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1 **Population declines of a widespread amphibian in agricultural landscapes**

2

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21 **Abstract.**

22 Modern agricultural practices are suspected to play a major role in the ongoing
23 erosion of biodiversity. In order to assess whether this biodiversity loss is linked to
24 past habitat modifications (e.g., land consolidation) or to current consequences of
25 modern agriculture (e.g., use of agrochemicals), it remains essential to monitor
26 species that have persisted in agricultural landscapes to date. In this study, we
27 assessed the presence, abundance and recent population trends of one such species,
28 the spined toad (*Bufo spinosus*) along a gradient of habitats from preserved (forests)
29 to highly agricultural sites in rural Western France. Our results showed that both
30 presence and abundance of spined toads were markedly lower in reproductive
31 ponds surrounded by intensive agriculture. The most salient result of our study is
32 the ongoing decline of this species in farmland habitats. Indeed, this result suggests
33 that unknown factors are currently affecting a widespread terrestrial amphibian
34 previously thought to persist in agricultural landscapes. These factors have recently
35 induced strong population declines over the course of a few years. Future
36 investigations are required to identify these factors at a time when anthropogenic
37 activities are currently leading to unprecedented rates of biodiversity loss.

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41 Key-words: abundance; agroecosystems; *Bufo spinosus*; biodiversity; presence

42

43

44 **Introduction**

45

46 Anthropogenic activities are currently leading to unprecedented rates of biodiversity
47 loss (Chapin III et al. 2000; Myers and Knoll 2001; Brooks et al. 2002). Indeed, human
48 activities are now recognized to be responsible of climate change (Vitousek 1994;
49 Steffen et al. 2007), major shifts in land use (Klein Goldewijk and Ramankutty 2004;
50 Young et al. 2005) and environmental contamination (Rudel et al. 2009; Saleh and
51 Aglan 2018); all of which can individually and/or interactively affect wildlife (de
52 Brito Rodrigues et al. 2019; Trudeau et al. 2020; Wagner 2020; Gunstone et al. 2021).

53

54 Among the various sources of anthropogenic disturbances to natural ecosystems,
55 modern agricultural practices are suspected to play a major role in the ongoing
56 erosion of biodiversity for several reasons (Altieri 1999; Dudley and Alexander 2017).
57 First, modern agriculture is responsible for the alteration and the reduction of natural
58 habitats and landscape homogenization (Fahrig 2003) as compared to ancestral
59 agricultural practices. For instance, in Europe, changes in land-use politics that
60 occurred post World War II (WWII) have induced a large scale land consolidation
61 (Benton et al. 2003; Tschardtke et al. 2005). The ancestral rural matrix of small plots
62 and meadows bordered by a dense network of hedges has been homogenized to
63 extended fields hosting monocultures (Benton et al. 2003; Tschardtke et al. 2005).
64 Concomitantly, this revolution has provoked a reduction in the spatial connectivity
65 between patches of favourable habitats among the agricultural landscape, limiting
66 therefore the persistence of wildlife (Benton et al. 2003). Second, detrimental effects
67 of modern agriculture are linked to the massive use of chemical substances that are

68 used to increase agricultural yields (Geiger et al. 2010). Many pesticides are used to
69 control weeds, insect and fungi in crops, but they have been suspected or shown to
70 detrimentally impact non-target species (Hasenbein et al. 2017; de Brito Rodrigues et
71 al. 2019), either directly through their toxic sublethal or lethal effects (Relyea 2004;
72 Slaninova et al. 2009; Williams et al. 2015) or indirectly through alterations of
73 ecosystem functioning (e.g., reduced food availability, Hart et al. 2006; Wagner 2020).

74
75 As a result, agricultural practices are expected to detrimentally affect wildlife at
76 different temporal scales. Indeed, at least in Western Europe, most of the landscape
77 changes linked to land consolidation have occurred after WWII (Antrop 2000) and
78 the subsequent habitat homogenisation and fragmentation have been mostly
79 achieved by the mid 70's or early 80's (Griffin 1979; Skole and Tucker 1993; Harper et
80 al. 2007; Rudel et al. 2009). As a consequence, it is expected that the effects of such
81 processes on the persistence of wild populations have already been acting for several
82 decades (Debinski and Holt 2000; Fuller et al. 2015). In contrast, the temporal scale of
83 the consequences of agrochemical use on wildlife is much more complex to assess.
84 Indeed, although the reliance on chemical inputs has progressively increased with
85 the development of modern agriculture, the type (fertilizers *versus* pesticides), the
86 quantity and the chemical composition (active compounds) of agrochemicals have
87 constantly changed over time; most notably to circumvent issues linked to the
88 adaptive resistance of pests and, more recently, in response to growing societal
89 concerns (Howden et al. 2007; Bhandari 2014; Prashar and Shah 2016; Hawkins et al.
90 2019; Sharma et al. 2019). Taken together, these ideas suggest that the consequences

91 of agriculture on wildlife linked to the reduction of natural habitats should have
92 already occurred and that impoverished biodiversity in agricultural areas should be
93 a ghost of past landscape changes (Harding et al. 1998; Cousins 2009; Surasinghe and
94 Baldwin 2014). In contrast, current negative trends of wildlife population should be
95 related to the consequences of other factors such as agrochemicals either directly or
96 due to their interactions with the constraints of habitat structure described above
97 (Potts et al. 2010; Oliver and Morecroft 2014) or current climatic modifications either
98 directly or due to their interactions with habitat structure and/or environmental
99 contamination (De Frenne et al. 2019; De Lombaerde et al. 2022). As a consequence,
100 it remains critical to continue to monitor the populations of species that have
101 persisted in agricultural landscapes.

102

103 In this study, we assessed the presence, abundance and recent population trends of
104 one such species, the spined toad (*Bufo spinosus*) in rural Western France. The spined
105 toad is a terrestrial amphibian that has been shown to persist in agricultural habitats
106 (Guillot et al. 2016, but see Meek 2022) when reproductive ponds are still present in
107 the environment. As most terrestrial amphibians, this species has a biphasic life
108 cycle with an extensive use of terrestrial habitats during most of the year and a short
109 breeding season in ponds where mating occurs and eggs and tadpoles develop
110 (Reading 1998; Semlitsch 2008; Kelleher et al. 2018). In order to describe the effects of
111 agriculture on the persistence of this species, we used three complementary
112 approaches. First, in 2021 and 2022, we assessed the presence of reproductive
113 individuals in ponds located along a gradient of habitats from preserved (forests) to

114 highly agricultural sites. Second, on a representative subsample of the same sites
115 and during the same years, we quantified abundances of reproductive individuals
116 (males and females) during the whole reproductive season. Finally, on a few sites
117 that have been monitored for other purposes since 2015, we used capture data of
118 reproductive males as an index of abundances to describe temporal trends.

119

120

121

122 **Material and methods**

123

124 *Study species*

125 Spined toad (*Bufo spinosus*) is one of the most common terrestrial amphibians in
126 Western Europe. This species can live in a wide variety of habitats and has been
127 shown to persist in agricultural areas (Guillot et al. 2016). Juveniles and adults are
128 terrestrial most of the year, but reproduce in aquatic sites (ponds) where eggs and
129 larvae develop during 2 to 3 months (Reading and Clarke 1983; Reading 1998;
130 Kelleher et al. 2018). At the beginning of the reproductive season, toads migrate to
131 breeding sites where males can remain for several weeks, while females occur shortly
132 for mating and egg-laying (Reading and Clarke 1983; Reading 1991, Reading 1998).

133

134 *Presence, abundance and recent population trends*

135 First, in 2021 and 2022, we assessed the presence of reproductive toads in 23 ponds
136 that were similar in term of size and depth (Appendix 1, Appendix 2). All these sites
137 were situated nearby the Centre d'Etudes Biologiques de Chizé (CEBC-CNRS;
138 coordinates 46.1475819, -0.4254604; see site 2 in Appendix 2). These sites were
139 monitored during 2 to 3 nights (separated by 2 to 4 days) during the peak of toad
140 abundance at their aquatic breeding sites. These peaks of toad abundance were
141 based on our abundance surveys (see below) as in our study area, the reproductive
142 phenology of study sites is simultaneous. During these surveys, ponds and their
143 surroundings were monitored at night (between 9 pm and 1 am) with headlamps to
144 locate individuals. All surveys were conducted by the same team in order to avoid
145 observers' effects. We recorded the presence (1 for presence and 0 for absence) of

146 breeding individuals and whether these individuals were males (1 for presence and 0
147 for absence) or females (1 for presence and 0 for absence) as the sexual dimorphism
148 (females being obviously larger than males) in this species allows straightforward
149 sexing without capture (Hemelaar 1988).

150

151 Second, in 2021 and 2022, we assessed the abundance of reproductive toads in 8 sites
152 from the ones surveyed for toad presence (Appendix 1, Appendix 2). These sites
153 were monitored three times a week (Monday, Wednesday and Friday) from late
154 January (25th in 2021 and 31st in 2022) before the arrival of the first reproductive
155 individuals, until the departure of the last reproductive individuals (April 9 in 2021
156 and April 11 in 2022). During these surveys, the ponds and their surroundings were
157 monitored at night (between 9pm and 1 am) with headlamps and the number of
158 males and females sighted were counted. From these nightly count data, we
159 extracted maximum number of individuals counted during a single night [total,
160 males or females] and mean number of individuals per nights during the whole
161 breeding season for each site [total, males or females]). Such monitoring could not be
162 carried out on all the 23 sites for logistical reasons. The 8 sites were selected because
163 they represent the variety of agricultural landscapes that could be found in the area
164 (Appendix 1, Appendix 2).

165

166 Finally, we used data collected for other studies to assess recent population trends in
167 4 sites situated in agricultural settings and 2 sites situated in preserved habitats
168 (Appendix 1, Appendix 2). On these sites, only males were monitored (Guillot et al.

169 2016; Brischoux et al. 2018; Brischoux and Cheron 2019; Brischoux et al. 2021; Renoirt
170 et al. 2021a; unpublished data). Since 2015, we aimed at capturing 30 to 40
171 individuals per study sites. Although such sample sizes were readily obtained at
172 agricultural sites during a single night during the reproductive peak at the beginning
173 of our projects, it became increasingly difficult to obtain these numbers during the
174 subsequent years in those given sites (see results). Importantly, this was the reason
175 why we included forest sites in subsequent years. This also explains why forest sites
176 were monitored posteriorly than initial (agricultural) sites included in these other
177 studies. We used these capture data (number of captured males during a single
178 night situated around the peak of reproduction) in order to monitor broad proxy of
179 abundances across years. Although we acknowledge that this dataset has not been
180 designed to thoroughly monitor toad abundances, we emphasize that the trends in
181 the number of captured individuals across years should describe, at least in a
182 qualitative way, the recent population trends in specific sites.

183

184 *Habitat classification*

185 The terrestrial part of the life cycle of toads occurs usually within 1 km from the
186 breeding ponds (Kovar et al. 2009; Janin et al. 2011; Guillot et al. 2016). As a
187 consequence, from aerial pictures of each study site (GoogleEarth), we drew buffers
188 with a radius of 1 km, corresponding to the potential distance travelled by an
189 individual to reach a breeding site (Kovar et al. 2009; Janin et al. 2011; Guillot et al.
190 2016). We extracted surface area of the main habitat types surrounding each study
191 site: forests and woods, hedges, agricultural fields, meadows and buildings (small
192 villages) using QGIS (3.22). We used the PC1 value from a principal component

193 analysis (PCA) of these five variables to attribute a habitat score to each site. The PC1
194 of the sites for which we assessed toad presence accounted for 51.9% of the total
195 variance and was positively correlated with agricultural fields ($r=0.82$) and
196 negatively correlated with forest ($r=-0.91$). The PC1 of the sites for which we
197 assessed toad abundances accounted for 65.0% of the total variance and was
198 positively correlated with agricultural fields ($r=0.90$) and negatively correlated with
199 forest ($r=-0.96$). For the sites used for assessing recent population trends, we used
200 habitat categories (agriculture *versus* forest, see Renoirt et al. 2021a for details).

201

202 *Statistical analyses*

203 We used Generalized Linear Mixed Models (GLMM) with a binomial distribution to
204 assess the influence of the habitat score on the presence (1) or absence (0) of toads
205 (overall, males or females). Some sites were monitored 2 years of our study ($N=10$),
206 while others were monitored once ($N=13$). Because including year and site identity as
207 random factors over-parametrized the models, both years were analysed separately.

208

209 We used Generalized Linear Mixed Models (GLMM) to assess the influence of
210 habitat score on toad abundance (total, maximum and mean number) for all
211 individuals and males or females, separately; with year added as a fixed factor. All
212 count data passed normality check.

213

214 Finally, we used GLMM to analyse our proxy of recent population trends with the
215 number of captured males as a response variable, and year and habitat category

216 (agriculture *versus* forest) as predictors, we used site identity as a random factor in
217 our models (the number of sites varied according to year).

218

219

220 **Results**

221

222 *Toad presence*

223 Models including overall toad presence were identical as those including the
224 presence of males solely, with only 4 sites (all agricultural with positive PC1 scores)
225 for which males were present but females absent.

226 We found a significant negative effect of the habitat score on the presence of both
227 males (2021: $X^2=5.557$, $df=1$, $p=0.018$; 2022: $X^2=4.147$, $df=1$, $p=0.041$) and females
228 (2021: $X^2=5.256$, $df=1$, $p=0.022$; 2022: $X^2=6.546$, $df=1$, $p=0.01$), with presence
229 decreasing with increasing agriculture (Fig. 1).

230

231 *Toad abundance*

232 We found a significant negative effect of the habitat score on the maximum number
233 of individuals (Estimate = -16.88, SE = 3.22, Wald = 27.55, $p < 0.001$), the maximum
234 number of males (Estimate = -14.41, SE = 3.18, Wald = 20.52, $p < 0.001$, Fig 1) and the
235 maximum number of females (Estimate = -2.50, SE = 0.47, Wald = 28.15, $p < 0.001$, Fig
236 1). These numbers varied between years in females (Estimate = -2.35, SE = 0.93, Wald
237 = 6.41, $p = 0.01$) but not in males ($p = 0.17$).

238 Finally, we found a significant negative effect of the habitat score on the mean
239 number of individuals (Estimate = -4.51, SE = 1.34, Wald = 11.32, $p < 0.001$), the mean
240 number of males (Estimate = -4.19, SE = 1.26, Wald = 10.99, $p < 0.001$) and the mean
241 number of females (Estimate = -0.54, SE = 0.24, Wald = 5.05, $p = 0.024$). These
242 numbers did not vary between years (all $p > 0.25$).

243

244 *Indices of population trends*

245 We found a significant interaction between the habitat type and the number of males
246 captured between years (Estimate = -0.11, SE = 0.04, Wald = 7.55, p = 0.006). Number
247 of captured males in agricultural sites decreased strongly between years ($F_{1,17}=32.88$,
248 $p<0.0001$, Fig. 2), while the number of captured individuals from forest sites
249 remained steady ($F_{1,6}=0.003$, $p=0.96$, Fig. 2).

250

251

252

253

254 **Discussion**

255 Overall, we found that both presence and abundances of a widespread terrestrial
256 amphibian were markedly lower in reproductive ponds surrounded by intensive
257 agriculture. The most salient, yet worrisome, result of our study is the apparent
258 ongoing decline of this species in such farmland habitats. Indeed, this result may
259 suggest that unknown factors are currently affecting amphibian populations and
260 have recently induced strong population decreases over the course of a few years.

261

262 Although spined toads have been earlier shown to persist in agricultural habitats
263 (Guillot et al. 2016, but see Meek 2022), our results show that, as for many other
264 farmland species, agriculture negatively influenced the presence of reproductive
265 individuals at breeding sites (Keller and Waller 2002; Williams et al. 2015; Tucker et
266 al. 2018). Importantly, although such effect was found in both males and females, in
267 4 of the study sites (25% of the sites with a positive habitat score and thus
268 characterized by intensive agriculture) we did not observe any reproductive female.
269 Such result dovetails relatively well with previous observations made on the same
270 species and which have highlighted the lack of reproductive females and subsequent
271 lack of eggs and developing larvae at some sites situated within agricultural
272 landscapes (Renoirt et al. 2021b). Although the putative sex-specific mechanisms
273 presumably affecting females more than males in agricultural habitats remain
274 unknown, the lack of reproductive females at some breeding ponds is likely to
275 jeopardize population persistence in agricultural habitats.

276

277 In sites where spined toads were present for reproduction, abundances of both males
278 and females were strongly reduced in agricultural habitats. Several hypotheses can
279 explain this result. First, for growing juveniles and adult individuals, agricultural
280 habitats may be characterized by lower carrying capacity, both in term of terrestrial
281 microhabitats availability (buffered and concealed retreat sites to evade predation
282 and decrease thermal and hydric constraints, Tuomainen and Candolin 2011; Oliver
283 and Morecroft 2014) during the terrestrial part of the life cycle and/or in term of
284 trophic resources availability (decreased abundances of prey, Hart et al. 2006;
285 Wagner 2020). Such constraints would inevitably increase intraspecific competition
286 for these limiting resources and thus decrease abundances of toads in agricultural
287 habitats. Second, for developing eggs and larvae, the quality of aquatic breeding
288 sites may be lower in agricultural landscapes. For instance, the presence of
289 environmental contaminants in such sites (Bókony et al. 2018; Leeb et al. 2020) may
290 well negatively influence the survival of embryos and larvae (Bókony et al. 2018;
291 Cheron et al. 2022a; Cheron et al. 2022b, but see Loman and Lardner 2006) and/or the
292 quality of metamorphic individuals (Boone et al. 2005). We emphasize that these
293 hypotheses are not mutually exclusive and it is likely that reduced abundances of
294 spined toads in agricultural landscapes may result from complex interactions
295 between various habitat-specific constraints. Deciphering the relative role of these
296 different constraints will require future investigations.

297

298 Importantly, our results on indices of recent population trends may give further
299 insights into these processes. Indeed, although we emphasize that these data were

300 not designed to thoroughly assess population abundances over time and thus need to
301 be handled with caution (Pechmann et al. 1991, Reid et al. 2011); the trends we
302 highlighted seem to indicate very recent population decreases in agricultural habitats
303 as compared with preserved forest habitats. In line with the ideas developed above
304 (see Introduction), such result may indicate that the structural constraints of
305 agricultural habitats (linked to previous landscape homogenization and
306 fragmentation) may not be the primary driver of the current decreased presence and
307 abundances of spined toads. Indeed, based on examination of aerial photographs
308 (GoogleEarth) and on our knowledge of the study area, no structural changes have
309 occurred since the beginning of our surveys (e.g., land consolidation). In
310 combination with recent population trend, such information suggests that the decline
311 we are currently witnessing may be linked either indirectly to a concomitant decline
312 in prey abundances (Hart et al. 2006; Wagner 2020) and/or to other factors affecting
313 directly spined toads. In this respect, the potential role of environmental
314 contamination seems a likely candidate knowing the detrimental effects of
315 agrochemicals on wildlife (Kendall and Akerman 1992) and more specifically on
316 amphibians (Baker et al. 2013; Trudeau et al. 2020). Alternatively, but not
317 exclusively, it is also plausible that recent changes in climatic conditions, which
318 apply more strongly in open habitats than under forest canopies (De Frenne et al.
319 2019; De Lombaerde et al. 2022), affected amphibian populations in agricultural areas
320 (e.g., due to increased temperature and reduced precipitation, Lawler et al. 2010).
321 Whatever the underlying mechanisms, the putative sex-specific mechanisms

322 presumably affecting females more than males (see above, Renoirt et al. 2021b) are
323 required to be deciphered.

324

325 Overall, our study potentially highlights a worrying recent decline in the populations
326 of a widespread terrestrial amphibian previously thought to persist in agricultural
327 landscape. We emphasize that unknown factors are currently affecting these
328 populations very rapidly. Future investigations are required to identify these factors
329 at a time when anthropogenic activities are currently leading to unprecedented rates
330 of biodiversity loss.

331

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333

334

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341

342 **Competing Interests**

343 The authors have no relevant financial or non-financial interests to disclose.

344

345 **Author Contributions**

346 MR, FA and FB conceived the ideas and designed methodology; MR, MC, LJ, ST and
347 FB collected the data; MR, FA, MC and FB analysed the data; MR, FA and FB led the
348 writing of the manuscript. All authors contributed critically to the drafts and gave
349 final approval for publication.

350

351

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604 Caption to figures

605

606 **Figure 1.** Left panels: presence (1) and absence (0) of males (A) and females (B)
607 spined toads during two years (2021 and 2022) in 23 breeding sites situated along a
608 gradient of habitats from preserved sites (negative scores) to intensive agriculture
609 (positive scores). Right panels: abundances (maximum number of individuals
610 counted during a single night during the breeding peak) of males (C) and females (D)
611 spined toads during two years (2021 and 2022) in 8 sites situated along a gradient of
612 habitats from preserved sites (negative scores) to intensive agriculture (positive
613 scores). All sites were not monitored during all years and details can be found in
614 Appendix 1.

615

616 **Figure 2.** Number of males captured during the course of other studies on spined
617 toads (see methods for details). We used these capture data (number of captured
618 males during a single night situated around the peak of reproduction) in order to
619 monitor broad proxy of abundances across years. Grey symbols are used for sites
620 surrounded by forest, while black symbols represent sites surrounded by intensive
621 agriculture. Site numbers refer to those indicated in Appendix 1 and Appendix 2. All
622 sites were not monitored during all years and details can be found in Appendix 1.

623

624

625

Figure 1.

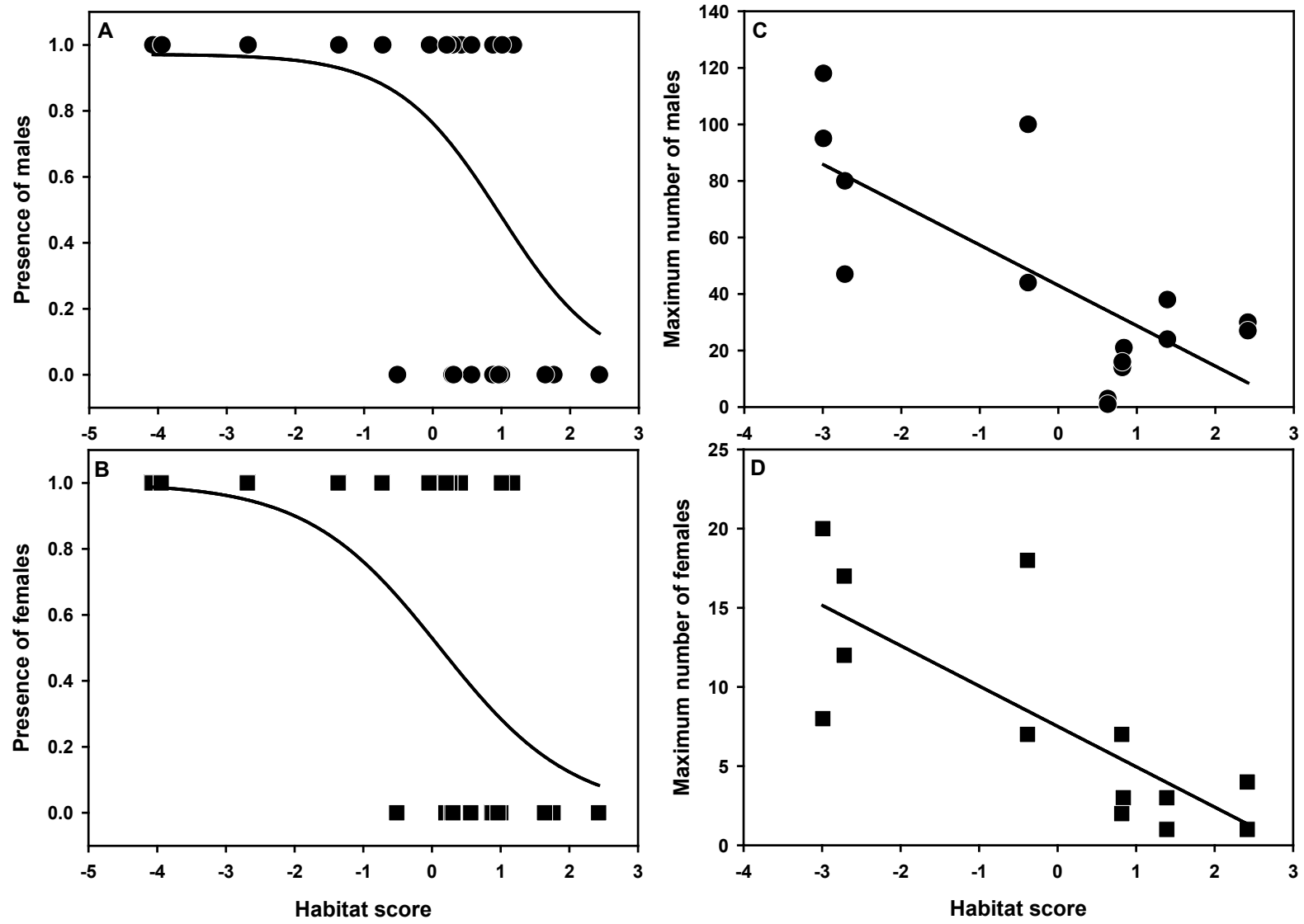
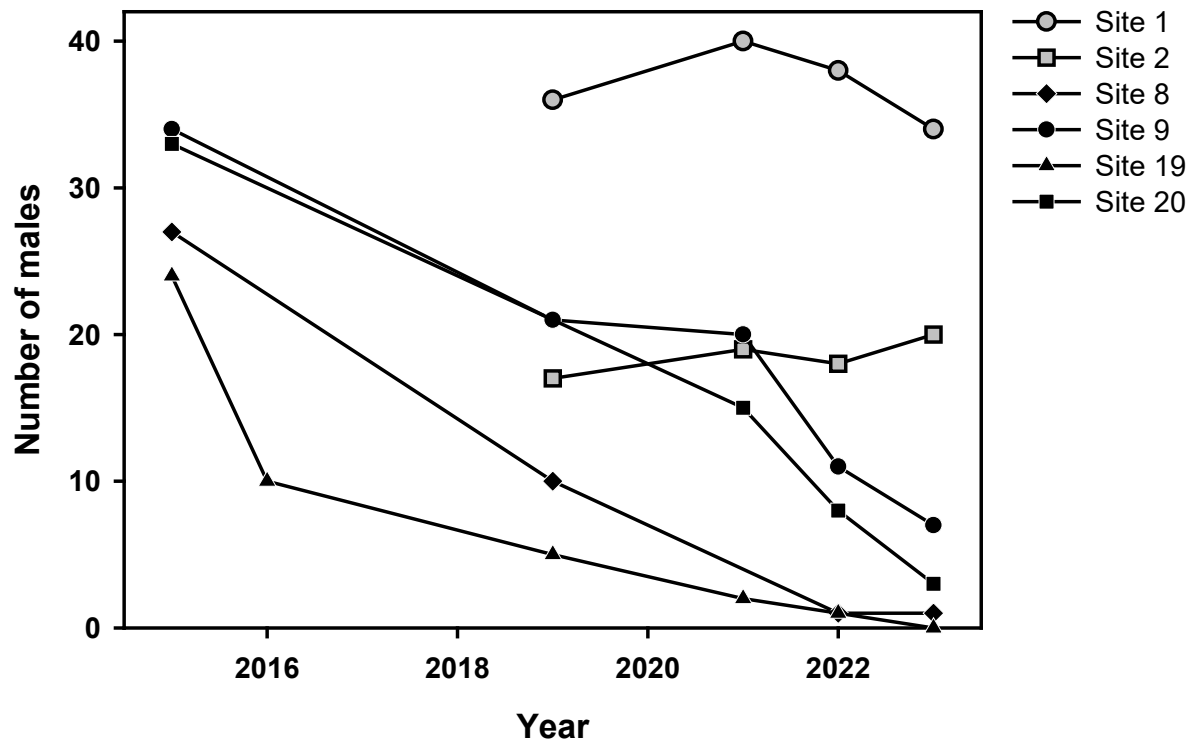


Figure 2.



Appendix 1. Description of the sampling details for our three complementary approaches (presence, abundance and index of population trends using capture data). Sites are numbered (Site#) according to ascending habitat score (Habitat score) from the PC1 values of a principal component analysis (see methods for details). Years monitored are indicated for each parameter (Presence, Abundance, Captures) used in our analyses.

Site#	Habitat score	Presence	Abundance	Captures
1	-4.07	2021-2022	2021-2022	2019, 2021-2023
2	-3.94	2021-2022	2021-2022	2019, 2021-2023
3	-2.69	2022		
4	-1.37	2021-2022	2021-2022	
5	-0.73	2022		
6	-0.51	2022		
7	-0.04	2022		
8	0.20	2021-2022	2021-2022	2015, 2019, 2021-2023
9	0.21	2021-2022	2021-2022	2015, 2019, 2021-2023
10	0.26	2021-2022	2021-2022	
11	0.28	2021-2022		
12	0.28	2022		
13	0.30	2021		
14	0.41	2021	2021	
15	0.56	2021-2022		
16	0.88	2021-2022		
17	0.96	2021		
18	1.00	2022		
19	1.01	2022		2015, 2016, 2019, 2021-2023
20	1.17	2021-2022	2021-2022	2015, 2019, 2021-2023
21	1.64	2022		
22	1.76	2022		
23	2.42	2021		

Appendix 2. Map of the study area showing each study sites surveyed. Numbers correspond to the sites detailed in Appendix 1. Site #2 is the Centre d'Etudes Biologiques de Chizé (CEBC-CNRS; coordinates 46.1475819, -0.4254604). Background image modified from GoogleEarth.

