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Unusual lack of reproduction in toad populations from agricultural habitats

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Anthropogenic alterations of habitats can have detrimental consequences for biodiversity. Documenting these effects require monitoring in multiple sites that vary in the degree of alterations over long temporal scales, a task that is challenging. Yet, simple naturalist observations can reveal major ongoing events affecting wild populations, and serve as a basis for further investigations. We quantified breeding parameters of spined toad (*Bufo spinosus*) populations from forested (preserved) and agricultural (altered) habitats. We found that reproduction did not occur at the sites surrounded by agriculture, while it occurred successfully in ponds from forests. Males were present at all sites, but females, amplexus, egg strings and tadpoles remained absent from agricultural sites. Observations made at the same sites indicated that breeding occurred during previous years. Our observations of habitat- and sex-specific lack of reproduction may have critical consequences for the persistence of populations of a widespread amphibian species in agricultural areas.

Keywords: Amphibian, *Bufo spinosus*, breeding, conservation, reproductive success

INTRODUCTION

Biodiversity is dramatically affected by human activities leading to an alteration of ecosystems (Chapin et al., 2000; Myers & Knoll, 2001; Brooks et al., 2002). Human activities, such as intensive farming, generate habitat alteration, fragmentation and simplification (e.g. Maron & Fitzsimons, 2007). In addition, agricultural landscapes often suffer from the massive use of pesticides, which contaminate the environment and the wildlife (Schäfer et al., 2007).

As a consequence, these modern agricultural practices can have detrimental impacts on fauna and flora (Myers & Knoll, 2001; Brooks et al., 2002; Fahrig, 2003; Relyea, 2009). In order to persist in these altered habitats, wildlife must adjust to these ongoing changes. However, the ability of a species to persist in agriculture landscapes can be jeopardised when critical elements necessary to perform its life-cycle are missing in the environment. For example, the lack of trees or shrubs can impair the ability of some bird species to breed in simplified landscapes (Newton, 1994; Verhulst et al., 2004). Similarly, amphibian populations will disappear if suitable breeding ponds are missing following habitat simplification (Smith & Green, 2005). In addition to habitat alteration, other effects can be linked to the increasing use of chemical inputs that aim to improve crop productivity in agricultural habitats (McLaughlin & Minneau, 1995; Köhler & Triebskorn, 2013). For instance, pesticides are used to control pests (e.g., weeds, insect, fungi) that negatively impact crop productivity. These pesticides can have toxic effects on non-target components. For example, they have been shown to negatively impact reproduction in wildlife species, through various mechanisms that spans from direct toxic or sublethal effects (Mnif et al., 2011; Cheron & Brischoux, 2020) to alterations of ecosystem functioning (e.g., disruption of the food web, Relyea & Hoverman, 2008).

The direct effects of habitat alteration on population persistence are relatively easy to assess (see above). Yet, assessments of indirect effects of agricultural practices on population persistence are more challenging and require population monitoring in multiple sites that vary in their habitat structure (i.e., degree of alteration and fragmentation). To document these effects, simple naturalist observations can be important because they often help to reveal major ongoing and detrimental events that affect wild populations (Sagarin & Pauchard, 2010; Sagarin & Pauchard, 2012; Mauz & Granjou, 2013).

During the course of a study that aimed to compare toad (*Bufo spinosus*) populations between forested (preserved) areas and agricultural (simplified) habitats, we opportunistically quantified breeding parameters (number of males, presence of amplexus, egg strings and tadpoles) in both types of habitat in Western France (Fig. 1). The toad (*Bufo spinosus*) is a widespread species that can live in a variety of habitats and has been previously shown to persist even in highly modified agricultural areas (Arntzen et al., 2014, Guillot et al., 2016). As in most anuran species, *B. spinosus* have a biphasic life-cycle with an extensive use of terrestrial habitats during
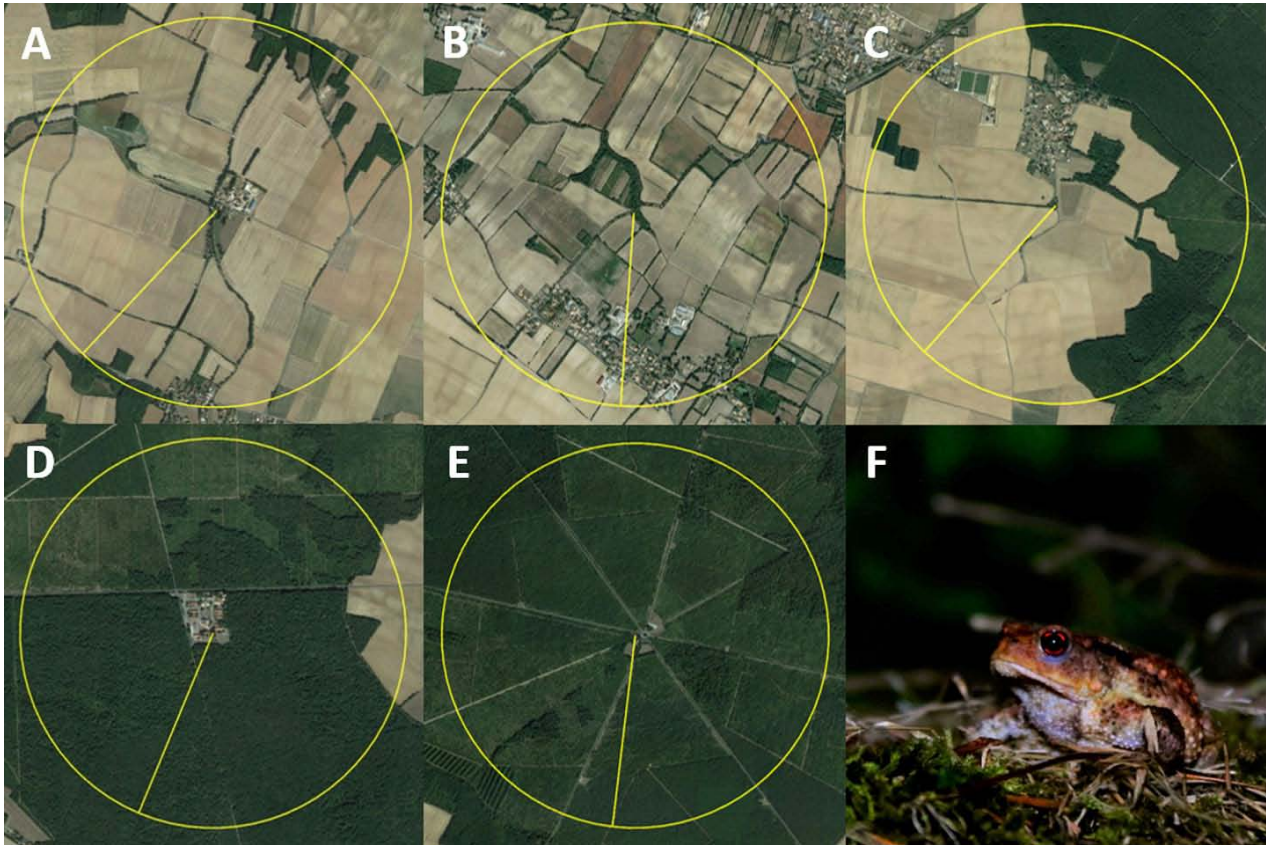
Figure 1. A-D: Aerial pictures (Google Earth) of the five study sites with the one km radius surrounding breeding ponds used to illustrate the contrast between three agricultural sites (A, B and C) and two forested sites (D and E). Letters in the pictures relate to site numbers in Table 1. **F**: Picture of an individual *Bufo spinosus* in the field in South Deux-Sèvres, France .

most of the year, and a short breeding season (~1 month) in aquatic sites (ponds) where mating occurs and eggs and tadpoles develop (Reading, 1998; Kelleher et al., 2018; Brischoux & Cheron, 2019). The breeding season occurs at the end of winter (February – March). During this period, male toads migrate towards aquatic breeding sites where they wait for females (Reading, 1998). Males can remain at the breeding site for several weeks, while females leave shortly after mating and egg-laying (Davies & Halliday, 1977). Eggs and tadpoles develop over three to four months before metamorphosis and subsequent dispersal in nearby terrestrial habitats. Reproductive events can be easily assessed later in the season (when breeders have left the breeding site) by monitoring the presence of egg strings and tadpoles.

The terrestrial part of the annual cycle of toads occurs in various environments usually within one km from the breeding pond (Janin et al., 2011; Guillot et al., 2016). Two of our study sites were located in forested areas where forest cover represented > 95 % within a circle of a one km radius centered on the breeding pond; while three sites were located in agricultural areas (composed mainly of large fields) where forest cover was always < 35 % within the same surface area (Fig. 1). Forest and agricultural sites were situated in close proximity (maximum distance 12 km) in order to avoid diverging climatic conditions that may affect timing of reproduction.

Observations were made from early January (week

one) to late June (week 26) 2020. At the onset of the reproductive period (from week one to week 11) all study sites were monitored every night. Observations were stopped from week 12 to week 16 because of the lockdown linked to the COVID-19 pandemic. Observations resumed on week 17 on a monthly basis until late June (week 26) in order to assess the presence of developing tadpoles.

Due to of the diverging reproductive behaviour of males and females (see above), we made the following observations. Males were individually counted when abundances were < 10 individuals and number of individuals was approximated by increment of 10 individuals when abundances were > 10 individuals. Females remain only briefly at the breeding pond, and amplexus occurs in areas where precise quantification is precluded (in highly vegetated areas or deeper water). As a consequence, we assessed female presence through the observation of amplexus and qualified for each site whether amplexus was observed of not (present/absent). When reproduction occurred, large numbers of egg strings and tadpoles precluded direct enumeration and successful reproduction was assessed with the presence/absence of egg strings and tadpoles.

We emphasise that our opportunistic observations are qualitative rather than quantitative for most parameters recorded as they were not directly linked to the primary goal of the surveys we performed (assessment of reproductive success across habitats).

Table 1. Summary of the data collected during our surveys. Male abundances show min-max number of individuals observed for each week. Female presence or absence was assessed through observations of amplexus. The presence of egg strings and developing tadpoles was also documented. "ND" stands for "no data". "NO" refers to absence of individuals at periods during which presence was expected, while "-" refers to absence of individuals at periods when absence was expected.

Observations	Sites	Habitat	1-4	5	6	7	8	9	10	11	12-16	17	21	26
								Week number						
Number of males	A	Agriculture	0	1-3	1	0	0	0	0	0	ND	-	-	-
	B	Agriculture	0	40	10-40	3-10	1-3	1	1	0	ND	-	-	-
	C	Agriculture	ND	ND	ND	100	70	50	50	50	ND	-	-	-
	D	Forest	0	10-20	20	20	10-20	10-20	10	10	ND	-	-	-
	E	Forest	0	30	30	20	10-20	20	20	10	ND	-	-	-
Presence of amplexus	A	Agriculture	NO	NO	NO	NO	NO	NO	NO	NO	ND	-	-	-
	B	Agriculture	NO	NO	YES	YES	NO	NO	NO	NO	ND	-	-	-
	C	Agriculture	ND	ND	ND	YES	NO	NO	NO	NO	ND	-	-	-
	D	Forest	NO	YES	YES	YES	YES	YES	YES	YES	ND	-	-	-
	E	Forest	NO	YES	YES	YES	YES	YES	YES	YES	ND	-	-	-
Presence of egg strings	A	Agriculture	NO	NO	NO	NO	NO	NO	NO	NO	ND	-	-	-
	B	Agriculture	NO	NO	NO	NO	NO	NO	NO	NO	ND	-	-	-
	C	Agriculture	ND	ND	ND	NO	NO	NO	NO	NO	ND	-	-	-
	D	Forest	NO	NO	YES	YES	YES	YES	YES	YES	ND	-	-	-
	E	Forest	NO	NO	YES	YES	YES	YES	YES	YES	ND	-	-	-
Presence of tadpoles	A	Agriculture	-	-	-	-	-	-	-	-	ND	NO	NO	NO
	B	Agriculture	-	-	-	-	-	-	-	-	ND	NO	NO	NO
	C	Agriculture	-	-	-	-	-	-	-	-	ND	NO	NO	NO
	D	Forest	-	-	-	-	-	-	-	-	ND	YES	YES	NO
	E	Forest	-	-	-	-	-	-	-	-	ND	YES	YES	NO

Observations are summarised in Table 1. Overall, we found that reproduction did not occur at the three sites from agricultural habitats, while it occurred successfully in breeding ponds from forested areas (presence of egg strings and tadpoles, Table 1).

At all of our study sites, breeding males were present, yet with variable abundances (Table 1). Mean number of adult males was 19.0±28.4 (range 0-100) for agricultural sites and 15.6±8.3 (range 0-30) for forest sites (Table 1). These numbers suggest that abundances of reproductive males did not seem to be related to the surrounding habitat structures. Indeed, some sites from agricultural areas displayed numbers of males that equaled or even exceeded those from forested habitats (Table 1). Importantly, the onset of the reproductive period (first observations of males occurring at the study sites) was similar between habitat types (occurring on week 5, Table 1), suggesting that climatic (micro-) conditions did not significantly influence reproduction between sites. These observations tend to further indicate that the lack of reproduction we recorded (see below) may not be linked to a lack of breeding males (although one agricultural site was characterised by lower abundances, Table 1), but rather to a lack of reproductive females.

Indeed, the most clear-cut difference between our study sites was linked to the presence of females (assessed through the presence of visible amplexus, Table 1) and their reproductive success (assessed through the presence of egg strings and developing tadpoles, Table 1). Amplexus was observed on very few nights (one or two nights) at two of the agricultural sites, and was not observed at the other agricultural site. Conversely, amplectant pairs were observed steadily almost every night over six weeks at the sites surrounded by forest. No egg-strings or developing tadpole were observed at all three sites from agricultural habitats, while egg strings and developing tadpoles were present at the two forest sites. Importantly, these observations suggest that females did not migrate to breed in sites surrounded by agricultural areas and, thus, that habitat-specific and sex-specific responses to habitat perturbations occurred in adult females.

It is important to stress that our observations are unreplicated and preliminary and that we have not observed this phenomenon in previous years. Therefore, these observations do not give any strong clue regarding the mechanisms through which habitat-specific and probably sex-specific lack of reproduction has occurred. Yet, previous observations made at the same study sites (Guillot et al., 2016; MC and FB unpublished data) indicate that breeding successfully occurred at some of these agricultural sites at least in 2015 and in 2019; 2 years during which we monitored reproduction at some of those sites and for which egg strings and developing

tadpoles were observed. Although we acknowledge the limitations of our observational study, we believe it is important to document, at least in a qualitative way, a potential problem for the persistence of the populations of a widespread amphibian species in agricultural areas (Guerry & Hunter, 2002, Boissinot et al., 2019); and we urge other researchers to share similar observations.

Author Contributions
F.B. and F.A. proposed the initial idea and together with M.R. and M.C. contributed to its development. M.R., M.C. and F.B. performed field work. M.R. and M.C. tabulated the resulting data. All authors discussed the results, and substantially contributed to the writing.

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