usage and competition with blue tits may be

220   affecting UK forest bird populations over time, the 25-year population trends of potential

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221   competitor forest bird species were analysed. Species were included based on the following criteria:

222   average body length less than twice a blue tit (<24cm), foraging substantially on foliage-gleaned

223   invertebrates during the breeding season, occupying wooded habitats, and with a substantial

224   enough UK population to have a 25-year BTO BirdTrends population trend estimate [43]. For each

225   species, the population trend was coded based on five categories (1 = > -50%, 2 = -11 - -50%, 3 = -10

226   - +10%, 4 = +11 - +50%, 5 = > +50%) [43], supplementary garden bird feeder usage was defined (1 =

227   rare or never, 2 = frequent (>5% occurrence in 2020 RSPB Big Garden Birdwatch

228   www.rspb.org.uk/get-involved/activities/birdwatch/results/)), competition status versus blue tit

229   categorised (1 = outcompeted (average lower mass and/or published evidence of outcompetition for

230   food or breeding sites [43–45]), 2 = not outcompeted (all others), and competition type valued (1 =

231   food, 2 = food and breeding site (if nesting in small cavities [46]); this yielded 21 species. A general

232   linear model [47] was conducted containing coded population trend as the response variable, and

233   feeder usage, competition status versus blue tit, and competition type as predictor variables. As the

234   sample size was small for linear modelling, three Welch's Two-sample T-tests were also conducted

235   to analyse independently how coded population trend varied with regards to i) supplementary

236   feeder usage, ii) competition status, and iii) competition type.

237

238   **Results**

239

240   Out of 788 active faecal samples, 53% (n = 414) contained evidence of artificial food consumption.

241   Five artificial foodstuffs were identified, with peanut (*Arachis*) by far the most common, present in

242   49% of total samples. Sunflower (*Helianthus*) was also highly prevalent (17%), with maize (*Zea*) (9%),

243   barley (*Hordeum*) (5%) and millet (*Panicum*) (1%) rarer. 63% of samples containing artificial food

244   contained only one type, with 37% showing evidence of more than one artificial foodstuff, and two

245   samples containing all five. The repeatability of finding evidence of either a peanut (Jaccard

246   similarity = 0.923) or artificial food in general (Jaccard similarity = 0.923) within a repeat sample was

247   very high.

248

249   Increasing distance to nearest human habitation predicted a significant reduction in artificial food

250   consumption (Table 1, Fig 1a). Different years also had significantly different artificial food

251   consumption rates, such that in 2014 the model predicted a 93% chance of a faecal sample

252   containing artificial foodstuffs at the closest site distances examined in our study (33m) reducing to

253   29% at 200m, 6% at 500m, and 1% at our furthest site distances examined (1384m), while in 2015

254   these figures were higher, with 97% chance at 33m, 51% chance at 200m, 15% chance at 500m, and

255   2% chance at 1384m to nearest human habitation (Fig 1a). The faeces collected at the site 1384m to

256   nearest human habitation did however show artificial food consumption in 75% of samples in 2015

257   (Fig 1a).

258

259   Artificial food use also significantly declined through the sampling period, in the run-up to breeding

260   (Table 1, Fig 1b). In 2014 the model predicted that there was a 65% chance of a faecal sample

261   containing artificial food at our earliest sampling times (70 days before mean first egg laying),

262   declining to 24% by 30 days to egg laying and 7% by egg laying (Fig 1b). For 2015 these figures were

263   elevated to 83% at the earliest times, 44% in the mid time frame and 17% at egg laying (Fig 1b).

264   Elevation and latitude showed no significant effect on artificial food consumption, and combining

265   more faeces per sample increased the likelihood of artificial food detection (Table 1). Site and

266   nestbox random effects explained similar amounts of variance (Table 1).

267

268   Increased artificial food consumption significantly predicted a large increase in nestbox occupation,

269   from a 20% likelihood with no artificial food consumption to a 75% likelihood with artificial food

270   present in every faecal sample (Table 2, Fig 2a). Artificial food consumption also significantly

271   advanced egg laying date by five days (from day 122 to day 117, Table 2, Fig 2b). However, it did not

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272   significantly affect clutch size, productivity or the mass of either male or female parent blue tits

273   (Table 2).

274

275   From the t-tests, it was shown that population trends of competitor bird species were significantly

276   more likely to be increasing for species using supplementary feeders and decreasing for species not

277   using them (t = -2.3, df = 18.0, p = 0.03, Fig 3). Additionally, population trends for species

278   outcompeted by blue tits were significantly more likely to be decreasing and those not outcompeted

279   by blue tits increasing (t = -2.4, df = 17.8, p = 0.03, Fig 3). While competition type did not significantly

280   predict population trends (t = 0.9, df = 17.7, p = 0.4), a non-significant trend of those species

281   competing with blue tits for both food and nesting sites declining more than those only competing

282   for food was observed (Fig 3). The linear model analysing population trends showed no significant

283   effects, but trends were strong and in the direction indicated by the t-tests (Table 3), with a species

284   that didn't use feeders, is outcompeted by blue tits, and competes for both food and nesting sites

285   with blue tits far more likely to be declining than a converse species (Fig 3, Table 3).

286

287   **Discussion**

288

289   Supplementary foodstuffs were shown by faecal metabarcoding to be present in the majority (53%)

290   of blue tit faecal samples immediately prior to breeding, with peanuts identified in more faecal

291   samples (49%) than any other single dietary item, natural or artificial, with the most frequent natural

292   prey item, *Argyresthia goedartella*, present in 34% of the same samples for comparison [29]. As the

293   study area incorporates some of the more remote parts of the UK and we show blue tits travelling

294   almost 1.4km to use supplementary bird feeders during a time of year when movement is thought to

295   be restricted around breeding territories [39], this study reveals just how prevalent and ubiquitous

296   supplementary food is in the diet of a wild bird species in a country with high provisioning rates

297   [2,4,32]. Indeed, these results reveal it likely that supplementary food is available to almost every

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298   blue tit (and other feeder-using bird species, as blue tits are relatively sedentary and short-winged

299   [39]) in the UK, with implications likely to extrapolate across large parts of the western world due to

300   similarly high supplementary feeding rates [11,12]. We infer from this that any impacts from

301   supplementary feeding will therefore be felt far wider than solely in urban environments as has

302   hitherto been assumed [1,33]. As we find that supplementary food usage is strongly associated with

303   a dramatic increase in nestbox occupation (a proxy of breeding density) and an advance in lay date,

304   it is perhaps unsurprising then that we find the national population trends of supplementary feeder-

305   using woodland bird species are increasing on average while the populations of competitor species

306   not benefitting from supplementary feeders are decreasing.

307

308   As predicted, supplementary food use declined with increasing distance to nearest human

309   habitation. While this relationship has previously been assumed [18,19], we believe this

310   quantification to be the first in a natural situation, made possible by faecal metabarcoding, with

311   inferences about diet composition proving highly repeatable. The usage rate was still considerable at

312   several hundred metres, however, beyond the cut-off distance used between treatments and/or

313   nearby human habitation in previous supplementary feeding experiments [19], which may

314   contribute a background or even confounding effect in many contexts. The distance travelled to

315   supplementary food, and overall usage rates, differed markedly between the two years in our study,

316   with 2015 having higher values than 2014. We believe this to be due to 2015 being considerably

317   colder across our study region, as natural food levels are lower in these conditions [29,48], and

318   benefits from supplementary feeding larger due to natural nutrient limitation, concurring with

319   previous studies [15]. In addition to a distance decay, supplementary food usage also declined over a

320   temporal gradient throughout our study period. This is presumably due to large increases in natural

321   invertebrate prey as spring progresses [29,49] alongside individuals being more restricted to

322   breeding territories as nesting commences [39]. There was no impact of the geographic gradients of

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323   latitude and elevation, which vary substantially over the study region, indicating a widespread

324   similarity in uptake.

325

326   Previous research has developed a mixed picture of the benefits and costs of supplementary feeding

327   conferred on the species using these extra resources, including blue tits. Using faecal metabarcoding

328   to identify definite rather than assumed supplementary food intake across a real-world gradient

329   without the need for additional experimentation has allowed us to demonstrate major fitness

330   benefits conferred upon blue tits at sites with higher supplementary food uptake. Supplementary

331   food use predicted an almost four-fold increase in nestbox occupation, an accurate proxy for

332   breeding density in our system due to sites having equal numbers of equally spaced nestboxes [36],

333   between lowest and highest values. We expect increasing breeding densities to extrapolate to other

334   feeder-using species as feeder presence increases local abundances of feeder-using species [34],

335   providing an explanation for bird breeding densities varying with human household densities [50].

336   The five-day advancement in egg laying we identify is very similar to that found in previous studies

337   [15,19] and may comprise the limit of the plastic response of phenology to the lifting of an energetic

338   constraint, with earlier laying associated with higher productivity [51]. Perhaps this is why individual

339   nest productivity didn't decline due to density effects as might be imagined [52], but instead showed

340   a minor increase. Clutch size not being significantly predicted by supplementary feeding agrees with

341   previous studies [19] and reinforces that environmental aspects seem to have little effect on clutch

342   size [36].

343

344   Many species utilising supplementary feeding, such as blue tits, are common, adaptable and already

345   at population carrying capacity [38,53]. Boosting the productivity, survival, fitness and breeding

346   densities of such species without any increase in available habitat or natural resources is likely to

347   negatively effect their competitors, particularly those not utilising the new artificial resources [38].

348   This may be particularly evident in forest species, as rather than replace natural resources that have

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349   been lost due to landscape intensification as might be the case for farmland species [54],

350   supplementary feeding is solely providing additional artificial resources to certain species. To this

351   end, we demonstrate that populations of UK forest bird species that don't use supplementary

352   feeders are likely to be declining whereas those that do are likely to be increasing over the last 25

353   years. Artificial feeding is therefore likely a driver of population change, in line with recent evidence

354   [33]. Species that are dominated in competition by blue tits are also likely to be declining whereas

355   those that are dominant over blue tits are likely to be increasing, while competing with blue tits for

356   limited breeding sites in addition to food resources appears to non-significantly increase the risk of

357   population decline.

358

359   While we do not analyse a causal link between supplementary feeding and the declines of these

360   competitor species due to the increased competitiveness of feeder-using species, the mechanisms

361   whereby increased blue tit densities could impact other species are clear. For example, blue (and

362   great) tits are known to frequently evict species such as willow tit and lesser spotted woodpecker

363   from nest holes that they have excavated [44,55], kill pied flycatchers when claiming nesting sites

364   [45], and dominate subordinate marsh and willow tits at food resources [39]. Abundant and

365   permanent feeding might also eliminate any competitive advantage other species (such as marsh

366   tits) exhibit in finding and exploiting natural resources first [56], before being outcompeted by

367   dominant species like blue tits, or migrating to warmer climes for winter to avoid winter starvation,

368   as for pied flycatchers [57]. Supplementary feeding therefore, although well-intentioned and

369   beneficial to the species partaking, may be shifting the competitive balance of natural ecosystems

370   and the structures enabling community coexistence, favouring certain species at the expense of

371   others, and contributing to human-mediated ecological homogenisation [37,38].

372

373   In conclusion, we reveal through faecal metabarcoding the pervasiveness of supplementary

374   foodstuffs in the diet of a wild bird and the large benefits conferred on breeding density and

375   phenology from using these substantial additional resources. We also show that the distances

376   travelled to utilise these resources are further than previously imagined, even in a largely sedentary

377   species at a time of year when movement is thought to be restricted. This indicates that the effects

378   of supplementary feeding on ecosystems are likely to extend far beyond just urban environments as

379   has hitherto been assumed. Finally, we demonstrate that species making use of supplementary

380   resources are likely to be increasing in population while those that do not are likely decreasing,

381   possibly due to shifting competition balances and ecosystem dynamics. As supplementary

382   provisioning of wildlife (both intentional and incidental) is hugely prevalent and increasing [2], it may

383   have large and widespread ramifications for biodiversity conservation, and we urge caution upon

384   policy makers advocating supplementary feeding for wildlife engagement.

385

386   **Acknowledgements**

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392

393   **Authors' Contributions**

394

395   JDS conceived and managed the study, conducted the fieldwork, contributed to data curation,

396   analysed the data and wrote the manuscript. UHT contributed to data curation. JAN devised and

397   conducted all laboratory protocols. All authors contributed to later manuscript editing.

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399   **Data Availability Statement**

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401   Data will be made publicly available via the Dryad digital repository upon acceptance.

402

403   **References**

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557   **Figures and Tables**

558

559   **Table 1** The effects of multiple predictors on whether artificial food has been consumed. Results are
560   taken from a Bayesian GLMM with categorical error structure and logit link function, showing slope
561   estimates and credible intervals for each fixed and random term, with significance asterisks for
562   significant and near-significant terms (pMCMC ≤0.1 ° ≤0.05* ≤0.01** ≤0.001***). Numeric predictor
563   variables are mean centred, distance to habitation is also logged, and date has been adjusted for
564   phenology by representing days before mean first egg laying at a given site within a given year. The
565   intercept year value is 2014 and number of faeces is one.

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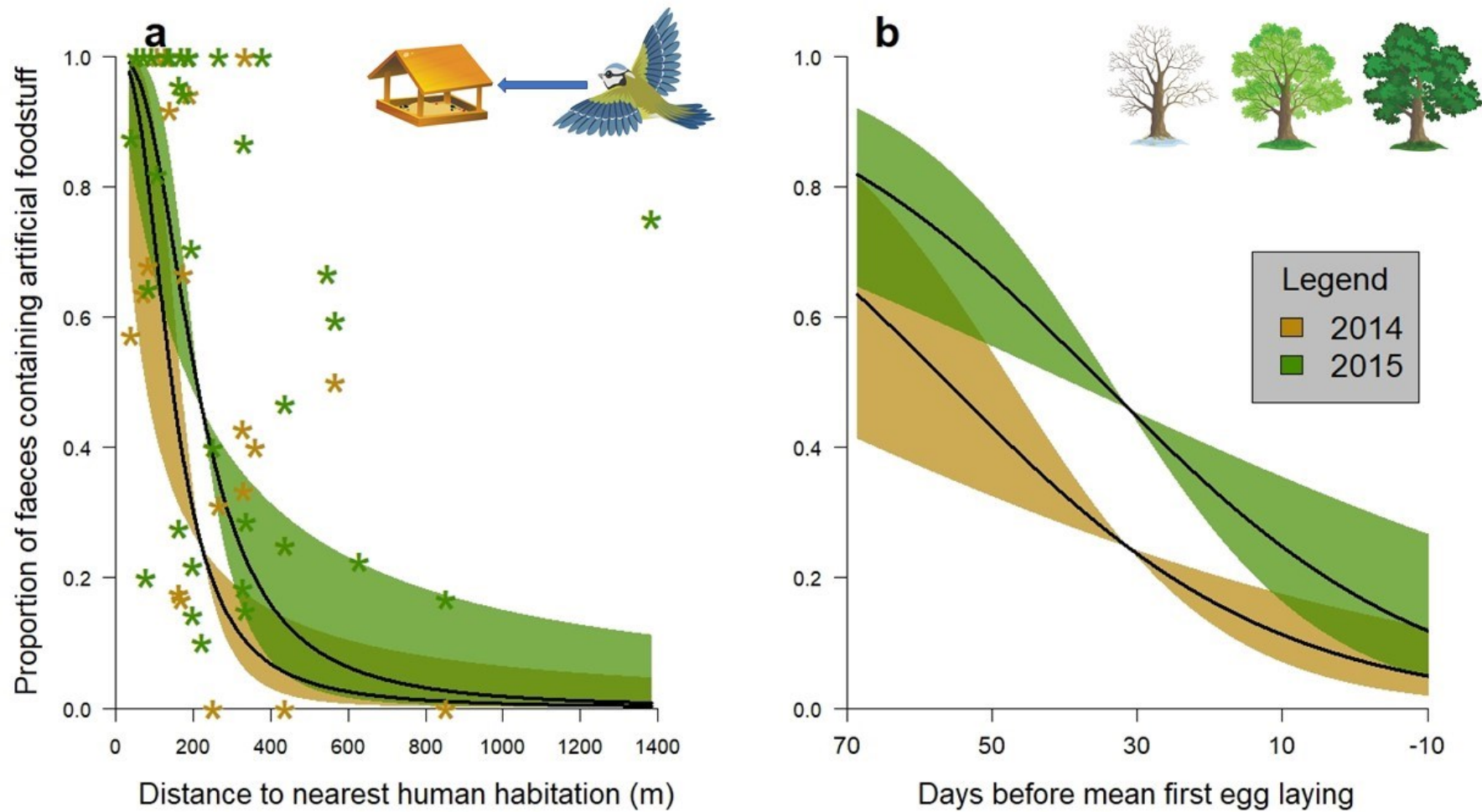| Fixed Effects	Coefficient (C.I.'s)
Intercept	-1.12 (-2.64 – 0.44)
Distance to habitation	-1.97 (-3.10 – -0.80) ***
Days before laying	0.04 (0.02 – 0.07) ***
Elevation	0.0005 (-0.0096 – 0.0104)
Latitude	-0.81 (-2.73 – 1.08)
Year 2015	0.95 (-0.12 – 2.03) °
Faeces = 2	-0.07 (-1.21 – 1.07)
Faeces = 3	1.37 (0.42 – 2.36) **
Faeces = unknown	2.25 (0.88 – 3.67) ***
Random Effects	
Site	5.85 (1.40 – 11.44)
Nestbox	5.01 (2.27 – 8.24)

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Figure 1: Illustrating the significant effects of a) distance to nearest human habitation and b) sampling date (adjusted for the phenology of the site within year by days before mean first egg laying within site and year) on the probability of artificial food consumption by blue tits, described in Table 1. The predicted response of each is shown in 2014 (gold) and 2015 (green), additionally showing the near-significant effect of year from the model (Table 1). Asterisks in a) show the proportion of faeces containing artificial foodstuff per site per year, not the 0/1 response analysed in the model.

Table 2 The effects of artificial food intake and other predictor variables on a range of blue tit breeding parameters. Results are taken from Bayesian GLMM's, showing slope estimates and credible intervals for each fixed and random term, with significance asterisks for significant terms (pMCMC ≤0.05* ≤0.01** ≤0.001***). Occupancy is presented on a binomial scale with the other response variables gaussian. Numeric predictor variables are mean centred, and the intercept year is 2014, with intercept values of first year adult and female for the mass model. Artificial food intake was calculated as a per site per year proportion of faeces containing artificial food and applied to all nests at that site in that year.

| Fixed Effects	Occupancy Coefficient (C.I.'s)	Egg Laying Coefficient (C.I.'s)	Clutch Size Coefficient (C.I.'s)	Productivity Coefficient (C.I.'s)	Mass Coefficient (C.I.'s)
Intercept	0.06 (-0.44 – 0.60)	119.33 (117.57 – 121.06)	8.70 (8.28 – 9.12)	6.96 (6.25 – 7.63)	10.71 (10.57 – 10.84)
Artificial Food	2.47 (1.14 – 3.70) ***	-4.39 (-8.50 – -0.17) *	-0.15 (-0.96 – 0.62)	0.87 (-0.47 – 2.11)	0.07 (-0.12 – 0.25)
Elevation	-0.01 (-0.01 – -0.01) ***	0.02 (0.01 – 0.03) **	0.001 (-0.002 – 0.004)	0.002 (-0.003 – 0.006)	-0.0006 (-0.0012 – 0.0002)
Latitude	-1.30 (-2.11 – -0.61) ***	0.79 (-1.82 – 3.20)	-0.55 (-0.99 – -0.09) *	-0.77 (-1.52 – -0.07) *	-0.13 (-0.25 – -0.03) *
Year 2015	1.20 (0.61 – 1.74) ***	4.41 (2.89 – 6.11) ***	-1.13 (-1.59 – -0.66) ***	-3.41 (-4.17 – -2.61) ***	0.003 (-0.14 – 0.12)
Year 2016	0.60 (0.08 – 1.18) *	8.19 (6.64 – 9.75) ***	-0.64 (-1.14 – -0.18) **	-1.54 (-2.34 – 0.74) ***	-0.13 (-0.27 – -0.002)
2nd Year +					0.06 (-0.04 – 0.16)
Sex Male					0.05 (-0.04 – 0.14)
Random Effects					
Site	0.71 (0 – 1.57)	12.41 (4.29 – 22.54)	0.24 (0 – 0.58)	0.60 (0 – 1.42)	0.008 (0 – 0.023)
Nestbox	0.74 (0 – 1.69)	2.76 (0 – 7.16)	0.15 (0 – 0.47)	0.15 (0 – 0.58)	0.003 (0 – 0.052)
Residual	0.5	30.98 (25.00 – 37.09)	2.90 (2.41 – 3.42)	8.19 (6.93 – 9.52)	0.23 (0.20 – 0.27)
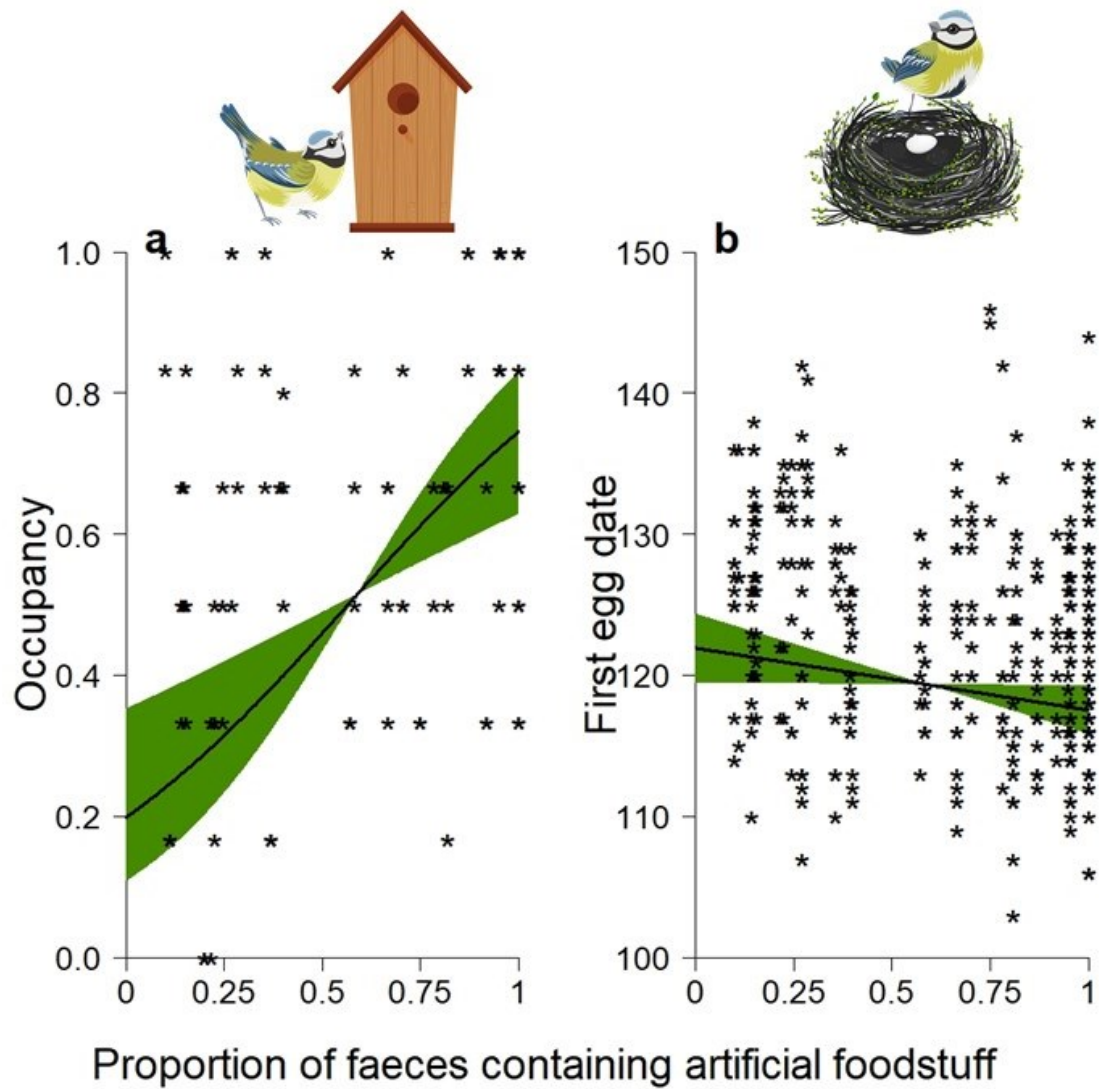
Figure 2 Illustrating the significant effects of site-level artificial food intake on a) nestbox occupancy probability and b) first egg laying date, taken from results shown in Table 2. Asterisks in a) depict site per year occupancy rates rather than the 0/1 occupied response analysed in the model. Predictions correspond to 2014.

Table 3 How 25-year population trend varies with use of garden feeders, competition status with blue tits, and whether only food or both food and nest sites are competed for. No terms are significant, the intercept value shows a species that doesn't use feeders, is outcompeted by blue tit and competes only for food.

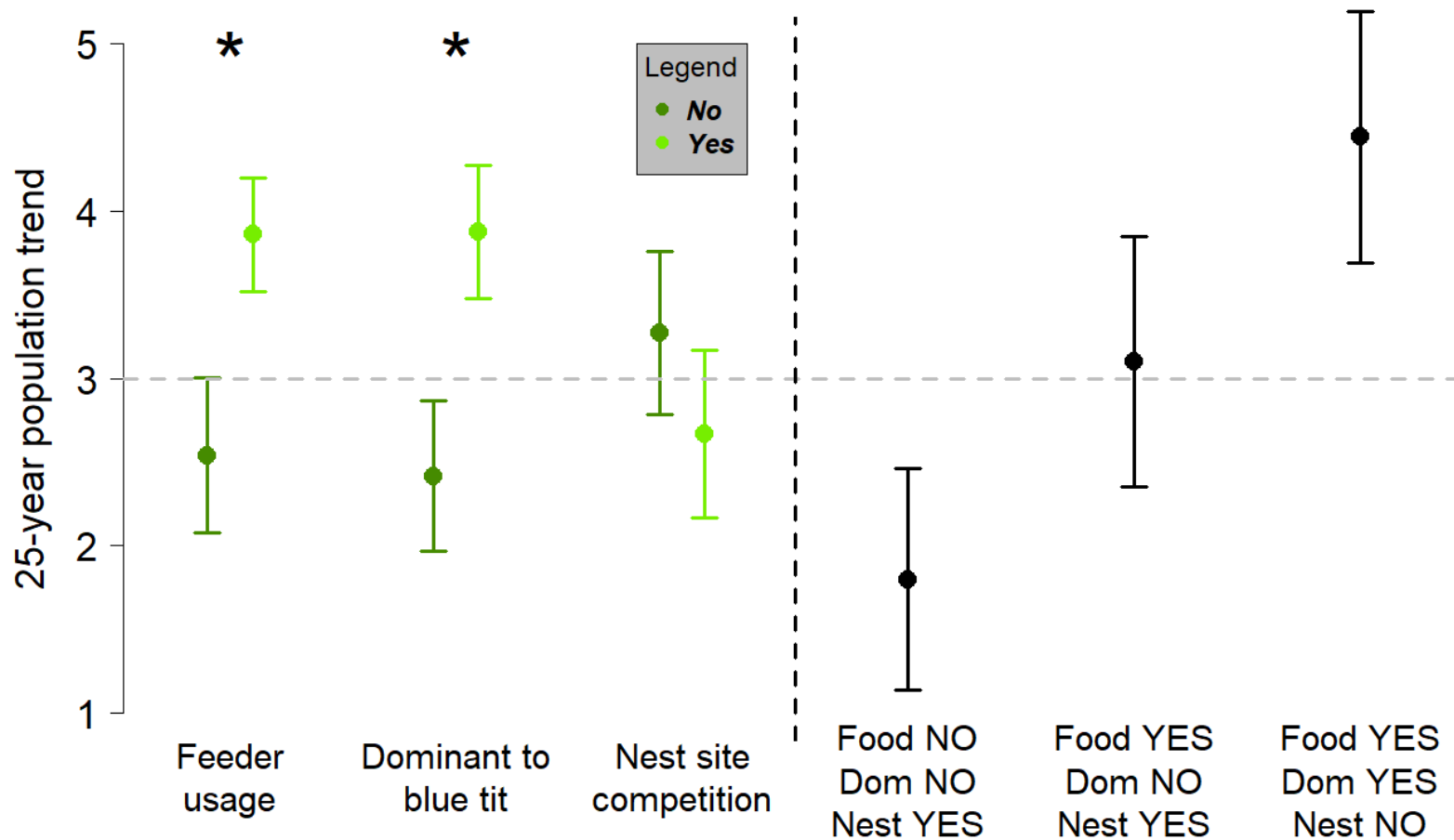| Fixed Effects	Estimate ± SE
Intercept	2.49 ± 0.50
Feeder use	1.30 ± 0.75
Not outcompeted by blue tit	0.65 ± 0.70
Nestbox competition	-0.69 ± 0.66

Figure 3 Differing population trends of twenty insectivorous woodland bird species in the UK with regards to i) supplementary garden feeder usage ii) behavioural dominance in comparison to blue tits and iii) whether the species competes for nest sites with blue tits, with values depicting mean ± standard error with significance asterisks above significant differences as determined by a t-test, all presented to the left of the horizontal dashed line. To the right of the horizontal dashed line are linear model estimates ± standard error depicting hypothetical species that vary in whether they use supplementary food, are dominant to blue tit and compete for nest sites with blue tit. A trend value of 1 represents a large population decline (> -50%) and 5 a large population increase (>50%) over 25 years, with a stable population (trend value = 3) shown by a grey dashed line.