

1 Spring temperature and land use change are associated with *Rana*  
2 *temporaria* reproductive success and phenology

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11

12 ABSTRACT

13 Chemical pollution, land cover change, and climate change have all been established as  
14 important drivers of amphibian reproductive success and phenology. However, little is  
15 known about the relative impacts of these anthropogenic stressors, nor how they may  
16 interact to alter amphibian population dynamics. Addressing this gap in our knowledge is  
17 important, as it allows us to identify and prioritise the most needed conservation actions.  
18 Here, we use long-term datasets to investigate landscape-scale drivers of variation in the  
19 reproductive success and phenology of UK Common frog (*Rana temporaria*) populations.  
20 Consistent with predictions, we found that increasing mean temperatures resulted in clear  
21 advancements in amphibian breeding phenology: earlier congregation of breeding *R.*  
22 *temporaria*, earlier initialisation of spawning, and earlier hatching. Temperature and number  
23 of frost days also affected rates of spawn mortality. However, temperature increases were  
24 also strongly correlated with increases in urban area, arable area, and nitrate levels in the  
25 vicinity of spawning grounds. None of these variables could explain variation in the total  
26 surface area of spawn present at breeding sites. These findings support previous work  
27 linking warming temperatures to shift in amphibian breeding phenology, but also highlight  
28 the importance of assessing the effect of land use change and pollution on wild amphibian  
29 populations. These results have implications for our understanding of the response of wild  
30 amphibian populations to climate change, and the management of human-dominated  
31 landscapes for declining wildlife populations.  
32

### 33 INTRODUCTION

34 Anthropogenic stressors are altering ecosystems across the world (Ceballos *et al.* 2015), with  
35 chemical pollution, land cover change, and climate change being highlighted as significant  
36 drivers of declines in ecosystem functioning (Wilcove *et al.* 1998; Walther *et al.* 2002;  
37 Howard *et al.* 2020). These altered ecosystems arise due to the effects of anthropogenic  
38 stressors on individual organisms, which can change community structures through  
39 decreased reproductive success and altered phenology (Nagelkerken & Munday  
40 2016). Although anthropogenic stressors influence a myriad of organisms, amphibians are  
41 particularly susceptible due to their unique life cycle and physiology (Blaustein & Kiesecker  
42 2002). Amphibia are subject to multiple threats including pathogens, land use change and  
43 climate change (Hof *et al.* 2011). The combined effects of these processes have led to  
44 amphibians becoming the most threatened vertebrate group (Hoffmann *et al.* 2010), and  
45 their phenologies advancing at a fast rate compared to other vertebrate taxa (Parmesan  
46 2006; Cohen *et al.* 2018). It is vital that we understand the drivers of these changes, and  
47 how these drivers may interact, as amphibians play many key roles in ecosystems (Burton &  
48 Likens 1975; Davic & Welsh Jr 2004; Mallory & Richardson 2005; Wood & Richardson 2010).  
49  
50 Climate change has affected amphibian reproductive success and phenology in both positive  
51 and negative ways. Increased winter precipitation resulting from climate change can benefit  
52 some amphibians (Benard 2015), but extreme weather events and advanced phenologies  
53 are likely to negatively impact others (Blaustein *et al.* 2010; Buss *et al.* 2021). In a review of  
54 the impacts of increased droughts and extreme precipitation, Walls *et al.* (2013) conclude  
55 that even amphibian species adapted to variable environmental conditions are not able to  
56 adapt fast enough to keep up with dramatic changes in precipitation patterns. Drought  
57 negatively affects the reproductive success of amphibians, whilst warming temperatures are  
58 responsible for trends towards earlier amphibian breeding (Ficetola & Maiorano 2016). For  
59 example, warmer winter temperatures caused earlier breeding in *Rana sylvatica*, but also  
60 lower female fecundity (Benard 2015), and have been associated with decreases in female  
61 body condition in female *Bufo bufo* (Reading 2007).  
62  
63 Chemical pollutants can increase mortality and alter rates of development in amphibian  
64 species (Carey & Bryant 1995), and the release of many chemical pollutants into the

65 environment is growing (Sharma *et al.* 2020). The application of nitrate and ammonium  
66 fertilisers in the United States has increased by 4000% since the 1940s (Cao *et al.* 2018),  
67 which has led to environmental nitrogen levels being high enough to impact amphibians  
68 (Rouse *et al.* 1999). Nitrogen fertilisers are toxic to amphibian embryos and larvae above  
69 certain levels, causing methemoglobinemia (Huey & Beitinger 1980), and can also lead to  
70 eutrophication (Boyer & Grue 1995). These effects can scale up to change reproductive  
71 success and phenology in amphibian populations. Ammonium nitrate exposure has been  
72 linked to species-specific survival in three Australian amphibian species (Hamer *et al.* 2004).  
73 Exposure of *Litoria aurea* larvae to 10-15 mg/l ammonium nitrate resulted in significantly  
74 reduced survival, but no such effect was seen in *Crinia signifera* or *Limnodynastes peronii*  
75 (Hamer *et al.* 2004). This suggests that the declines solely seen in *L. aurea* populations were  
76 due to the effect of ammonium nitrate on this species alone. Ammonium nitrate may also be  
77 responsible for declines in European species; *Hyla arborea*, *Discoglossus galganoi*, and *Bufo*  
78 *bufo*, all of which have reduced survival in <200 mg/l of ammonium nitrate (Ortiz *et al.*  
79 2004). Ammonium nitrate lowers larval development rate of *Pleurodeles waltl*, *Bufo*  
80 *calamita*, and *Pelobates cultripes*, suggesting that sub-lethal doses of ammonium and nitrate  
81 ions could alter amphibian phenology by delaying metamorphosis (Ortiz *et al.* 2004).

82  
83 The most likely driver of increased exposure to chemicals like ammonium nitrate is land  
84 cover change. Many amphibian species use terrestrial habitats to disperse from natal ponds  
85 (Semlitsch 2008), meaning high-quality terrestrial habitat is needed to prevent isolation  
86 (Marsh & Trenham 2001). Isolation can lead to reduced reproductive success in amphibian  
87 populations (Allentoft & O'Brien 2010), so both habitat quality and connectivity are vital, but  
88 land cover change can alter both these factors. Previous studies have shown that  
89 urbanisation reduces the area of suitable habitat available to many amphibians (Price *et al.*  
90 2012), as well as significantly increasing fragmentation and isolation (Natuhara & Zheng  
91 2022). Expansion of intensive agriculture can also lead to amphibian declines, with areas of  
92 Mediterranean cropland having significantly lowered amphibian abundance (Beja & Alcazar  
93 2003) compared to the surrounding countryside. The mechanisms driving these changes are  
94 likely the combined effects of reduced connectivity of habitat fragments, alongside  
95 increased exposure to chemicals.

96

97 Despite this abundance of research on the impacts of individual anthropogenic stressors on  
98 amphibian reproductive success and phenology, few studies have evaluated the relative  
99 impacts of these stressors simultaneously. This deficit can lead to difficulties in identifying  
100 the most effective actions needed to conserve amphibian species and the ecosystems they  
101 belong to (Frick *et al.* 2020). Here we investigate the relative impacts of chemical pollutants,  
102 land cover change, and climate change on the reproductive success and phenology of one  
103 amphibian species: *Rana temporaria*.

104

105 *R. temporaria* is an Anuran species widely distributed across Europe (Dabagyan & Sleptsova  
106 1991; Sillero *et al.* 2014) that breeds in still, fresh water (Haapanen 1982) but spend the  
107 majority of time in terrestrial habitats (Dabagyan & Sleptsova 1991). Although widespread,  
108 many *R. temporaria* populations are declining (Cooke 1972; Loman & Andersson 2007;  
109 Guarino *et al.* 2008) and their breeding phenology is advancing (Scott *et al.* 2008). Like other  
110 amphibian species, it seems that chemical pollutants, land cover change and climate change  
111 are altering *R. temporaria* reproductive success and phenology.

112 Although there are conflicting results as to whether ecologically relevant levels of  
113 ammonium and nitrate are directly lethal to *R. temporaria* spawn and larvae (Oldham *et al.*  
114 1997; Johansson *et al.* 2001), multiple studies demonstrate that high levels of these ions can  
115 reduce larval *R. temporaria* fitness, as well as delay metamorphosis (Johansson *et al.* 2001;  
116 Manson 2002; Oromí *et al.* 2009). In contrast, land cover change has been shown to cause *R.*  
117 *temporaria* population declines due to reduced reproductive success. For example, Cooke  
118 (1972) attributes the national declines in UK *R. temporaria* populations between 1940 and  
119 1970 to the draining of wetlands to make way for intensive agriculture and urbanisation.

120 Similar impacts of agricultural expansion have been seen in Sweden, where cropland  
121 populations are declining (Loman & Andersson 2007). These declines are not due to  
122 increased chemical pollutants (Loman & Lardner 2006) but could be due to lack of the  
123 suitable terrestrial refugia that adults need (Marnell 1998), leading the reduced gene pools  
124 and reproductive success (Allentoft & O'Brien 2010). There is little evidence to suggest that  
125 climate change is causing similar declines, with the only example being a period of high  
126 temperatures leading to reduced female fecundity in France (Neveu 2009). On the other  
127 hand, warming temperatures have been widely associated with earlier breeding phenology

128 in *R. temporaria* (Neveu 2009), with Scott *et al.* (2008) finding that dates of breeding  
129 congregations, spawning, and hatching advanced in correlation with warming temperatures.

130

131 Here, we use a 21-year long-term monitoring dataset of *R. temporaria* breeding populations  
132 in the UK to quantify the relative importance of chemical pollutants, land cover change, and  
133 climate change on *R. temporaria* reproductive success and phenology. We test the  
134 predictions that:

- 135 1. Increasing average temperatures associated with climate change will drive earlier  
136 breeding phenology, including congregation to breed, spawning and hatching.
- 137 2. Land cover change, measured as the expansion of arable and urban areas, will be  
138 associated with decreased reproductive success.
- 139 3. Increased levels of pollutants such as ammonium and nitrate will be associated with  
140 greater degrees of spawn mortality

141

142

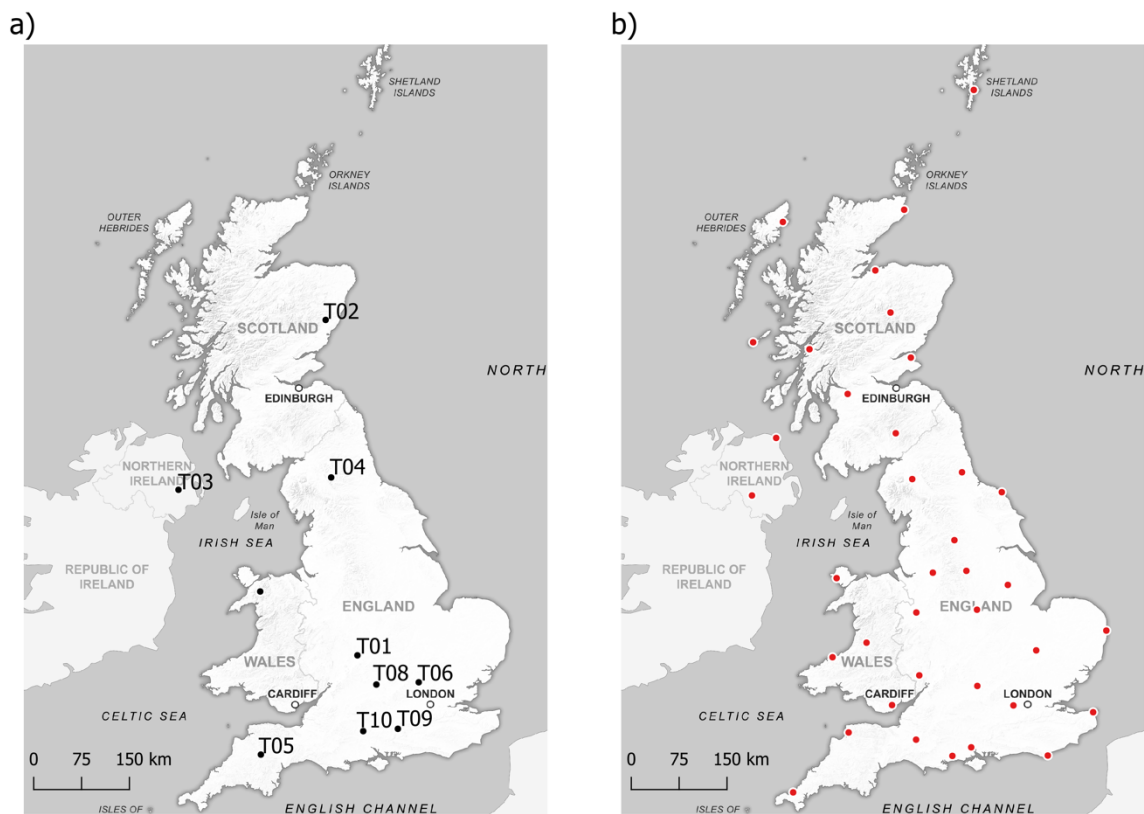
143 METHODS

144

145 *Data Sources*

146 We obtained data on *R. temporaria* reproductive success and phenology, as well as  
147 ammonium and nitrate ion concentrations, from the Environmental Change Network (ECN)  
148 (Rennie 2017). Across the UK, there are 10 ECN sites which collect this data (Figure 1a). The  
149 ECN sites differed in the number of ponds sampled and the duration they were sampled for  
150 (Table 1).

151



152

153 **Figure 1.** a) Locations of ponds sampled by the Environmental Change Network to obtain  
154 data on *R. temporaria* reproductive success and phenology, and agricultural pollutants, b)  
155 Locations of weather stations the Met Office use to measure climatic variables

156

157

ECN site	Co-ordinates	Number of ponds	First sampling year	Last sampling year
T01	52.19361, -1.76417	1	1994	2012
T02	56.90917, -2.55333	2	1994	2014
T03	54.45333, -6.07806	2	1994	2011

T04	54.695, -2.38778	7	1994	2015
T05	50.78167, -3.91778	1	1994	2015
T06	51.80333, -0.3725	1	1994	2010
T08	51.781111, -1.33583	1	1994	1994
T09	51.15444, -0.86306	1	1995	1995
T10	51.12694, -1.63972	1	2001	2001
T11	53.07455, -4.03351	2	1995	1995

158

159 **Table 1:** The number of ponds per ECN site and the years each ECN site was  
160 sampled for.

161

162 From 1<sup>st</sup> January every year, ponds were sampled weekly until males first congregated at the  
163 pond. This date was recorded, and subsequent sampling frequencies increased to daily until  
164 spawn first hatched. The dates of spawning and hatching were recorded. After hatching,  
165 sampling frequencies decreased to weekly until 16 weeks after spawning or when froglets  
166 were seen leaving the ponds. The date of leaving was recorded. The areas of spawn present  
167 and the percentage of spawn found dead were recorded when each pond was sampled. The  
168 concentrations of ammonium and nitrate ions were measured from the date of spawning by  
169 taking 250ml of pond water to analyse in a laboratory when each pond was sampled.

170 We obtained data on land cover change from the UK Centre for Ecology & Hydrology  
171 (UKCEH) (Rowland 2020a, b). Using satellite data, the dominant land cover type in each  
172 25x25m square of the UK in 1990 and 2015 were classified into 6 classes: woodland, arable,  
173 grassland, water, built-up areas, and other.

174

175 We obtained data on climate change from 37 historic Met Office stations across the UK  
176 (Figure 1b), which have been collecting climate data for at least 44 years (Met Office 2022).  
177 Climate data consists of monthly mean daily maximum temperature (°C), mean daily  
178 minimum temperature (°C), days with air frost, and total rainfall (mm).

179

### 180 *Data Processing*

181 To quantify the land cover change surrounding each ECN location and the distances between  
182 each ECN location and each Met Office station, we used QGIS (QGIS Development Team  
183 2022). Coordinates for the ECN locations were provided by the ECN, and the coordinates for  
184 the Met Office stations were taken from the Historic station data webpage (Met Office



185 2022), using the coordinate reference system OSGB 1996/British National Grid. To calculate  
186 the land cover change surrounding each ECN location we created a buffer zone with a radius  
187 of 2.5km surrounding each ECN location, as some common frogs can disperse over 2km  
188 (Kovar *et al.* 2009). We then used the land cover data obtained from UKCEH to calculate the  
189 area of each land cover class within these buffers in both 1990 and 2015. To calculate the  
190 distances between each ECN location and each Met Office station, we used the Distance  
191 matrix tool.

192

193 We used the software *R* (R Core Team 2020) to create a dataframe for each reproductive  
194 success and phenological response variable measured by the ECN (Table 2). Here, each row  
195 represented a pond at an ECN location in a single year from 1994-2015. The reproductive  
196 success data frames contained the largest surface area of spawn and percentage of dead  
197 spawn at each pond in each year. The phenology data frames contained the earliest date of  
198 either congregation, spawning, hatching, or leaving at each pond in each year, all converted  
199 to Julian days.

200

<b>Extracted variable</b>	<b>Abbreviation</b>	<b>Unit of measurement</b>	<b>Data source</b>
Congregation date	cong_date	Julian days	Rennie <i>et al.</i> , 2017
Spawning date	spawn_date	Julian days	Rennie <i>et al.</i> , 2017
Hatching date	hatch_date	Julian days	Rennie <i>et al.</i> , 2017
Leaving date	leave_date	Julian days	Rennie <i>et al.</i> , 2017
Surface area of spawn	surf_area	m <sup>2</sup>	Rennie <i>et al.</i> , 2017
Percentage of spawn dead	perc_dead	%	Rennie <i>et al.</i> , 2017
Ammonium concentration	spawn_nh4n	mg/l	Rennie <i>et al.</i> , 2017

Nitrate concentration	spawn_no3n	mg/l	Rennie <i>et al.</i> , 2017
Woodland area	woodland_area	m <sup>2</sup>	Rowland, 2020a Rowland, 2020b
Arable area	arable_area	m <sup>2</sup>	Rowland, 2020a Rowland, 2020b
Grassland area	grassland_area	m <sup>2</sup>	Rowland, 2020a Rowland, 2020b
Freshwater body area	freshwater_area	m <sup>2</sup>	Rowland, 2020a Rowland, 2020b
Built-up area	urban_area	m <sup>2</sup>	Rowland, 2020a Rowland, 2020b
Other land cover area	other_area	m <sup>2</sup>	Rowland, 2020a Rowland, 2020b
Mean daily maximum temperature per month	tmax	°C	Met Office, 2022
Mean daily minimum temperature per month	tmin	°C	Met Office, 2022
Number of days with air frost per month	af	days	Met Office, 2022
Total rainfall per month	rain	mm	Met Office, 2022

201

202 **Table 2:** The variables extracted for analysis and their sources. Abbreviations are  
 203 used in the Principal Components Analysis plots

204

205

206 To the data frame containing information on the date of spawning, we added the data on  
 207 maximum ammonium and nitrate ion concentrations recorded in each pond in each year  
 208 whilst breeding adults were present. To the data frames containing information on the  
 209 surface area of spawn, the percentage of spawn dead, and the date of hatching, we added  
 210 data on mean ammonium and nitrate ion concentrations recorded in each pond in each year  
 211 whilst spawn was present. To the data frame containing information on the date of leaving,

212 we added data on the mean ammonium and nitrate ion concentrations recorded in each  
213 pond in each year whilst tadpoles were present.

214

215 To calculate land cover change, we made assumption that the *rate* of land cover change was  
216 constant between years, allowing us to estimate the area of each land cover class  
217 surrounding each ECN location in each year from 1994-2015 (Table 2). We identified the  
218 nearest Met Office station to each ECN location and used the climate data from these  
219 stations as estimates for the climate at the ECN locations. As congregation and spawning  
220 dates depend on winter climate (Carroll *et al.* 2009; Benard 2015), we calculated the average  
221 of each climatic variable at each ECN location in each year over the winter months  
222 (December-March). We calculated the average of each climatic variable at each ECN location  
223 in each year during the months whilst spawn was present (January-May) and added these  
224 values to the data frames containing information on the surface area of spawn, the  
225 percentage of spawn dead, and the date of hatching. Finally, we calculated the average of  
226 each climatic variable at each ECN location in each year during the months whilst tadpoles  
227 were present (February-September) and added these values to the data frame containing  
228 information on the date of leaving.

229

### 230 *Data Analysis*

231 All data and code to reproduce these analyses is provided at

232 <https://github.com/xavharrison/FrogSpawn2023>

233

234 As landscape-scale variables such as land cover, temperature and chemical use are often  
235 correlated, we used a Principal Components Analysis (PCA) on the anthropogenic stressor  
236 variables for each of the reproductive success and phenology data frames using the  
237 factoextra and FactoMineR packages (Sebastien Le 2008; Mundt 2020). We extracted  
238 Principal Component 1 (PC1) and Principal Component 2 (PC2) for each reproductive success  
239 and phenological variable to use as predictors in our modelling. We used rotation plots to  
240 identify the anthropogenic stressors explained by each Principal Component (Simko 2021),  
241 and so crucially the biological interpretation of the importance of each PC changes for each  
242 model.

243

244 We used Generalised Linear Models (GLMs) and General Linear Mixed Effects Models  
245 (GLMM) to investigate drivers in variation in date of congregation (n=88), spawning (n=23),  
246 and hatching (n=25), as well as the surface area of spawn (n=34), and the percentage of  
247 dead spawn (n=33). Variation in the size of the datasets is a result of ensuring all rows have  
248 complete information on all traits, including land use change and pollution. For these  
249 datasets, we fitted GLMMs with Gaussian errors, with PC1 and PC2 as the explanatory  
250 variables, and the ECN location and/or pond as a random intercept. We logit-transformed  
251 the percentage of dead spawn prior to model fitting. These models also controlled for  
252 temporal autocorrelation in residuals. We used the 'Leave One Out' Information Criterion  
253 (LOO-IC, Vehtari *et al.* 2017) to identify the model(s). We performed 'full model tests',  
254 comparing the full model against a null model, to minimise bias in standard errors / credible  
255 intervals and control the Type I error rate (Forstmeier & Schielzeth 2011).

256

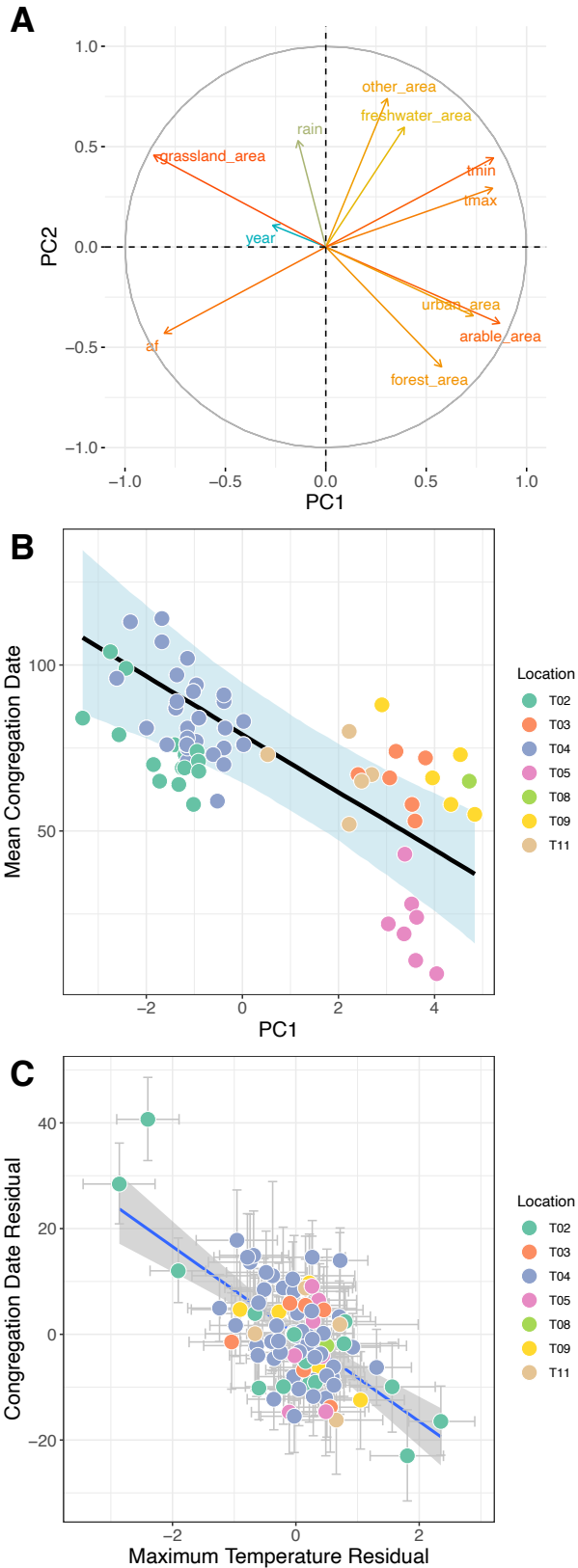
257 Finally, where we identified reproductive success and phenological variables to be  
258 associated with temperature (i.e. temperature loaded significantly into PC1 or PC2), we used  
259 bivariate mixed effect models to estimate the posterior correlation between temperature  
260 and phenological outcome variables. That is, variation in temperature arises due to  
261 latitudinal gradients as well as climate change, and so a higher standard of evidence is  
262 required to link shifts in phenology specifically to climate change rather than differences in  
263 site location. These bivariate models quantify correlation among the *residuals* of the two  
264 responses (e.g. mean maximum temperature and hatch date), whilst also accounting for  
265 fixed effects such as year/trends over time (see Houslay & Wilson 2017). Here we would  
266 predict that higher than average maximum temperatures (a positive residual) would be  
267 associated with earlier than average hatch dates (a negative residual). This manifests as a  
268 negative correlation in the residuals of the model, assessed as significant/important  
269 depending on whether the credible intervals cross zero. This approach is similar to  
270 'detrending' residuals to identify causal effects (e.g. see Votier *et al.* 2008)).

271

272 RESULTS

273 *Congregation Date*

274 We detected a negative relationship between PC1 and congregation date, meaning that  
275 higher maximum temperatures, and higher proportions of urban and arable area were  
276 associated with earlier congregation to breed (Fig. 2A,B). There was no support for an effect  
277 of PC2 (credible intervals crossed zero). The full model was a superior fit to a null model  
278 ( $\Delta$ LOO-IC 49.7). The model explained 81.5% of variation in congregation date [95% CI 77.2 =  
279 84.3%]. A bivariate model estimating the strength of correlation between maximum  
280 temperature and congregation date whilst controlling for temporal autocorrelation  
281 supported these patterns, where higher than average temperatures are associated with  
282 earlier than average congregation to breed (mean correlation -0.52, 95% CI -0.01 - -0.85; Fig  
283 2C).  
284



285

286 **Figure 2.** (A) Loadings of input variables on the first 2 axes of a Principal Component Analysis

287 explaining variation in congregation date. (B) Significant negative relationship between PC1

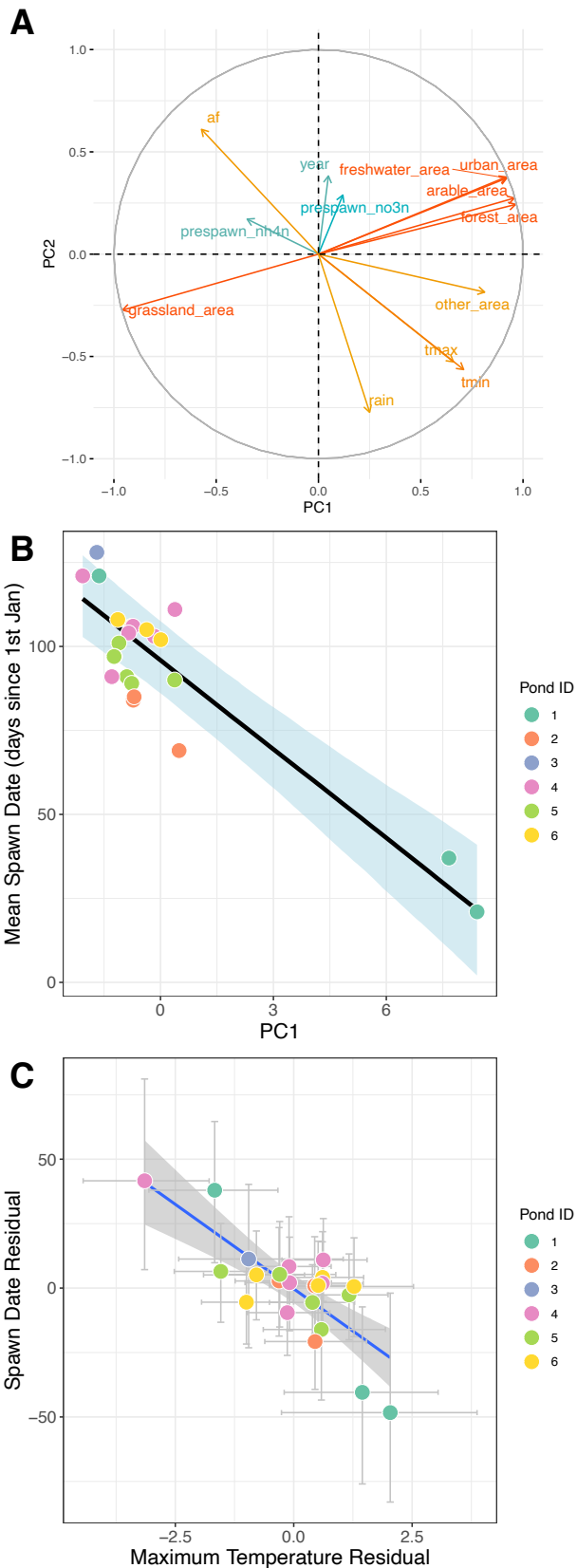
288 and congregation date (C) Significant negative correlation between maximum temperature  
289 at site and congregation date.

290

291 *Spawn Date*

292 As for congregation date, we uncovered a negative relationship between PC1 and spawn  
293 date. Higher maximum temperatures, and higher proportions of urban and arable area were  
294 associated with earlier spawning (Fig. 3A,B). There was no support for an effect of PC2  
295 (credible intervals crossed zero). The full model was a superior fit to a null model ( $\Delta$ LOO-IC  
296 40.3). The model explained 87% of variation in spawn date [95% CI 77.3 = 92%]. A bivariate  
297 model estimating the strength of correlation between maximum temperature and spawn  
298 date whilst controlling for temporal autocorrelation supported these patterns (mean  
299 correlation -0.67, 95% CI -0.34 - -0.87; Fig 3C).

300



301

302 **Figure 3.** (A) Loadings of input variables on the first 2 axes of a Principal Component

303 Analysis. (B) Significant negative relationship between PC1 and spawn date (C) Significant

304 negative correlation between maximum temperature at site and spawn date.



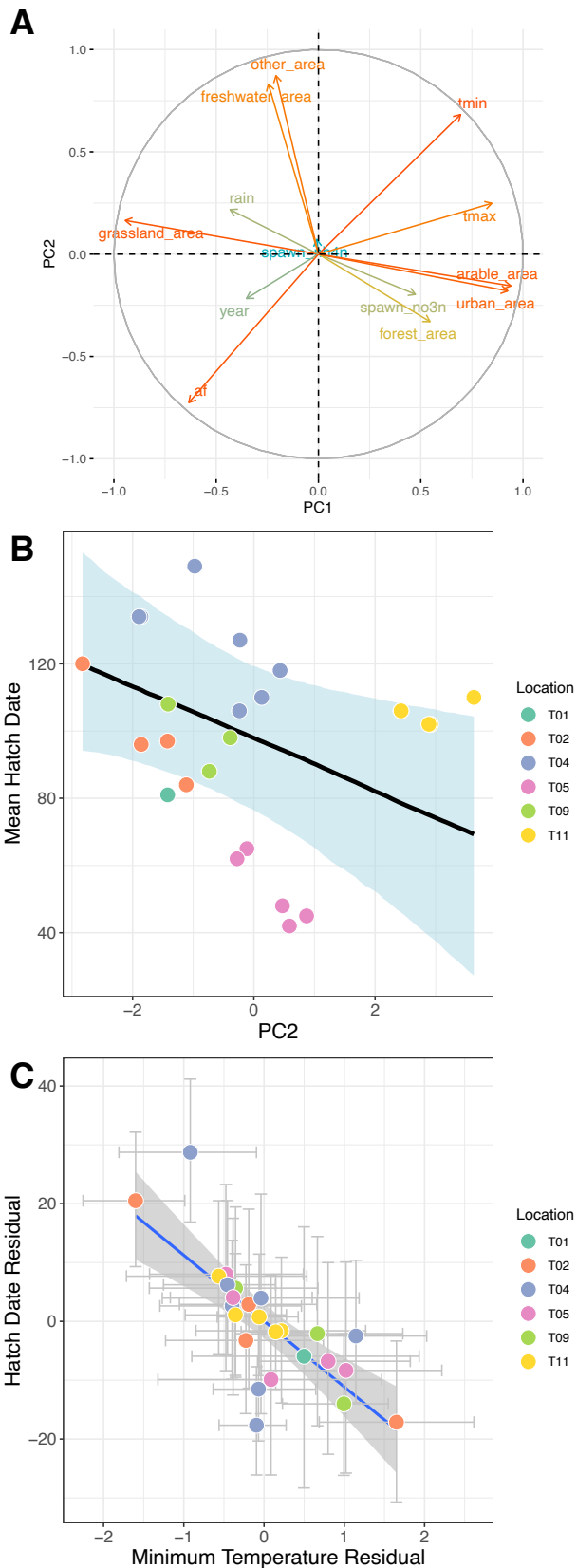
305 *Hatch Date*

306 For hatch date, we detected significant effects of PC2, where increases in freshwater area  
307 and higher *minimum* temperatures were associated with earlier hatching, and an increased  
308 in the number of Air Frost days was associated with later hatching (Fig. 4A; Fig. S1). A model  
309 containing effects of PC1 and PC2 was marginally superior fit to an intercept only model  
310 ( $\Delta\text{LOO-IC} = 6.8$ ; Fig. 4B). There was no support for an effect of PC1 (credible intervals crossed  
311 zero).

312 The model explained 89% of variation in hatch date [95% CI 77.3 = 92%]. A bivariate model  
313 estimating the strength of correlation between minimum temperature and hatch date whilst  
314 controlling for temporal autocorrelation supported these patterns (mean residual correlation  
315 -0.65, 95% CI -0.27 - -0.88; Fig 4C). A bivariate model with maximum temperature as a  
316 predictor returned similar results.

317

318



319

320 **Figure 4.** (A) Loadings of input variables on the first 2 axes of a Principal Component

321 Analysis. (B) Significant negative relationship between PC2 and hatch date (C) Significant

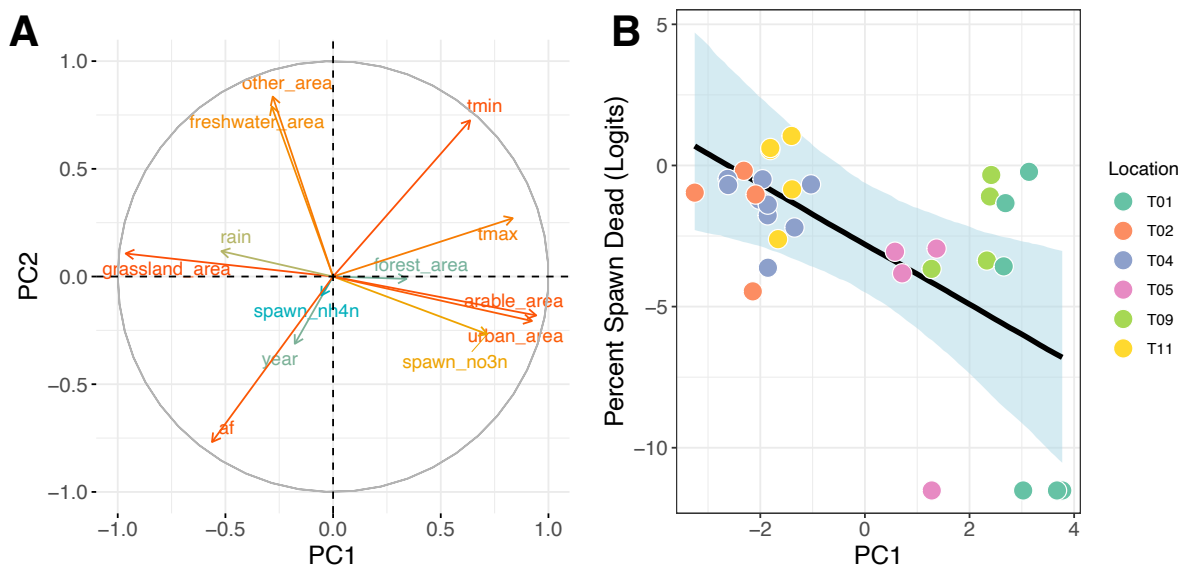
322 negative correlation between maximum temperature at a site and hatch date.

323

### 324 *Reproductive Success*

325 We detected a negative association between PC1 and the percentage of dead spawn  
326 observed (Fig 5A,B). PC1 represents a metric of the area of arable, freshwater, grassland, and  
327 built-up land cover, and the mean maximum daily temperatures spawn was exposed to (Fig  
328 5A). Higher cumulative days of air frost and higher grassland area were associated increased  
329 percentages dead spawn (Fig. S2), whilst increases in arable area, built-up area, and  
330 maximum temperature led to reductions in the percentage of dead spawn (Fig 5B; Fig S2).  
331 This model explained 45% of the variance in percentage of dead spawn (95% CI 22.5 –  
332 60.8%). Unlike previous traits, there was no support for a relationship between maximum  
333 temperature and % dead spawn (credible intervals crossed zero, though the relationship was  
334 negative (mean correlation -0.28, 95% CI -0.58 – 0.09). Finally, there was no statistical  
335 support for an effect of any of variable on the maximum surface area of spawn observed.

336



337

338 **Figure 5.** (A) Loadings of input variables on the first 2 axes of a Principal Component  
339 Analysis. (B) Significant negative relationship between PC1 and the percentage of spawn  
340 dead.

341

342

## 343 DISCUSSION

344 Here we used long-term data on the breeding behaviour of UK *Rana temporaria* to identify  
345 associations with land use change, pollution, and climate. We found that higher maximum  
346 temperatures were associated with earlier congregation & spawning, whilst higher minimum  
347 temperatures were linked to earlier hatching. No predictors could be linked to the surface  
348 area of spawn present at a site, but higher numbers of frost days were associated with  
349 higher proportions of dead spawn. All reproductive traits we measured could also be linked  
350 to traits of land use change and pollution (via Principal Component Analysis), which  
351 themselves correlated with temperature variation. This study highlights the important role  
352 of winter temperature in driving variation in amphibian breeding phenology, but also the  
353 complexity of disentangling the relative significance of multiple correlated environmental  
354 variables in wild phenology studies.

355

### 356 *Phenology, Land Use Change and Temperature*

357 This work is consistent with previous studies linking temperature shifts to alteration in  
358 amphibian breeding phenology (Reading 2007; Blaustein *et al.* 2010; Benard 2015).  
359 Consistent with our predictions, we found that warmer average temperatures correlated  
360 with advancements in the timings of adult congregation to breed, spawning, and hatching.  
361 Crucially, our modelling approach allows us to disentangle the relative effects of latitudinal  
362 variation in breeding phenology and *within location* variation caused by local shifts in  
363 climate, such as mean winter temperature. These bivariate models revealed that within  
364 sites, warmer than average winter temperatures were associated with earlier than average  
365 metrics of breeding phenology. Taken together these data suggest that *R. temporaria*  
366 breeding phenology shows a degree of plasticity, and responds to local variation in  
367 temperature among years by shifting their timing of congregation, spawning and hatching.  
368 Similar patterns have recently been observed in two newt species (Hubáček & Gvoždík  
369 2023), suggesting this many amphibian species are capable of exhibiting such individual  
370 plasticity.

371

372 Warmer winters have been shown to advance phenology in congeners like wood frogs (*Rana*  
373 *sylvatica*), but also delay larval development time (Benard 2015). Crucially earlier breeding  
374 does not compensate for this developmental delay. In common toads *Bufo bufo* in the

375 United Kingdom, warmer than average temperatures correlated with decreased body  
376 condition and survival (Reading 2007). Recent work on wood frogs showed that phenological  
377 shifts can expose individuals to colder temperatures and resulted in lower tolerance of  
378 offspring to pollutants like NaCl (Buss et al 2021). Collectively these data suggest that in  
379 some species, warming can induce cryptic cost of breeding plasticity in multiple life stages  
380 that are not immediately apparent if looking at phenological variables alone (see Blaustein  
381 et al 2010).

382

383 We also uncovered associations between land use change and breeding phenology.  
384 Increased arable and built-up land cover is associated with earlier hatching, whilst grassland  
385 is associated with later hatching. Arable and built-up areas tend to be warmer than the  
386 surrounding natural habitats due to unvegetated ground, whilst grassland areas, with higher  
387 vegetation levels, are cooler (Lembrechts *et al.* 2019; Schmidt *et al.* 2019). The warming  
388 effects of arable and built-up areas can even increase the temperature of the wider  
389 landscape, leading to earlier breeding phenology in the areas surrounding human-  
390 dominated land (Tian *et al.* 2020). These effects could be reduced by increasing vegetated  
391 areas in these land cover types, thus providing cooler microclimates (Greenwood *et al.*  
392 2016).

393

#### 394 *Land Use Change, Temperature and Spawn Mortality*

395 We found no clear association between mean winter maximum or minimum temperatures  
396 and spawn mortality, when temperature was used as the sole predictor in models. Instead,  
397 we found that the percentage of dead spawn was linked to a composite measure of frost  
398 days, rainfall, land use change, and temperature. Increases in the proportion of arable and  
399 urban areas, nitrate levels, and increased mean maximum temperature, were associated  
400 with higher spawn survival. Conversely, increased numbers of frost days, rainfall and  
401 grassland areas were associated with decreasing reproductive success.

402

403 These associations did not align with our predictions, where we expected increased  
404 farmland (and associated pollution such as nitrates), and increased urbanisation to be  
405 detrimental rather than associated with higher spawn survival. However, though human-  
406 driven land use change is often associated with lower reproductive success, some studies

407 have shown that they can support declining populations. For example, the average  
408 reproductive success of multiple amphibian species in America breeding in arable ponds was  
409 no different to in natural wetlands (Knutson *et al.* 2004). Though *some* species did respond  
410 negatively to arable land use (Knutson *et al.* 2004), *R. temporaria* have been found to use  
411 arable ponds more than other amphibians (Hartel *et al.* 2011). Therefore, arable land cover  
412 may be able to support robust populations of *R. temporaria*, leading to higher spawn  
413 survival in the ECN sampling locations. Additionally, arable ditches have been shown to  
414 confer landscape connectivity for amphibians, reptiles, and mammals (Maisonneuve & Rioux  
415 2001; Jobin *et al.* 2004). Maes *et al.* (2008) even found that ditches in agricultural  
416 environmental schemes supported similar *Rana esculenta* abundances as nature reserves,  
417 demonstrating how these features of arable land cover can serve as important avenues for  
418 dispersal between breeding sites. This is crucial in preventing population isolation, perhaps  
419 explaining why we found a negative relationship between the arable area and the maximum  
420 percentage of spawn that died (Allentoft & O'Brien 2010).

421  
422 Built-up areas have also been shown to increase the fitness of some wildlife populations,  
423 with some threatened birds (Kettel *et al.* 2019) and amphibians (Iglesias-Carrasco *et al.*  
424 2017) having greater reproductive success and body condition respectively in built-up areas  
425 than in the countryside. There are multiple theories for why this could be. Saenz *et al.* (2015)  
426 suggest that the occurrence of chytridiomycosis, an amphibian disease that can reduce  
427 fitness, could be lower in urban areas. However, chytridiomycosis is not common in British  
428 amphibians (Garner *et al.* 2005) so this is unlikely to explain our findings. Hall and Warner  
429 (2017) suggest that the high densities of prey insects in urban areas or lower predation  
430 pressures that allow adults to spend more time hunting, could increase fitness. Further  
431 evidence for this is from Germany, where *R. temporaria* adults were found to be bigger in  
432 urban greenspace than in the surrounding countryside (Niemeier *et al.* 2020). Larger adult  
433 body size is likely to increase offspring survival (Hall & Warner 2017), providing an  
434 explanation as to why built-up areas are associated with lower *R. temporaria* spawn  
435 mortality.

436  
437 Many of the studies that demonstrate the value of human-dominated land cover types also  
438 acknowledge the need for management. In arable areas, vegetation complexity in ditches

439 should be encouraged (Maisonneuve & Rioux 2001) to allow ditches to decrease *R.*  
440 *temporaria* population isolation. Similarly, in built-up areas, it is important to reduce barriers  
441 to movement by connecting urban greenspace (Mazgajska & Mazgajski 2020; Niemeier *et al.*  
442 2020). These suggestions highlight that local management for *R. temporaria* could be more  
443 important than the broad-scale land cover type in determining this amphibian's reproductive  
444 success.

445  
446 Landscape management practices could also explain why grassland is negatively associated  
447 with *R. temporaria* reproductive success. In the UK, only 2% of grassland is classed as diverse  
448 (Bullock *et al.* 2011), due to widespread "improvement" (Vickery 1999) and livestock grazing  
449 (Fuller 1987; Bullock *et al.* 2011). However, grazing can be detrimental to amphibians.  
450 Livestock can cause high levels of wetland bank erosion (Trimble 1994), leading to increased  
451 sediment deposition. When investigating the impact of cattle on *Bufo achalensis*, a toad  
452 species endemic to Argentina, Jofré *et al.* (2007) found that increased sediment levels  
453 reduced algal growth, a key food source for larval amphibians, leading to higher mortality in  
454 the *B. achalensis* larvae. This could result in increased isolation between *R. temporaria*  
455 populations, potentially leading to the increased percentage of spawn death observed in  
456 ECN locations surrounded by grassland. High densities of livestock can also directly reduce  
457 amphibian spawn reproductive success through disruption and trampling (Knutson *et al.*  
458 2004).

459  
460 The negative relationship between freshwater area and reproductive success seems  
461 counterintuitive, however, predator presence could explain this relationship. The land cover  
462 data used in this study had a resolution of 25x25m, so only large water bodies were present  
463 in the data (Rowland 2020a, b). Previous studies have found that these are often avoided by  
464 amphibians as they are more likely to contain a high density of predators (Pearman 1995).  
465 Knutson *et al.* (2004) even found that the presence of predatory fish was one of the most  
466 important factors in determining the reproductive success of an American amphibian  
467 community. Therefore, the presence of large areas of freshwater surrounding the ECN  
468 sampling locations may increase the isolation of ECN breeding populations, resulting in  
469 reduced spawn survival and higher spawn mortality.

470

471 Our modelling could not conclusively implicate temperature regime changes as a potential  
472 driver of difference in amphibian reproductive success, though maximum temperature and  
473 number of frost days were part of the composite measure (PC1) associated with spawn  
474 survival. Although climate change is leading to warmer temperatures, it is also causing  
475 extreme weather conditions (Huber & Gullede 2011). One example is the occurrence of  
476 spring cold-snaps, a phenomenon that has been shown to have detrimental effects on a  
477 wide range of organisms (Augspurger 2013; Benard 2015; Turner & Maclean 2022). Benard  
478 (2015) observed an increase in *Rana sylvatica* larvae being exposed to cold-snaps from 2006  
479 to 2012, leading to altered development. Freezing is known to kill *R. temporaria* (Pasanen &  
480 Karhapää 1997), making it likely that cold-snaps could lead to high spawn mortality. Warmer  
481 springs and fewer frost days likely explain the lower proportions of dead spawn observed in  
482 this dataset under these conditions.

483

#### 484 *Conclusions and Future Work*

485 Here we have demonstrated associations between climate, land use change and parameters  
486 of amphibian breeding success and phenology at the landscape scale. Our results are  
487 consistent with investigations at the scale of individual ponds that uncovered similar  
488 relationships between temperature and the timing of amphibian breeding and larval  
489 development (Benard 2015). Future work on *R. temporaria* in the UK should prioritise pond-  
490 scale approaches to these questions, which will permit measurement of microhabitats  
491 experienced by breeding adults. Microhabitat temperature measurements could shed  
492 further light on the frequency and consequences of freezing temperature (i.e. lower winter  
493 minima) on *R. temporaria*, as well as their effect on larval development and survival.  
494 Similarly, fine-scale land-use data could aid in identifying the habitats most beneficial for  
495 successful *R. temporaria* breeding, allowing the efficacy of management practices to be  
496 optimised. The need for fine-scale data is particularly important due to the small size and  
497 limited dispersal ability of *R. temporaria* (Kovar *et al.*, 2009), and thus may also be applicable  
498 to other such species. For the ongoing survival of *R. temporaria* it is vital to reduce the harm  
499 of extreme weather events due to climate change, which may be achieved through the  
500 management of microclimates. Afforestation and re-flooding drained wetlands, for example,  
501 could help maintain stable and favourable microclimatic envelopes. These practices could



502 also aid *R. temporaria* by increasing vegetation complexity and providing additional breeding  
503 locations and their connectivity, both of which could improve *R. temporaria* survival.

504

505

## 506 REFERENCES

507

508 Allentoft, M.E. & O'Brien, J. (2010). Global amphibian declines, loss of genetic diversity and  
509 fitness: a review. *Diversity*, 2, 47-71.

510

511 Augspurger, C.K. (2013). Reconstructing patterns of temperature, phenology, and frost  
512 damage over 124 years: spring damage risk is increasing. *Ecology*, 94, 41-50.

513

514 Beja, P. & Alcazar, R. (2003). Conservation of Mediterranean temporary ponds under  
515 agricultural intensification: an evaluation using amphibians. *Biological Conservation*,  
516 114, 317-326.

517

518 Benard, M.F. (2015). Warmer winters reduce frog fecundity and shift breeding phenology,  
519 which consequently alters larval development and metamorphic timing. *Global  
520 Change Biology*, 21, 1058-1065.

521

522 Blaustein, A.R. & Kiesecker, J.M. (2002). Complexity in conservation: lessons from the global  
523 decline of amphibian populations. *Ecology Letters*, 5, 597-608.

524

525 Blaustein, A.R., Walls, S.C., Bancroft, B.A., Lawler, J.J., Searle, C.L. & Gervasi, S.S. (2010).  
526 Direct and indirect effects of climate change on amphibian populations. *Diversity*, 2,  
527 281-313.

528

529 Boyer, R. & Grue, C.E. (1995). The need for water quality criteria for frogs. *Environmental  
530 Health Perspectives*, 103, 352-357.

531

532 Bullock, J.M., Jefferson, R.G., Blackstock, T.H., Pakeman, R.J., Emmett, B.A., Pywell, R.J. *et al.*  
533 (2011). Semi-natural grasslands.

534

535 Burton, T.M. & Likens, G.E. (1975). Salamander populations and biomass in the Hubbard  
536 Brook experimental forest, New Hampshire. *Copeia*, 541-546.

537

538 Buss, N., Swierk, L. and Hua, J., (2021). Amphibian breeding phenology influences offspring  
539 size and response to a common wetland contaminant. *Frontiers in Zoology*, 18(1),  
540 p.31.

541

542 Cao, P., Lu, C. & Yu, Z. (2018). Historical nitrogen fertilizer use in agricultural ecosystems of  
543 the contiguous United States during 1850–2015: application rate, timing, and  
544 fertilizer types. *Earth System Science Data*, 10, 969-984.

545

- 546 Carey, C. & Bryant, C.J. (1995). Possible interrelations among environmental toxicants,  
547 amphibian development, and decline of amphibian populations. *Environmental*  
548 *Health Perspectives*, 103, 13-17.  
549
- 550 Carroll, E., Sparks, T., Collinson, N. & Beebee, T. (2009). Influence of temperature on the  
551 spatial distribution of first spawning dates of the common frog (*Rana temporaria*) in  
552 the UK. *Global Change Biology*, 15, 467-473.  
553
- 554 Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M. & Palmer, T.M. (2015).  
555 Accelerated modern human-induced species losses: entering the sixth mass  
556 extinction. *Science Advances*, 1, e1400253.  
557
- 558 Cohen, J.M., Lajeunesse, M.J. & Rohr, J.R. (2018). A global synthesis of animal phenological  
559 responses to climate change. *Nature Climate Change*, 8, 224-228.  
560
- 561 Cooke, A. (1972). Indications of recent changes in status in the British Isles of the frog (*Rana*  
562 *temporaria*) and the toad (*Bufo bufo*). *Journal of Zoology*, 167, 161-178.  
563
- 564 Dabagyan, N. & Sleptsova, L. (1991). The common frog *Rana temporaria*. In: *Animal Species*  
565 *for Developmental Studies: Vertebrates*. Springer, pp. 283-305.  
566
- 567 Davic, R.D. & Welsh Jr, H.H. (2004). On the ecological roles of salamanders. *Annu. Rev. Ecol.*  
568 *Evol. Syst.*, 35, 405-434.  
569
- 570 Ficetola, G.F. & Maiorano, L. (2016). Contrasting effects of temperature and precipitation  
571 change on amphibian phenology, abundance and performance. *Oecologia*, 181, 683-  
572 693.  
573
- 574 Forstmeier, W. & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models:  
575 overestimated effect sizes and the winner's curse. *Behavioral Ecology and*  
576 *Sociobiology*, 65, 47-55.  
577
- 578 Frick, W.F., Kingston, T. & Flanders, J. (2020). A review of the major threats and challenges to  
579 global bat conservation. *Annals of the New York Academy of Sciences*, 1469, 5-25.  
580
- 581 Fuller, R.M. (1987). The changing extent and conservation interest of lowland grasslands in  
582 England and Wales: a review of grassland surveys 1930–1984. *Biological*  
583 *Conservation*, 40, 281-300.  
584
- 585 Garner, T.W., Walker, S., Bosch, J., Hyatt, A.D., Cunningham, A.A. & Fisher, M.C. (2005).  
586 Chytrid fungus in Europe. *Emerging Infectious Diseases*, 11, 1639.  
587
- 588 Greenwood, O., Mossman, H.L., Suggitt, A.J., Curtis, R.J. & Maclean, I.M. (2016). Using in situ  
589 management to conserve biodiversity under climate change. *Journal of Applied*  
590 *Ecology*, 53, 885-894.  
591

- 592 Guarino, F., Di Già, I. & Sindaco, R. (2008). Age structure in a declining population of *Rana*  
593 *temporaria* from northern Italy. *Acta Zoologica Academiae Scientiarum Hungaricae*,  
594 54, 99-91.  
595
- 596 Haapanen, A. (1982). Breeding of the common frog (*Rana temporaria* L.). In: *Annales*  
597 *Zoologici Fennici*. JSTOR, pp. 75-79.  
598
- 599 Hall, J.M. & Warner, D.A. (2017). Body size and reproduction of a non-native lizard are  
600 enhanced in an urban environment. *Biological Journal of the Linnean Society*, 122,  
601 860-871.  
602
- 603 Hamer, A.J., Makings, J.A., Lane, S.J. & Mahony, M.J. (2004). Amphibian decline and  
604 fertilizers used on agricultural land in south-eastern Australia. *Agriculture,*  
605 *Ecosystems & Environment*, 102, 299-305.  
606
- 607 Hartel, T., Băncilă, R. & Cogălniceanu, D. (2011). Spatial and temporal variability of aquatic  
608 habitat use by amphibians in a hydrologically modified landscape. *Freshwater*  
609 *Biology*, 56, 2288-2298.  
610
- 611 Hof, C., Araújo, M.B., Jetz, W. and Rahbek, C., 2011. Additive threats from pathogens, climate  
612 and land-use change for global amphibian diversity. *Nature*, 480, 516-519.  
613
- 614 Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T.M., Butchart, S.H. *et al.*  
615 (2010). The impact of conservation on the status of the world's vertebrates. *Science*,  
616 330, 1503-1509.  
617
- 618 Houslay, T.M. & Wilson, A.J. (2017). Avoiding the misuse of BLUP in behavioural ecology.  
619 *Behavioral Ecology*, 28, 948-952.  
620
- 621 Howard, C., Flather, C.H. & Stephens, P.A. (2020). A global assessment of the drivers of  
622 threatened terrestrial species richness. *Nature Communications*, 11, 993.  
623
- 624 Hubáček, J. & Gvoždík, L. (2023). Terrestrial amphibians respond to rapidly changing  
625 temperatures with individual plasticity of exploratory behaviour. *Journal of Thermal*  
626 *Biology*, 103757.  
627
- 628 Huber, D.G. & Gullede, J. (2011). *Extreme weather and climate change: understanding the*  
629 *link, managing the risk*. Pew Center on Global Climate Change Arlington.  
630
- 631 Huey, D.W. & Beiting, T.L. (1980). Hematological responses of larval *Rana catesbiana* to  
632 sublethal nitrite exposures. *Bulletin of Environmental Contamination and Toxicology*,  
633 25, 574-577.  
634
- 635 Iglesias-Carrasco, M., Martín, J. & Cabido, C. (2017). Urban habitats can affect body size and  
636 body condition but not immune response in amphibians. *Urban Ecosystems*, 20,  
637 1331-1338.  
638

- 639 Jobin, B.t., Bélanger, L., Boutin, C. & Maisonneuve, C. (2004). Conservation value of  
640 agricultural riparian strips in the Boyer River watershed, Québec (Canada).  
641 *Agriculture, Ecosystems & Environment*, 103, 413-423.  
642
- 643 Jofré, G., Reading, C. & di Tada, I. (2007). Habitat selection in the Pampa de Achala toad,  
644 *Bufo achalensis*. *Amphibia-Reptilia*, 28, 129-138.  
645
- 646 Johansson, M., Räsänen, K. & Merilä, J. (2001). Comparison of nitrate tolerance between  
647 different populations of the common frog, *Rana temporaria*. *Aquatic Toxicology*, 54,  
648 1-14.  
649
- 650 Kettel, E.F., Gentle, L.K., Yarnell, R.W. & Quinn, J.L. (2019). Breeding performance of an apex  
651 predator, the peregrine falcon, across urban and rural landscapes. *Urban Ecosystems*,  
652 22, 117-125.  
653
- 654 Knutson, M.G., Richardson, W.B., Reineke, D.M., Gray, B.R., Parmelee, J.R. & Weick, S.E.  
655 (2004). Agricultural ponds support amphibian populations. *Ecological Applications*,  
656 14, 669-684.  
657
- 658 Kovar, R., Brabec, M., Vita, R. & Bocek, R. (2009). Spring migration distances of some Central  
659 European amphibian species. *Amphibia-Reptilia*, 30, 367-378.  
660
- 661 Lembrechts, J.J., Nijs, I. & Lenoir, J. (2019). Incorporating microclimate into species  
662 distribution models. *Ecography*, 42, 1267-1279.  
663
- 664 Loman, J. & Andersson, G. (2007). Monitoring brown frogs *Rana arvalis* and *Rana*  
665 *temporaria* in 120 south Swedish ponds 1989–2005. Mixed trends in different  
666 habitats. *Biological Conservation*, 135, 46-56.  
667
- 668 Loman, J. & Lardner, B. (2006). Does pond quality limit frogs *Rana arvalis* and *Rana*  
669 *temporaria* in agricultural landscapes? A field experiment. *Journal of Applied Ecology*,  
670 43, 690-700.  
671
- 672 Maes, J., Musters, C. & De Snoo, G.R. (2008). The effect of agri-environment schemes on  
673 amphibian diversity and abundance. *Biological Conservation*, 141, 635-645.  
674
- 675 Maisonneuve, C. & Rioux, S. (2001). Importance of riparian habitats for small mammal and  
676 herpetofaunal communities in agricultural landscapes of southern Québec.  
677 *Agriculture, Ecosystems & Environment*, 83, 165-175.  
678
- 679 Mallory, M.A. & Richardson, J.S. (2005). Complex interactions of light, nutrients and  
680 consumer density in a stream periphyton–grazer (tailed frog tadpoles) system.  
681 *Journal of Animal Ecology*, 74, 1020-1028.  
682
- 683 Manson, P.S. (2002). The sub-lethal effects of ammonium nitrate fertiliser on the common  
684 frog '*Rana temporaria*'.  
685

- 686 Marnell, F. (1998). Discriminant analysis of the terrestrial and aquatic habitat determinants  
687 of the smooth newt (*Triturus vulgaris*) and the common frog (*Rana temporaria*) in  
688 Ireland. *Journal of Zoology*, 244, 1-6.  
689
- 690 Marsh, D.M. & Trenham, P.C. (2001). Metapopulation dynamics and amphibian conservation.  
691 *Conservation Biology*, 15, 40-49.  
692
- 693 Mazgajska, J. & Mazgajski, T. (2020). Two amphibian species in the urban environment:  
694 changes in the occurrence, spawning phenology and adult condition of common and  
695 green toads. *The European Zoological Journal*, 87, 170-179.  
696
- 697 Met Office. (2022). Historic station data. Available at:  
698 <https://www.metoffice.gov.uk/research/climate/maps-and-data/historic-station-data>  
699 [Accessed 20/10/2022].  
700
- 701 Mundt, A.K.A.F. (2020). Factoextra: extract and visualize the results of multivariate data  
702 analyses. R package version 1.0.7.  
703
- 704 Nagelkerken, I. & Munday, P.L. (2016). Animal behaviour shapes the ecological effects of  
705 ocean acidification and warming: moving from individual to community-level  
706 responses. *Global Change Biology*, 22, 974-989.  
707
- 708 Natuhara, Y. & Zheng, X. (2022). Effects of advance and retreat of agricultural landscapes on  
709 *Rana japonica* and *R. ornativentris*. *Landscape and Ecological Engineering*, 18, 493-  
710 503.  
711
- 712 Neveu, A. (2009). Incidence of climate on common frog breeding: long-term and short-term  
713 changes. *Acta Oecologica*, 35, 671-678.  
714
- 715 Niemeier, S., Mueller, J., Struck, U. & Rödel, M.O. (2020). Superfrogs in the city: 150 year  
716 impact of urbanization and agriculture on the European common frog. *Global*  
717 *Change Biology*, 26, 6729-6741.  
718
- 719 Oldham, R., Latham, D., Hilton-Brown, D., Towns, M., Cooke, A. & Burn, A. (1997). The effect  
720 of ammonium nitrate fertiliser on frog (*Rana temporaria*) survival. *Agriculture,*  
721 *Ecosystems & Environment*, 61, 69-74.  
722
- 723 Oromí, N., Sanuy, D. & Vilches, M. (2009). Effects of nitrate and ammonium on larvae of  
724 *Rana temporaria* from the Pyrenees. *Bulletin of Environmental Contamination and*  
725 *Toxicology*, 82, 534-537.  
726
- 727 Ortiz, M.E., Marco, A., Saiz, N. & Lizana, M. (2004). Impact of ammonium nitrate on growth  
728 and survival of six European amphibians. *Archives of Environmental Contamination*  
729 *and Toxicology*, 47, 234-239.  
730
- 731 Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu.*  
732 *Rev. Ecol. Evol. Syst.*, 37, 637-669.

733  
734 Pasanen, S. & Karhapää, M. (1997). Can boreal common frog (*Rana temporaria* L.) survive in  
735 frost? In: *Annales Zoologici Fennici*. JSTOR, pp. 247-250.  
736  
737 Pearman, P.B. (1995). Effects of pond size and consequent predator density on two species  
738 of tadpoles. *Oecologia*, 102, 1-8.  
739  
740 Price, S.J., Browne, R.A. & Dorcas, M.E. (2012). Evaluating the effects of urbanisation on  
741 salamander abundances using a before-after control-impact design. *Freshwater*  
742 *Biology*, 57, 193-203.  
743  
744 QGIS Development Team. (2022). QGIS Geographic Information System.  
745  
746 R Core Team. (2020). R: a language and environment for statistical computing. Vienna,  
747 Austria: R Foundation for Statistical Computing.  
748  
749 Reading, C. (2007). Linking global warming to amphibian declines through its effects on  
750 female body condition and survivorship. *Oecologia*, 151, 125-131.  
751  
752 Rennie, S., Adamson, J., Anderson, R., Andrews, C., Bater, J., Bayfield, N. *et al.* (2017). UK  
753 Environmental Change Network (ECN) frog data: 1994-2015. (ed. Centre, NEID).  
754  
755 Rouse, J.D., Bishop, C.A. & Struger, J. (1999). Nitrogen pollution: an assessment of its threat  
756 to amphibian survival. *Environmental Health Perspectives*, 107, 799-803.  
757  
758 Rowland, C.S., Marston, C.G., Morton, R.D. & O'Neil, A.W. (2020a). Land Cover Change 1990-  
759 2015 (25m raster, GB).  
760  
761 Rowland, C.S., Marston, C.G., Morton, R.D. & O'Neil, A.W. (2020b). Land Cover Change 1990-  
762 2015 (25m raster, N. Ireland).  
763  
764 Saenz, D., Hall, T.L. & Kwiatkowski, M.A. (2015). Effects of urbanization on the occurrence of  
765 *Batrachochytrium dendrobatidis*: do urban environments provide refuge from the  
766 amphibian chytrid fungus? *Urban Ecosystems*, 18, 333-340.  
767  
768 Schmidt, M., Lischeid, G. & Nendel, C. (2019). Microclimate and matter dynamics in  
769 transition zones of forest to arable land. *Agricultural and Forest Meteorology*, 268, 1-  
770 10.  
771  
772 Scott, W.A., Pithart, D. & Adamson, J.K. (2008). Long-term United Kingdom trends in the  
773 breeding phenology of the common frog, *Rana temporaria*. *Journal of Herpetology*,  
774 42, 89-96.  
775  
776 Sebastien Le, J.J. & Husson, F. (2008). FactoMineR: an R package for multivariate analysis.  
777 *Journal of Statistical Software*, 25, 1-18.  
778

- 779 Semlitsch, R.D. (2008). Differentiating migration and dispersal processes for pond-breeding  
780 amphibians. *The Journal of Wildlife Management*, 72, 260-267.  
781
- 782 Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A. *et al.* (2020). Global trends in  
783 pesticides: a looming threat and viable alternatives. *Ecotoxicology and Environmental*  
784 *Safety*, 201, 110812.  
785
- 786 Sillero, N., Campos, J., Bonardi, A., Corti, C., Creemers, R., Crochet, P.A. *et al.* (2014).  
787 Updated distribution and biogeography of amphibians and reptiles of Europe.  
788 *Amphibia-Reptilia*, 35, 1-31.  
789
- 790 Simko, T.W.A.V. (2021). R package 'corrplot': visualization of a correlation matrix (version  
791 0.91).  
792
- 793 Tian, J., Zhu, X., Shen, Z., Wu, J., Xu, S., Liang, Z. & Wang, J. (2020). Investigating the urban-  
794 induced microclimate effects on winter wheat spring phenology using Sentinel-2 time  
795 series. *Agricultural and Forest Meteorology*, 294, 108153.  
796
- 797 Trimble, S.W. (1994). Erosional effects of cattle on streambanks in Tennessee, USA. *Earth*  
798 *Surface Processes and Landforms*, 19, 451-464.  
799
- 800 Turner, R.K. & Maclean, I.M. (2022). Microclimate-driven trends in spring-emergence  
801 phenology in a temperate reptile (*Vipera berus*): evidence for a potential “climate  
802 trap”? *Ecology and Evolution*, 12, e8623.  
803
- 804 Vehtari, A., Gelman, A. & Gabry, J. (2017). Practical Bayesian model evaluation using leave-  
805 one-out cross-validation and WAIC. *Statistics and Computing*, 27, 1413-1432.  
806
- 807 Vickery, J.A., Tallwin, J.T., Feber, R.E., Atkinson, P.W., Asteraki, E.J., Fuller, R.J., & Brown, V.K.  
808 (1999). Changes in lowland grassland management: implications for invertebrates &  
809 birds. *BTO Reserach Report*.  
810
- 811 Votier, S.C., Bearhop, S., Attrill, M.J. & Oro, D. (2008). Is climate change the most likely driver  
812 of range expansion for a critically endangered top predator in northeast Atlantic  
813 waters? *Biology Letters*, 4, 204-205.  
814
- 815 Walls, S.C., Barichivich, W.J. & Brown, M.E. (2013). Drought, deluge and declines: the impact  
816 of precipitation extremes on amphibians in a changing climate. *Biology*, 2, 399-418.  
817
- 818 Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J. *et al.* (2002).  
819 Ecological responses to recent climate change. *Nature*, 416, 389-395.  
820
- 821 Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. (1998). Quantifying threats to  
822 imperiled species in the United States. *BioScience*, 48, 607-615.  
823
- 824 Wood, S.L. & Richardson, J.S. (2010). Evidence for ecosystem engineering in a lentic habitat  
825 by tadpoles of the western toad. *Aquatic Sciences*, 72, 499-508.