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2   DR. DARA ASHLEY SATTERFIELD (Orcid ID : 0000-0003-3036-5580)

3   PROF. MARK D HUNTER (Orcid ID : 0000-0003-3761-8237)

4   DR. TYLER FLOCKHART (Orcid ID : 0000-0002-5832-8610)

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13   **Migratory monarchs that encounter resident monarchs show life-history changes and**

14   **higher rates of parasite infection**

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16   *Dara A. Satterfield,*[1] *John C. Maerz,*[2] *Mark D. Hunter,*[3] *D.T. Tyler Flockhart,*[4] *Keith A.*

17   *Hobson,*[5] *D. Ryan Norris,*[4] *Hillary Streit,*[3] *Jacobus C. de Roode,*[6] *and Sonia Altizer*[1]

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19

20

21   [1]Odum School of Ecology, University of Georgia, Athens, GA 30602, USA;

22   dara.satterfield@gmail.com; saltizer@uga.edu

23   [2]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602,

24   USA; jcmaerz@uga.edu

25   [3]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI

26   48109, USA; mdhunter@umich.edu; hbstreit@umich.edu

27   [4]Departmment of Integrative Biology, University of Guelph, Guelph, ON N1G2W1, Canada;

28   dflockha@uoguelph.ca; rnorris@uoguelph.ca

29   [5]Department of Biology, Western University, London, ON N6A5B7, Can.; khobson6@uwo.ca

30   [6]Department of Biology, Emory University, Atlanta, GA 30322, USA; jderood@emory.edu

31   *Corresponding author: Smithsonian National Zoo, PO Box 37012, MRC 5503, Washington, DC

32   20013; 770-584-4965

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MDH, HS, DAS and JR conducted cardenolide analyses, KAH, DTTF, DRN and DAS conducted

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41   **ABSTRACT**

Environmental change induces some wildlife populations to shift from migratory to resident

behaviours. Newly formed resident populations could influence the health and behaviour of

remaining migrants. We investigated migrant-resident interactions among monarch butterflies

and consequences for life history and parasitism. Eastern North American monarchs migrate

annually to Mexico, but some now breed year-round on exotic milkweed in the southern U.S.

and experience high infection prevalence of protozoan parasites. Using stable isotopes (δ^2H,

δ^{13}C) and cardenolide profiles to estimate natal origins, we show that migrant and resident

monarchs overlap during fall and spring migration. Migrants at sites with residents were 13 times

more likely to have infections and three times more likely to be reproductive (outside normal

breeding season) compared to other migrants. Exotic milkweed might either induce these states

or attract migrants that are already infected or reproductive. Increased migrant-resident

interactions could affect monarch parasitism, migratory success, and long-term conservation.

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INTRODUCTION

Wildlife populations that engage in partial migration include both migrant and resident

individuals, with migrants moving between habitats seasonally and residents remaining in the

same area throughout the year (Newton 2008; Chapman *et al.* 2011a, b). Migrants and residents

often differ in reproductive behaviour, body size, predation risk, and in some cases, pathogen

infection (Adriaensen & Dhondt 1990; Hendry 2004; Hebblewhite & Merrill 2009; Altizer *et al.*

2011). Seasonal migrants and residents can interact and share habitat for part of the year, as

reported for Canada Geese (*Branta canadensis*), wildebeest (*Connochaetes taurinus*), and other

species (Caccamise *et al.* 2000; Estes 2014; Chapman *et al.* 2012), and such interactions are

likely widespread across taxa, given the high incidence of partial migration in wildlife

populations (Chapman *et al.* 2011a). However, the ecological implications of migrant-resident

interactions represent a critical knowledge gap in migration biology (Chapman *et al.* 2011a, b;

Brodersen *et al.* 2008). Migratory animals that share habitat with residents could encounter

additional resources or mates, but they might also experience greater exposure to natural enemies

or factors that alter their behaviour and movement.

Examining the ecological consequences of migrant-resident relationships is important for

the conservation of migratory species (Chapman *et al.* 2011a), many of which are now

threatened (Wilcove & Wikelski 2008). Residency is becoming more common in some

populations (Berthold 1999; Griswold *et al.* 2011), as birds, ungulates, and other animals

establish or expand resident sub-populations due to habitat alteration, climate change, or

supplemental feeding (Sutherland 1998; Fiedler 2003; Partecke & Gwinner 2007; Jones *et al.*

2014). For instance, a partially migratory population of Great Bustards (*Otis tarda*) in Europe

has increasingly shown resident behaviours, a change linked to high mortality of migrants on

power lines (Palacín *et al.* 2016). Bats, storks, waterfowl, and numerous other species are

showing similar increases in residency (Baskin 1993; Tortosa *et al.* 1995, 2002; Van Der Ree *et*

al. 2006). Quantifying the extent to which migrants overlap with and respond to growing resident

sub-populations could help improve population projections and inform whether interactions with

residents require mitigation.

92   One critical question is whether residents increase pathogen infection risk for migrants

93   that encounter them. Theoretical models and empirical studies have demonstrated greater

94   infection prevalence for residents compared to migrants in some cases (Cross *et al.* 2010; Akbar

95   *et al.* 2012; Poulin *et al.* 2012; Qviller *et al.* 2013; Hall *et al.* 2014). Seasonal migration can

96   reduce pathogen transmission through several mechanisms, including by periodically enabling

97   migrants to escape parasite-contaminated habitat (*migratory escape*; Folstad *et al.* 1991; Loehle

98   1995) and by causing disproportionate mortality or loss of infected individuals during strenuous

99   journeys (*migratory culling*; Bartel *et al.* 2011). In contrast, resident populations would not

100   experience these processes and, as a result, can suffer higher parasite burdens – with the potential

101   for transmission to migrants (Hines *et al.* 2007; Cross *et al.* 2010; Hill *et al.* 2012).

102   Another important question is whether resident animals and their habitats also alter

103   migrant behaviour, particularly movement. This might occur if resident areas induce migrants to

104   curtail their journeys or modulate the physiological states that facilitate migration. For instance,

105   changes in climate and food have enabled some bird populations to shorten their migrations by

106   using new wintering sites closer to breeding grounds (Elmberg *et al.* 2014; Teitelbaum *et al.*

107   2016). Sites with year-round residents (providing mates and breeding habitat) might similarly

108   allow shortened migrations. Further, resources at resident sites could modify the physiological

109   states that help animals undertake and survive strenuous journeys (e.g., atrophy of non-essential

110   organs; Dingle 2014). Past work suggested that many migrants initially ignore environmental

111   stimuli that could interrupt migration (Kennedy 1985; Dingle 2014), but this remains

112   understudied and may be distinctly different for males (Gatehouse 1997). Moreover, persistent

113   exposure to attractive resources and heightened risks of migratory journeys might modify this.

114   Here, we focus on the widely recognized monarch butterfly (*Danaus plexippus*), whose

115   annual migration has been well studied (Figure S1), to investigate whether migrants in Texas

116   encounter residents *en route*, and to ask whether differences in infection status or reproductive

117   behaviour are associated with these interactions. To conserve energy for migration, most

118   (although not all) monarchs postpone reproduction during fall and enter a hormonally-induced

119   state called reproductive diapause (Herman 1973; Brower *et al.* 1977) as they travel to

120   overwintering sites in central Mexico (Urquhart & Urquhart 1978). In spring, these same

121   monarchs break reproductive diapause, mate, and return to the southern U.S. to lay eggs on

122   milkweed; their progeny and grand-progeny continue northward to recolonize the breeding range

123 (Malcolm *et al.* 1993; Miller *et al.* 2012; Flockhart *et al.* 2013). Past work indicated that this

124 annual journey reduces monarchs' infection prevalence from the specialist protozoan

125 *Ophryocystis elektroscirrha* (OE), through migratory culling and migratory escape (Bartel *et al.*

126 2011; Altizer *et al.* 2015; Flockhart *et al.* 2017). However, some monarchs no longer migrate and

127 now breed year-round in the southern U.S. (Howard *et al.* 2010; Batalden & Oberhauser 2015).

128 Surveys of volunteers indicate monarch winter-breeding occurs almost exclusively on exotic

129 tropical milkweed (*Asclepias curassavica;* Satterfield *et al.* 2016), which is often planted in

130 gardens, does not senesce during fall like most native milkweeds, and can provide food year-

131 round for larval monarchs in warm climates (Batalden & Oberhauser 2015; Satterfield *et al.*

132 2015, 2016). Reports from citizen scientists (Howard *et al.* 2010) and a survey of historical

133 documents (Satterfield et al. 2015 Supplementary Material) suggest that the planting of tropical

134 milkweed and year-round monarch breeding has become more common in recent decades,

135 potentially linked to warmer winters. Previously, we found that resident monarchs in the

136 southern coastal U.S. experience significantly higher OE infection prevalence compared to

137 migrants, likely because of loss of the migratory mechanisms that typically control disease

138 (Satterfield *et al.* 2015, 2016). The impacts of resident monarchs on the infection risk and

139 movement behaviour of migrants have not previously been investigated.

140   We conducted field sampling and chemical analyses of wild butterflies to ask: (1) Do

141 migrant and resident monarchs share habitat during fall and spring migrations? (2) Are fall

142 migrants that encounter sites with resident monarchs more likely to harbour parasite infections?

143 (3) Are fall migrants at resident sites more likely to be reproductively active (typically associated

144 with non-migratory behaviour), and do they show evidence of abandoning migration to remain at

145 these locations? We assigned resident and migrant status based on analyses of stable isotope

146 composition to estimate natal origins (using isoscapes based on Malcolm *et al.* 1993; Hobson *et*

147 *al.* 1999; Dockx *et al.* 2004; Flockhart *et al.* 2013) and cardenolide fingerprints (milkweed

148 secondary compounds) to infer natal host plant species (Malcolm *et al.* 1989). We also collected

149 data on OE infection status, morphometrics, and reproductive behaviour. We hypothesized that if

150 migratory monarchs pass through resident sites *en route* to and from overwintering locations,

151 migrants could acquire parasites from residents; migrants that are reproductively active

152 (primarily in spring but also sometimes in fall) could also lay eggs on parasite-laden tropical

153 milkweed, leading to high infection risk for offspring. Further, encounters with resident

154   monarchs or tropical milkweed might prompt fall migrants in diapause to become reproductive

155   and halt their journeys (Batalden & Oberhauser 2015).

156   **MATERIAL AND METHODS**

157   *Parasite biology*

158   Transmission of OE in monarchs occurs from adults to caterpillars, when infected butterflies

159   (covered with millions of dormant spores on the outside of their bodies) scatter parasites onto

160   eggs or milkweed leaves (McLaughlin & Myers 1970). Caterpillars ingest the spores, and

161   parasites replicate internally. Infected adults can transfer dormant spores to other adults (e.g.,

162   during mating), although spores must be consumed by a larva to initiate infection. Infections can

163   lower pupal survival and reduce adult lifespan, body size, mating success, and flight performance

164   (de Roode *et al.* 2009; Bradley & Altizer 2005; de Roode *et al.* 2007).

165

166   *Field collections and capture-mark-recapture study*

167   To investigate migrant-resident interactions, we sampled a total of 508 adult monarchs and 56

168   larval monarchs in Texas across 9 sites (Figure 1; Figure S1), exhibiting either: (a) seasonal

169   monarch activity, where migrants stop to refuel but where monarch breeding does not occur

170   during Dec-Feb (hereafter called *seasonal stopover sites*), or (b) year-round monarch activity,

171   where residents are known to breed during winter on tropical milkweed (hereafter called *year-*

172   *round breeding sites*). Monarchs inhabit year-round breeding sites throughout the year, but not

173   always continuously if food depletion or hard freezes cause local extinction-recolonization

174   cycles. Both site types provide flowering nectar plants as stop-over resources for migrants, which

175   travel primarily either along the central flyway (extending from the Midwest through central

176   Texas) or the coastal flyway (extending from the Atlantic and Gulf coasts through coastal

177   Texas), where resident monarchs reside (Calvert & Wagner 1999; Howard & Davis 2008). In

178   fall, we collected 345 adult monarchs across four seasonal stopover sites (N=200; average of

179   50/site) and three year-round breeding sites (N=145; average of 48/site) during Oct-Dec 2014.

180   We also tagged and released an additional 113 adults in a capture-mark-recapture study at three

181   year-round breeding sites (Oct 14-Dec. 5) to observe whether migrants halted migration. Adults

182   were tagged before, during, and after peak migration period to estimate monarchs' duration of

183   stay, changes in mass, and site fidelity (Supporting Information B). During spring migration

184   (April 2015), we collected 50 adult monarchs and 56 immature stages from two seasonal

185   stopover sites (N=12 adults from one site; N=29 larvae/singly-laid eggs from *A. viridis* or *A.*

186   *asperula* from two sites) and three year-round breeding sites (N=38 adults; N=27 pupae or larvae

187   from *A. curassavica* across three sites). Monarchs collected as eggs/larvae were reared in

188   individual containers and fed greenhouse-grown, parasite-free *A. incarnata* (after consuming

189   their natal leaves). Forty eggs/larvae (of 56) survived to adulthood. As detailed below, we

190   assessed captured-and-released butterflies (N=113) for infection status, sex, and forewing length.

191   We assessed collected monarchs (N=395) for infection status, sex, forewing length, natal origin,

192   and reproductive status (for fall butterflies), except where noted in Supporting Information A.

193

194   *Infection status*

195   We examined all adult monarchs non-destructively for OE infection by pressing clear adhesive

196   tape (1.5cm) against the abdomen (as in Altizer *et al.* 2000). We viewed samples at 60X to

197   observe parasite spores. Based on prior laboratory work, samples with ≥100 spores were

198   classified as infected, indicating infections acquired as larvae. Samples with <100 spores were

199   classified as uninfected, and most likely resulted from adults acquiring dormant spores from

200   other infected adults during mating or other contact (as opposed to monarchs that ingested spores

201   as larvae, which develop much higher infection loads; Altizer *et al.* 2004; de Roode *et al.* 2009).

202   We assessed immature monarchs for infection at adulthood.

203

204   *Reproductive status*

205   We evaluated reproductive status for a subset of fall-collected monarchs (N=300 of 345 fall

206   monarchs). We expected that most fall monarchs would be in reproductive diapause, but that a

207   small fraction would exhibit reproductive activity, as shown previously (Calvert 1999; Zalucki &

208   Rochester 1999; Goehring & Oberhauser 2002; Borland, *et al.* 2004); these individuals could be

209   older summer-breeding monarchs, or migrants (bound for the southern U.S. or Mexico) in a

210   reproductive state. We examined reproductive activity across both site types, allowing us to

211   compare the background level of reproductive activity for monarchs sampled at seasonal

212   stopover sites to those at year-round breeding sites. Within 5 days of capture, wild-caught

213   females were dissected (N=106 across seven sites) to observe the presence or absence of mature

214   eggs in ovaries (Oberhauser & Hampton 1995). Wild-caught males were placed in mesh cages

215   either outdoors (N=163) or in incubators set to outdoor photoperiod and temperatures (N=31) to

216   observe mating with lab-reared females over 8-10 days, or until monarchs experienced seven

217   days at >21.1ºC. We categorized females with mature eggs and males that mated as

218   reproductively active (Supporting Information C).

219

220   *Natal origins: Stable isotope and cardenolide analyses*

221   We used chemical markers to assign wild butterflies (N=390, of 395 total adults collected in fall

222   and spring) as "migrant" or "resident" and to obtain natal origin information (Figure 2). Stable

223   hydrogen (δ^2H) and carbon (δ^{13}C) isotope composition from wing chitin has been used to

224   estimate geographic regions of natal origin (Wassenaar & Hobson 1998; Miller *et al.* 2012;

225   Flockhart *et al.* 2013; Altizer *et al.* 2015). Mean δ^2H patterns in precipitation (δ^2H$_p$; amount-

226   weighted mean growing season values) decrease with increasing latitude; these patterns are

227   integrated into the plant tissue eaten by larvae and retained in monarch wing membranes

228   (Hobson *et al.* 1999). Monarchs from northern latitudes have more depleted (negative) values of

229   δ^2H. Mean δ^{13}C values vary longitudinally in milkweeds, and δ^{13}C measurements enhance

230   geospatial natal assignment maps (Wassenaar & Hobson 1998; Hobson *et al.* 1999). Wings were

231   stored at -20ºC and prepared (as in Flockhart *et al.* 2013) by washing right hindwings with 2:1

232   chloroform-methanol and weighing and loading wing pieces into capsules. We used an elemental

233   analyser coupled with a continuous-flow isotope ratio mass spectrometer to obtain wing δ^2H and

234   δ^{13}C following calibration with laboratory standards (Supporting Information D).

235   Next, we examined cardenolide profiles in wings to determine whether natal host plants

236   were native or tropical milkweed (*A. curassavica,* which feed resident monarchs). In North

237   America, monarch larvae can feed on dozens of milkweed species with varying toxic

238   cardenolides (cardiac glycosides) that are retained in wing tissue (Zalucki *et al.* 2001; Agrawal *et*

239   *al.* 2012); thus, chromatography can determine natal host plant species and inform resident and

240   migrant classifications (Malcolm *et al.* 1993; Dockx 2012). *A. curassavica* has high

241   concentrations of diverse cardenolides compared to native milkweeds, such as *A. incarnata* or *A.*

242   *syriaca,* which support the vast majority of migrants (Seiber *et al.* 1986). To obtain cardenolide

243   profiles, we pulverized right forewings, extracted cardenolides in methanol, dried samples, and

244   re-suspended extracts in methanol with a known cardenolide standard (digitoxin). We then used

245   Acquity ultra-performance liquid chromatography (UPLC; Waters Corp, Milford, MA, USA)

246   with a Luna C(18) column (Phenomenex, Torrance, CA, USA) and a photodiode array detector

247 to assess cardenolide concentration, non-polarity (retention time per peak), and diversity

248 (Supporting Information E). We used non-metric multidimensional scaling (NMDS) to represent

249 each cardenolide profile with two Cartesian coordinates. Monarchs with cardenolide

250 concentrations of zero were automatically assigned as migrants (and excluded from NMDS

251 analyses), as these butterflies could not have originated from *A. curassavica*.

252

253 ***Resident and migrant classifications***

254 Based on δ^2H and cardenolide profiles (N=390), we classified monarchs as residents or migrants

255 with two approaches: (1) decision rules developed from previous knowledge about monarch

256 biology and chemical patterns; and (2) a discriminant analysis developed from known resident

257 and migrant monarchs, using δ^2H values, cardenolides, and wing length. This dual approach

258 allowed us to assess monarchs based on previously established findings as well as recent data

259 from wild-caught individuals. In subsequent analyses, we only included individuals for which

260 both classification methods agreed (96.7% of samples; N=377 of 390).

261 In the decision-rules method, we classified monarchs using the following assumptions:

262 (i) Monarchs were assigned as migrants if they originated from northern latitudes, defined here

263 as corresponding to wing δ^2H <-111‰. This value was informed by previously described

264 monarch δ^2H isoscapes (Hobson *et al.* 1999) and is three standard deviations below the mean

265 δ^2H value for a set of known resident monarchs (N=25 individuals collected from Texan year-

266 round-breeding sites as late-instar larvae/pupae, with average δ^2H=-91‰; range: -76‰ to -

267 104‰; Figure S5). (ii) Of the remaining (southern) individuals, monarchs with cardenolide

268 profiles matching *A. curassavica* were residents. To meet this criterion, a butterfly's NMDS

269 cardenolide coordinates fell within a defined "*A. curassavica* polygon," previously constructed

270 from a separate set of lab-raised and field-collected monarchs known to be fed *A. curassavica*

271 (N=134 monarchs; Figure S6). (iii) Monarchs with cardenolide NMDS coordinates falling

272 outside the *A. curassavica* polygon (>3 SD's from the cluster means) were considered migrants,

273 as they likely originated from native milkweed. This assumption was tested previously with

274 additional lab-raised and field-collected monarchs from known milkweed species (N=214

275 monarchs; Figure S6). (iv) Any remaining wild monarchs (N=11) were deemed unclassifiable

276 and removed from further analyses.

277   For the discriminant analysis approach, we used 25 known residents (described above)
278 and 84 known migrants (collected at Mexican overwintering sites in Feb 2013 and for which we
279 had previously attained δ^2H values; A. Fritzsche McKay and S.A. Altizer, unpublished) as
280 training data to build discriminant functions including total cardenolide concentration,
281 cardenolide NMDS coordinates, δ^2H values, and forewing length (using the *MASS* package in R
282 3.2.3). Values of δ^{13}C were not available for known migrants. Results indicated that cardenolide
283 concentration was the strongest predictor of resident versus migrant status (Wilk's lambda=0.52,
284 $F_{1,107}$=262.5, p<0.001). Wing δ^2H was informative although not significant (Wilk's
285 lambda=0.15, $F_{1,107}$=2.00, p=0.16, NS). Cardenolide NMDS coordinates and forewing length
286 were not significant predictors of migratory status. Next, to classify wild monarchs, we pre-
287 grouped individuals as residents if cardenolide NMDS values fell within the *A. curassavica*
288 polygon and δ^2H values indicated southern origins (>-111‰; see above). We used the
289 discriminant functions to classify remaining butterflies, placing monarchs into groups with high
290 posterior probabilities (>0.9). One monarch with a posterior probability <0.7 was unclassifiable.
291 We proceeded with the 377 monarchs (of 390) for which assignments agreed using both
292 methods.

293 *Geospatial natal assignment maps*
294 We created geospatial natal origin maps using both δ^2H and δ^{13}C values (Figure 2). We used a
295 multivariate normal probability assignment to calculate posterior probability densities of natal
296 origin for geographically indexed cells across eastern North America (described in Flockhart *et*
297 *al.* 2013); expected values were based on previously developed δ^2H and δ^{13}C isoscapes for
298 monarchs (Hobson *et al.* 1999). We then reclassified the probability surface to a binary surface
299 (pixels assigned 1 or 0) for each individual, using a 2:1 odds ratio whereby the upper third of the
300 probability surface was deemed the region of natal origin.

301

302 *Data analysis*
303 We used logistic regression to examine how migratory status (migrant vs. resident) varied by site
304 type (seasonal stopover vs. year-round breeding) and time period during fall (divided into five
305 14-day intervals). Next, we examined differences in reproductive and infection status between
306 migrants and residents. We used a generalized linear mixed model (GLMM) with binomial error
307 distribution to test effects of migratory status, sex, and a migratory-status-by-sex interaction on

308 reproductive state during the fall (reproductive or in diapause), with site as a random variable

309 (for N=286 fall monarchs for which all needed data were available). A second GLMM with the

310 same model structure examined predictors of binary infection status (N=329). Sample sizes for

311 analyses are described in Supporting Information A.

312   We next focused only on monarchs assigned as migrants, to ask whether fall migrants

313 were more likely to be reproductive or parasitized at year-round breeding sites compared to

314 stopover sites. We assessed predictors of reproductive status using a third GLMM with binomial

315 error and fixed factors for site type, sex, and their interaction (N=237). We also included δ^2H (a

316 proxy for latitude) as a continuous variable to observe from which regions reproductive migrants

317 originated. Site was a random variable. Infection status of fall migrants was analysed using a

318 fourth GLMM with the same model structure (N=273). Non-significant terms were eliminated.

319   For monarchs in the capture-mark-recapture study, we recorded duration of stay and used

320 Bayesian hierarchical Cormack-Jolly-Seber models to estimate site fidelity of presumed migrants

321 versus residents at year-round breeding sites (Supporting Information B). We conducted

322 statistical analyses in R 3.2.3.

323

324

325 **RESULTS**

326 *Co-occurrence of residents and migrants*

327 Across all sites and sampling periods, we detected 290 migrant and 87 resident monarchs (total

328 N=377). During fall, the proportion of migrants vs. residents differed significantly by site type

329 (χ^2=46.19, df=1, p<0.0001) and changed nonlinearly with time (χ^2=37.35, df=4, p<0.0001). At

330 seasonal stopover sites, we detected only migrants (N=198, Figure 1A), and at year-round

331 breeding sites, we assigned 57% of sampled monarchs as migrants and 43% as residents (N=131;

332 Figure 1A). The proportion of migrants at year-round breeding sites increased from Oct through

333 mid-Nov, before sharply declining (Figure 1B).

334   Small sample sizes collected during spring (when monarchs disperse and are more

335 difficult to capture) again showed that migrants and residents shared habitat (Figure 1C). At

336 year-round breeding sites in April, we assigned 24% of sampled monarchs as migrants and 76%

337 as residents (N=38). At the single seasonal stopover site sampled for adults in spring, we

338 detected eight migrants and two residents.

339   *Reproductive activity*

340   Resident monarchs were more likely to be classified as reproductive (47%; N=49) than were

341   migrants (18%; N=237; χ^2=6.08, df=1, p=0.01; Figure 3A) during fall. Across all samples, males

342   were more likely to be reproductive than were females (χ^2=7.55, df=1, p=0.006). Sex differences

343   in reproductive status were especially strong among migrants, with 4% of females and 26% of

344   males being reproductive. Sex differences were present but less pronounced among residents

345   (migratory-status-sex interaction: χ^2=4.63, df=1, p=0.03).

346   Fall migrants sampled at year-round breeding sites were nearly three times more likely to

347   be reproductively active (35%; N=69) than migrants at seasonal stopover sites (11%; N=169;

348   χ^2=5.06, df=1, p=0.02; Figure 3A). Male reproductive activity was again significantly higher

349   compared to females (χ^2=9.98, df=1, p=0.002; Figure 4). Migrants sampled at year-round

350   breeding sites were predominantly male (unlike at seasonal stopover sites), but the interaction

351   term between site type and sex was not a significant predictor of reproductive status. Hydrogen

352   isotope values (correlated with natal origin latitude) did not predict reproductive status.

353

354   *Infection risk*

355   During fall, 95% of resident monarchs (N=56) and 9% of migrants (N=273) were infected with

356   OE. Thus, migratory status was the strongest predictor of infection in fall (χ^2=21.51, df=1,

357   p<<0.001; Figure 3B). Infection status did not differ by sex. Importantly, migrants were 13 times

358   more likely to be infected at year-round breeding sites (27%; N=75) than at seasonal stopover

359   sites (2%; N=198; χ^2=14.03, df=1, p=0.0002; Figure 3B), and infected migrants were more likely

360   to originate from southern latitudes (less negative δH^2; χ^2=16.12, df=1, p<0.001). As fall

361   progressed, the total proportion of butterflies infected at year-round breeding sites initially

362   decreased, as healthy migratory monarchs arrived and "diluted" site prevalence, and later

363   increased, as migrants departed and infected residents remained (Figure 1D).

364   In spring, resident monarchs continued to show high infection prevalence (71%; N=31)

365   relative to migrants (24%; N=17). For larvae sampled during spring, infection prevalence was

366   higher at year-round breeding sites (41%; N=27) compared to sites with seasonal milkweed only

367   (0%; N=13).

368

369   *Monarch movement behaviour at year-round breeding locations*

370   The capture-mark-release study included 113 monarchs not used in natal origin assignments.

371   Because most of these monarchs were not recaptured, migratory status could not be confirmed,

372   and we proceeded with capture-mark-recapture analyses (Supporting Information B) by

373   assuming that infected monarchs were likely residents (N=100) and uninfected monarchs were

374   likely migrants (N=37), based on infection patterns noted earlier. We recaptured 40% of

375   'presumed residents' and 8% of 'presumed migrants' at least once. This limited dataset suggests

376   that most migrants continued migrating, but a small fraction halted their journeys.

377   An additional 24 individuals were marked, recaptured, collected, and later used in natal

378   origin analyses (described above); we assigned 12 as migrants, nine as residents, and three as

379   unclassifiable. Of the 12 migrants, 11 stayed at the same year-round breeding site for seven days

380   or more (five remained >20 days) and had presumably terminated migration. Migrants that

381   stayed at year-round breeding sites were all male; they also tended to be reproductively active (6

382   out of 9 assessed), infected with OE (10 of 12), and originated from more southern latitudes

383   (mean δ^2H=-95‰, range=-126 to -80‰).

384

385   **DISCUSSION**

386   Migratory monarchs sampled in Texas during both spring and fall shared habitat with resident

387   monarchs, which breed year-round and harbour high protozoan infection prevalence. Although

388   most migrants were non-reproductive and free of infections (conditions that support successful

389   migration), migratory monarchs captured at year-round breeding sites showed a 3-fold higher

390   propensity for reproduction and a 13-fold higher probability for infection, compared to migrants

391   sampled at seasonal stopover sites (with no resident monarchs). Most migrants that visited year-

392   round breeding sites (with resident monarchs) continued to migrate; however, a small fraction

393   remained in these gardens for days or weeks. In spring, monarchs migrating northward to lay

394   eggs shared breeding habitat with residents, both at seasonal sites with native milkweed and at

395   year-round breeding sites with exotic milkweed, where infection risk for larval monarchs is high.

396   Two possibilities could explain why migrants sampled at year-round breeding locations

397   were more likely to show reproductive activity. First, exposure to tropical milkweed in the fall

398   might induce monarchs to break reproductive diapause (Batalden & Oberhauser 2015), which is

399   thought to be induced and maintained by decreasing day length and temperatures combined with

400   exposure to aging milkweed (Goehring & Oberhauser 2002). Unlike the vast majority of native

401 milkweeds that senesce during fall, tropical milkweed continues to grow during winter in some

402 areas. It is unclear whether exposure to actively growing milkweed over a matter of days could

403 induce a physiological change as strong as reproductive development (Batalden & Oberhauser

404 2015), although adult monarchs can break diapause quickly following exposure to warm

405 temperatures and longer photoperiods (Herman 1981). A second explanation could be that these

406 sites attract the small proportion of migrants that are not in diapause and already reproductively

407 active (Herman 1981; Brower 1985; Goehring & Oberhauser 2002; Borland, e*t al.* 2004).

408 Habitats with warm temperatures and viable host plants might recruit these reproductive

409 migrants to join resident populations. Our results cannot distinguish between these explanations.

410 The higher infection probability among migratory monarchs sampled at year-round

411 breeding sites (compared to seasonal stopover sites) could result from butterflies acquiring

412 parasite spores, possibly from heavily infected residents attempting to mate with them (which

413 can, in some cases, cause moderate spore loads, as shown in captive experiments; de Roode et al.

414 2009) or from contact with contaminated milkweeds. We observed, for instance, eight confirmed

415 migrants nectaring or landing on tropical milkweeds, which are often covered in parasites at

416 year-round breeding locations (Altizer *et al.* 2004). Alternatively, higher infection prevalence

417 among migrants at year-round breeding sites could occur if tropical milkweed gardens

418 disproportionately attract migrants that are already infected. Past work showed that infected

419 females preferentially oviposit on tropical milkweeds, which offer highly toxic cardenolides that

420 reduce parasite load in larval offspring (Lefèvre *et al.* 2010). Moreover, infected monarchs

421 cannot fly as well as healthy monarchs (Bradley & Altizer 2005) and are less likely to migrate

422 successfully to Mexico (Bartel *et al.* 2011; Altizer *et al.* 2015), and thus, might possibly abandon

423 migration when given opportunities for immediate reproduction.

424 Of particular concern is whether spring migratory monarchs returning from Mexico lay

425 eggs on milkweeds contaminated with parasites from infected residents. This could increase

426 infection risk for migrants' offspring. Our results suggest that shared habitat use creates the

427 potential for pathogen spillover from resident to migrant butterflies. At year-round breeding

428 sites, where infection risk is extremely high (Satterfield *et al.* 2015), we observed six resident

429 and four migrant females (chemically confirmed) ovipositing on *A. curassavica*. Additional data

430 are needed to assess movements of infected residents in spring, when monarchs are more

431 dispersed and thus the frequency of resident-migrant interactions likely changes. Some spring

432 residents appear to visit seasonal sites with native milkweeds (Figure 1C), on which they may

433 deposit eggs and potentially parasites; we observed one confirmed resident ovipositing on *A.*

434 *asperula*. If migrants and residents share breeding habitat frequently, infection levels could rise

435 among the first generation of spring monarchs, most of which are produced along the Gulf coast

436 before traveling north to recolonize the breeding range (Malcolm *et al.* 1993; Miller *et al.* 2012).

437   Migratory monarchs have undergone an 84% decline in eastern North America (1996-

438 2015), thought to be caused by multiple factors, including habitat loss, throughout their annual

439 migratory cycle (Semmens *et al.* 2016; Thogmartin *et al.* 2017; Vidal & Rendon-Salinas 2014;

440 Flockhart *et al.* 2015; Brower *et al.* 2012; Akbar *et al.* 2012; Marini & Zalucki 2017). Threats for

441 monarchs during fall migration are particularly difficult to measure but could be significant (Ries

442 *et al.* 2015; Inamine *et al.* 2016). Understanding the types of habitats through which migratory

443 monarchs travel, and how these influence their health, behaviour, and migratory success, could

444 inform conservation actions. Results here indicate potential consequences for migratory

445 monarchs that share habitat with residents, and represent the first quantification of migrant-

446 resident interactions within the monarchs' core migratory range. Previous work showed that fall

447 migrant monarchs can enter areas with resident monarchs in Cuba and South Florida, but these

448 locations are peripheral to the major flyways, and monarchs from these locations are unlikely to

449 interact with migrants that reach Mexico (Dockx *et al.* 2004; Dockx 2012; Knight & Brower

450 2009). Our study presents evidence that either (A) year-round breeding sites with tropical

451 milkweed disproportionately attract infected and reproductively active migrants, in which case

452 migrants' offspring produced at these sites will face high infection risk, or alternatively, (B)

453 year-round breeding sites induce migrants to break reproductive diapause, which could interrupt

454 migration or lower its success. In either case, these findings and other studies (Satterfield *et al.*

455 2015; Batalden & Oberhauser 2015) collectively provide evidence that native, seasonal

456 milkweeds – rather than exotic, year-round milkweeds – could best support monarch migration.

457 We recommend that future efforts in eastern North America to restore pollinator habitat focus on

458 native species and, when possible, avoid further planting of tropical milkweed. In locations

459 where tropical milkweed is already present, it should be cut back monthly throughout fall and

460 winter to limit monarch winter-breeding and its associated parasite transmission risk.

461   While we concentrated on monarchs, the co-occurrence of resident and migrant

462 conspecifics is likely common in wildlife populations across taxa. Further, as many migratory

463 species shift towards shorter migrations or non-migratory behaviours in response to human
464 activities (Satterfield *et al.* 2018), migrant-resident interactions may become more frequent in the
465 future. This study addresses an imperative question in light of these changes: What are the
466 consequences of expanding resident populations for migratory animals already facing multiple
467 stressors? Our work suggests that, for some populations, the health and migratory success of
468 migrants might be influenced by interactions with conspecific residents. Our findings underscore
469 growing scientific support for prioritizing the preservation not only of migratory species
470 themselves, but of their behaviours and propensities for migration, which can reduce infectious
471 disease risk and contribute to ecosystem function (Altizer *et al.* 2011; Bauer & Hoye 2014).

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718

719 **FIGURE CAPTIONS**

720 **Figure 1.** Map of sampling locations in Texas, USA from (A) fall 2014 and (C) spring 2015,
721 with the proportion of sampled adult monarchs that were assigned migrant status (black) or
722 resident status (blue) at *year-round breeding sites* (blue points) and *seasonal stopover sites* (pink
723 points). (B) Temporal changes in the proportion of migrants vs. residents at year-round breeding
724 sites during the fall. (D) Temporal changes in infection prevalence of adult monarchs at year-
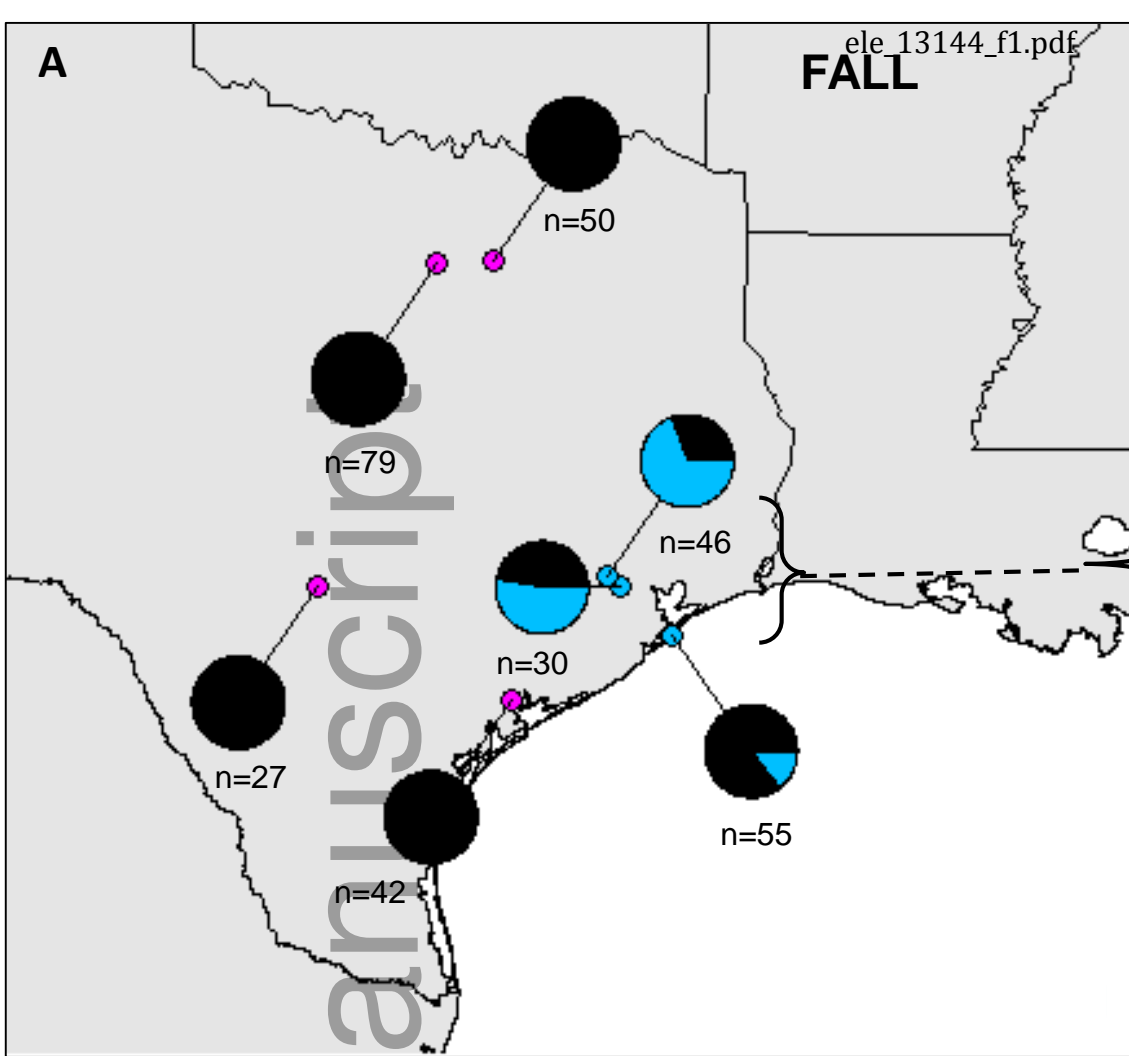725 round breeding sites during the fall.

726

727 **Figure 2.** Assigned natal origins based on δ^2H and δ^{13}C values for (A) monarchs classified as
728 migrants in our analyses (N=273), captured in Texas during fall 2014; (B) monarch #933, an
729 individual classified as a migrant and shown here as an example of a migrant that departed a
730 northern area and was sampled at a year-round breeding location; (C) monarchs classified as
731 residents in our analyses (N=56), captured in Texas during fall 2014; and (D) monarch #746, an
732 individual classified as a resident and shown here as an example.

733

734 **Figure 3.** (A) Reproductive activity and (B) infection prevalence among fall migrants and

735 residents sampled at seasonal stopover sites and year-round breeding sites. Resident monarchs

736 were more likely to be reproductive and to be infected than were migrants; residents were only

737 observed at year-round breeding sites during fall, and no residents were observed at seasonal

738 stopover sites. Migratory monarchs sampled at year-round breeding sites were significantly more

739 likely to show reproductive activity and OE infections than were migrants sampled at seasonal

740 stopover sites.

741

742 **Figure 4.** Proportion of fall migrant monarchs that were reproductively active by sex and site

743 type. Migrants were more likely to be reproductive at year-round breeding locations with tropical

744 milkweed as compared to seasonal stopover locations. Males were significantly more likely to be
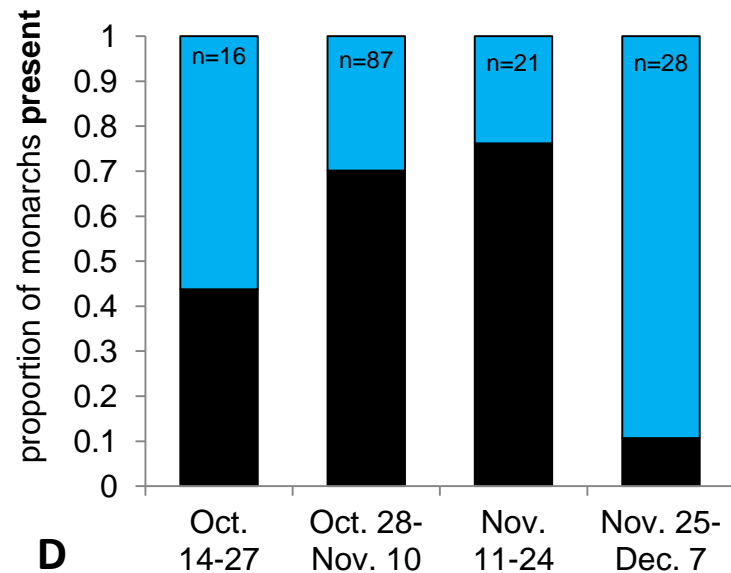
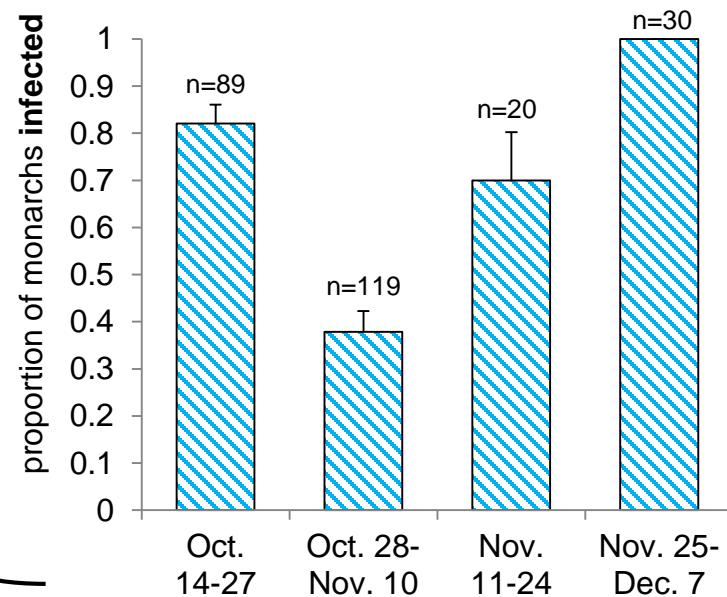745 reproductive, regardless of site type.
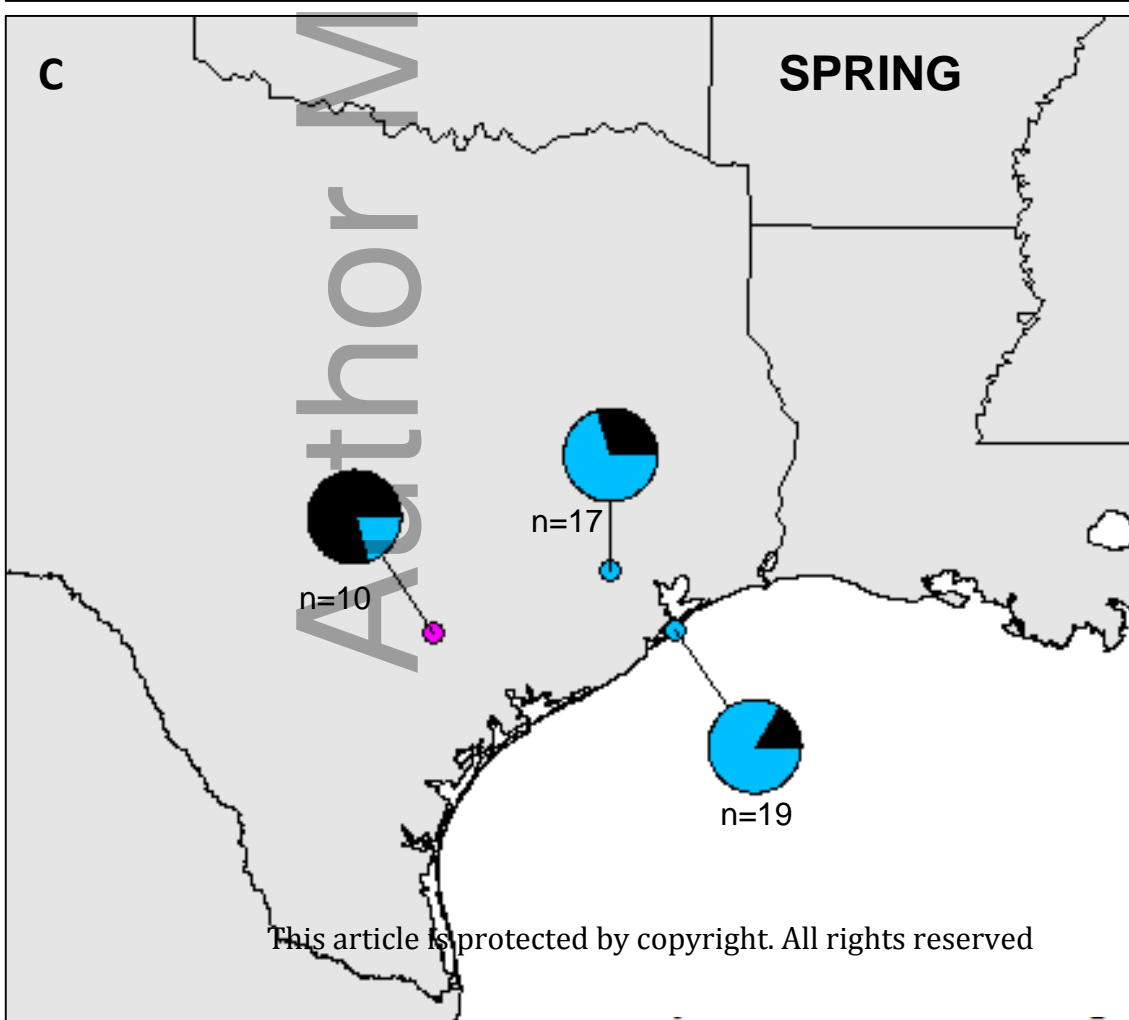
A FALL

B year-round breeding sites

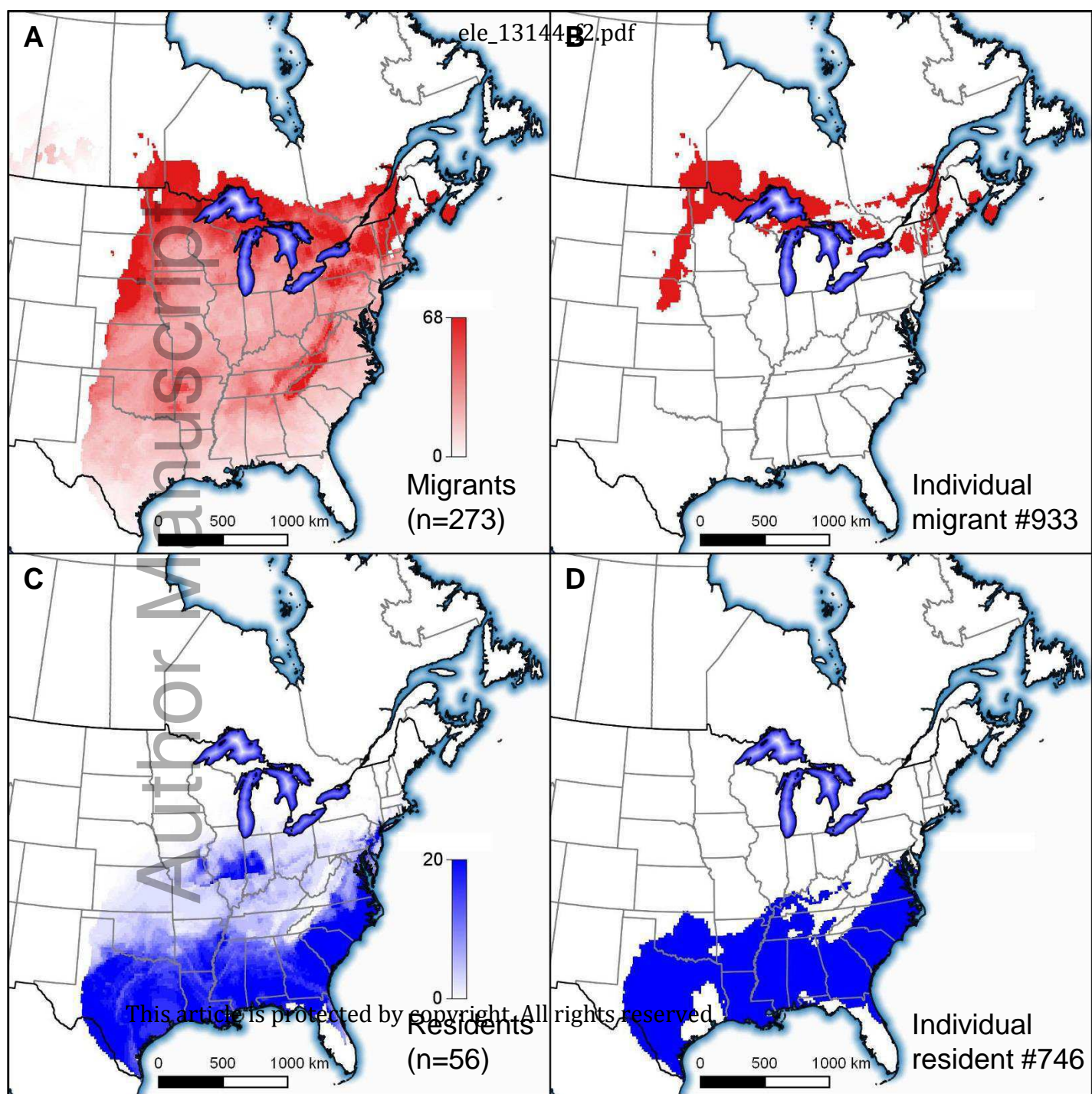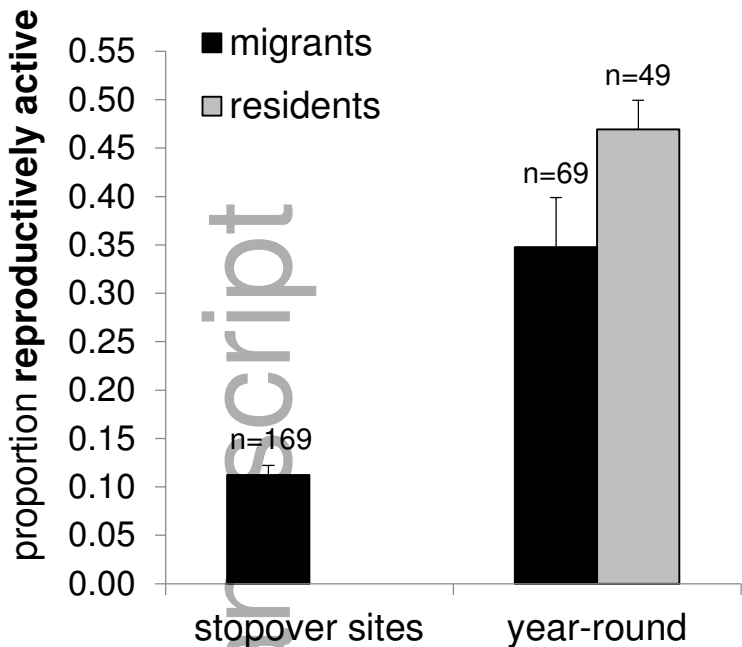**C** SPRING

D

residents
migrants
year-round breeding sites
stopover sites

A Migrants (n=273)

B Individual migrant #933

C Residents (n=56)

D Individual resident #746