SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ANIMAL MIGRATION
BEHAVIOURAL ECOLOGY
AGRI-ECOLOGY
BIODIVERSITY

Received
14 March 2013

Accepted
23 August 2013

Published
10 September 2013

Correspondence and requests for materials should be addressed to G.B. (gberger@zalf.de)

Glyphosate applications on arable fields considerably coincide with migrating amphibians

Gert Berger, Frieder Graef & Holger Pfeffer

Leibniz Centre for Agricultural Landscapes Research, Institute of Land Use Systems, Eberswalder Str. 84, 15374 Müncheberg, Germany.

Glyphosate usage is increasing worldwide and the application schemes of this herbicide are currently changing. Amphibians migrating through arable fields may be harmed by Glyphosate applied to field crops. We investigated the population-based temporal coincidence of four amphibian species with Glyphosate from 2006 to 2008. Depending on a) age- and species-specific main migration periods, b) crop species, c) Glyphosate application mode for crops, and d) the presumed DT50 value (12 days or 47 days) of Glyphosate, we calculated up to 100% coincidence with Glyphosate. The amphibians regularly co-occur with pre-sowing/pre-emerging Glyphosate applications to maize in spring and with stubble management prior to crop sowing in late summer and autumn. Siccation treatment in summer coincides only with early pond-leaving juveniles. We suggest in-depth investigations of both acute and long-term effects of Glyphosate applications on amphibian populations not only focussed on exposure during aquatic periods but also terrestrial life stages.

The global decline of lowland amphibian populations around the world is clearly associated with habitat loss and fragmentation due to both urbanisation of landscapes including increasing traffic and agricultural expansion and intensification[1–3]. Agrochemical pollution, often interacting with other causes such as climate change, UV-B radiation, diseases and alien species, is detrimental to amphibians[4]. For instance, impacts of chemical fertilisers on amphibians in water bodies[5] and on land[6–8] have been well documented. Herbicide impacts on amphibians have been even more thoroughly studied, in particular Glyphosate formulations[9–11]. Those Glyphosate formulations containing the common surfactant POEA (polyethoxylated tallow amine) have been shown to be considerably more toxic than the active ingredient (ai) Glyphosate alone[9], in particular for aquatic organisms. The effects of Glyphosate include osmotic instability, delayed or accelerated development, reduced size at metamorphosis, malformations, stress, and death[7,12]. For terrestrial juvenile stages of *Rana temporaria* (Linnaeus, 1758) severe toxic effects of direct overspray with several pesticides were documented under a 'realistic worst-case' exposure scenario[13].

Glyphosate formulations are the most common herbicides used worldwide, and also in Germany, covering a wide range of crops and agronomic measures[14–16]. The application schemes of pesticides and particularly Glyphosate are currently changing. The Glyphosate usage is continuously increasing for controlling not only weeds but also the entire production process including reduced soil tillage and seed bed preparation, erosion prevention, controlled ripening (siccation) and harvesting of crops, and stubble management[16,17]. Outside Germany and the EU, Glyphosate application is closely linked to growing shares of genetically modified plants[14]. Modified herbicide application schemes, however, may change the exposure risk to amphibian populations. Furthermore, while adverse Glyphosate effects on the aquatic amphibian stages are well studied, less is known about its effects and particularly its exposure on terrestrially active amphibians moving among breeding ponds and terrestrial habitats during non-breeding periods of the year[18].

Based on a field study from 2006 to 2008 on amphibian activity in a pond-rich agricultural landscape, we quantitatively assessed the temporal exposure of amphibian populations to Glyphosate applied to arable fields, documenting their migrations between terrestrial sites and breeding ponds. We selected those species covering a wide range of different activity periods: fire-bellied toad (*Bombina bombina* (Linnaeus, 1761)), moor frog (*Rana arvalis* (Nilsson, 1842)), spadefoot toad (*Pelobates fuscus* (Laurenti, 1768)) and crested newt (*Triturus cristatus* (Laurenti, 1768)).

Results

Glyphosate applications to crop species. Pre-sowing or pre-emerging application of glyphosate in spring was used only with maize, and siccation for harvest preparation only with winter barley. For seed bed preparation in late summer and autumn prior to crop sowing, Glyphosate was applied on the stubbles to all six crops but not to every field. Stubble application was done on about 20% of the fields in 2006 and $< 10\%$ in 2007 (Table 1). Glyphosate application on stubble fields depends on the type of previous crop and its applicability for reduced soil tillage. Of all stubble fields treated with Glyphosate, 45% were winter rape and 30% winter rye (data not shown). Stubble fields regularly treated in late summer or autumn were usually followed by winter crops (barley, wheat, rye and triticale). The Glyphosate application frequency to crops varied between years. While in late summer and autumn 2006 at least 40% of the fields were treated prior to sowing winter barley, triticale and maize, in 2007 less than 25% or even no application took place.

Overall coincidence of Glyphosate with amphibian populations. Coincidences of amphibian populations with Glyphosate treatment of crops ranged from zero to 100% (Table 2). For pre-sowing/pre-emerging application in spring and stubble management in late summer and autumn prior to crop sowing, we found coincidences with amphibian populations for almost all fields treated. For siccation with winter barley in summer, seven out of 12 treated fields coincided with amphibians.

Amphibian species- and crop-specific coincidence with Glyphosate. The amphibian species as well as adult and/or juvenile amphibians, respectively, showed various migration periods (Figure 1), leading to a highly diverse picture of coincidence with Glyphosate (Table 2). The application of glyphosate as a pre-emerging application to maize in May 2008 coincided only with *B. bombina* adults, whereas the application in March 2007 coincided with all four species. Siccation during summer did not coincide with *T. cristatus* but did with the other three species. Stubble management in late summer and autumn prior to crop sowing coincided with juveniles of all four species and the adults of only *T. cristatus* and *B. bombina*, while the adults of *P. fuscus* and *R. arvalis* did not leave ponds at that time. Depending on the date of Glyphosate application prior to crop sowing, the level of coincidence differed between species. For instance, only 3.4% of the *T. cristatus* juveniles leaving ponds in summer and autumn 2006 coincided with stubble management prior to winter rape, compared to 39.3% of the population with winter wheat (12-day DT50, specifying the half-life of Glyphosate in soils). Specifically for stubble management in 2006 and for amphibians migrating from ponds we tested for species differences. The coincidence levels decreased in the following order: *B. bombina* (juveniles) > *T. cristatus* (juveniles) > *R. arvalis* (juveniles) > *T. cristatus* (adults) > *P. fuscus* (juveniles)(12-day DT50, Kruskal-Wallis-Test; $p < 0.01$).

Effect of DT50-values. The proportions of populations coinciding with Glyphosate application to crops differed considerably depending on which of the two DT50-values was used (Table 2). Both the average and maximum coincidences per field increased with the 47-day DT50, in some cases attaining 100% of the population. Moreover, coincidences occurred where none were present with the 12-day DT50. The latter was particularly true for siccation of winter barley in 2006 and 2007. The number of fields treated and coinciding with amphibians also increased with the 47-day DT50. For instance, stubble management with Glyphosate prior to winter rye sowing applied on six fields in 2006 completely coincided with *T. cristatus* juveniles using the 47-day DT50 but involved only three fields with the 12-day DT50. The Kruskal-Wallis-Test for species differences for the 47-day DT50 for stubble management in 2006 revealed a modified ranking of the coincidence levels: *T. cristatus* (juveniles) > *T. cristatus* (adults) > *B. bombina* (juveniles) > *R. arvalis* (juveniles) > *P. fuscus* (juveniles)($p < 0.001$).

Discussion

Our results provide the first assessment of the potential temporal coincidences of amphibians on fields with Glyphosate application to crops. We used a standard daily migration distance of 100 m. The real daily migration distances of individuals, however, are species- and age-specific, and also depend on surface roughness and the morphology, the fitness of individuals and their behavioural patterns, for instance to directly migrate through field plots or to rest in cultivated and noncultivated sites during foraging[6]. Our results are based on the assumption that amphibians had to cross treated field plots during their migration to and from breeding ponds or during direct movements between them. We did not consider field sojourns in crops around ponds for more or less stationary foraging. The average distance from pond to next non arable land in the investigation area was estimated to be 300 m. This is in line with results from the north-eastern plain of Germany, but it may differ between different landscapes[19].

The temporal coincidences were high but depended on crop species, herbicide management measures, amphibian species and age, and the DT50 values assumed. Most Glyphosate applications coincided with amphibian terrestrial activity. We identified particularly high coincidences with a wide range of species during stubble management/seed bed preparation in late summer and autumn. This is the predominant application mode of Glyphosate in Germany and is designed to reduce soil tillage of crops following winter rape and winter cereals[17]. In situ DT 50 values of Glyphosate may vary depending on specific soil type and/or weather conditions. The two DT 50 values we applied were used to cover varying conditions within a range of different environments.

Amphibian species and age classes have different activity periods (Figure 1) and, depending on the timing of Glyphosate application, its effects will vary. In general, early Glyphosate applications (pre-sowing) in early spring coincide with adults of all species investigated. Later spring applications (pre-emerging) are likely to coincide with later active adults of *P. fuscus* and *B. bombina*. In summer the

Table 1 | Analysed fields and proportion of fields treated with Glyphosate with respect to application types

	N of investigated fields/proportion of treated fields (%) per crop						
Application type	all crops	maize	triticale	winter barley	winter rape	winter rye	winter wheat
pre-sowing/pre-emerging application in spring	106/0.9	19/5.3	9/0	12/0	19/0	34/0	13/0
	108/1.9	18/11.1	7/0	15/0	28/0	24/0	16/0
siccation in summer	89/2.2	5/0	7/0	5/40	28/0	23/0	21/0
	106/6.6	19/0	9/0	12/58.3	19/0	34/0	13/0
stubble management in late summer/autumn prior to crop	89/21.3	5/80.0	7/42.9	5/60.0	28/7.1	23/26.1	21/4.8
	106/7.5	19/0	9/0	12/25.0	19/0	34/14.7	13/0

Table 2 | Temporal coincidence of migrating amphibians and Glyphosate application and/or presence with $DT50_{Glyphosate}$ = 12 and 47 days

application period	half-life periods DT50 (d)	maize		triticale		winter barley		winter rape		winter rye		winter wheat	
		species_age/migration direction*: N of coinciding glyphosate applications/median and maximum values of proportion of migrating amphibians (%)											
Pre-sowing or pre-emerging application in spring													
15.03.-15.03.07	12	R_ad/tp*	1/40.6/-	n.a.		n.a.		n.a.		n.a.		n.a.	
	47		1/42.6/-										
	12	T_ad/tp	1/63.9/-										
	47		1/71.1/-										
	12	P_ad/tp	1/17.1/-										
	47		1/59.7/-										
	12	B_ad/tp	1/37.8/-										
	47		1/92.0/-										
02.05.-02.05.08	12	B_ad/tp	2/39.7/39.7	n.a.		n.a.		n.a.		n.a.		n.a.	
	47		2/62.4/62.4										
	12	P_ad/fp	2/48.2/48.2										
	47		2/89.7/89.7										
siccation in summer													
30.06.-01.07.06	12	n.a.		n.a.		R_jv/fp	2/8.7/8.8	n.a.		n.a.		n.a.	
	47						2/50.7/51.7						
	12					P_jv/fp	2/24.4/24.9						
	47						2/100/100						
	12					B-jv/fp	0/0/-						
	47						2/23.2/25.6						
20.06.-25.06.07	12	n.a.		n.a.		R_jv/fp	2/0.0/7.6	n.a.		n.a.		n.a.	
	47						7/76.8/79.2						
stubble management in late summer/autumn prior to crop sowing													
30.07.-23.10.06	12	T_ad/fp	4/23.6/37.1	R_jv/fp	3/15.8/25.3	R_jv/fp	3/20.3/20.3	R_jv/fp	2/21.4/21.4	R_jv/fp	6/22.6/25.3	R_jv/fp	1/25.3/-
	47		4/52.2/76.5		3/16.7/32.1		3/25.6/25.6		2/48.2/48.2		6/50.6/70.9		1/32.1/-
	12	T_jv/fp	2/9.5/34.6	T_ad/fp	3/17.7/17.9	T_ad/fp	3/12.8/12.8	T_ad/fp	2/9.2/9.2	T_ad/fp	3/8.9/17.7	T_ad/fp	1/17.7/-
	47		2/9.5/50.1		3/50.7/78.1		3/46.5/46.5		2/39.8/39.8		6/36.3/51.5		1/50.7/-
	12			T_jv/fp	3/35.9/39.3	T_jv/fp	3/35.7/35.7	T_jv/fp	2/3.4/3.4	T_jv/fp	3/18.6/39.3	T_jv/fp	1/39.3/-
	47				3/84.3/100		3/95.3/95.3		2/78.8/78.8		6/69.4/100		1/100/-
	12			B_jv/fp	2/27.5/49.9	B_jv/fp	3/34.0/34,0	B_jv/fp	2/47.8/47.8	P_jv/fp	3/15.5/31.6	B_jv/fp	1/49.9/-
	47				2/27.5/49.9		3/34.0/34.0		2/83.9/83.9		3/15.5/31.6		1/49.9/-
	12									B_jv/fp	6/28.1/49,9		
	47										6/74.9/100		
10.08.-26.10.07	12	n.a.		n.a.		R_jv/fp	3/6.3/6.3	n.a.		R_jv/fp	5/13.9/14.6	n.a.	
	47						3/6.3/6.3				5/21.7/23.2		
	12					T_ad/fp	3/15.4/15.4			T_ad/fp	5/9.3/16.7		
	47						3/87.0/87.0				5/56.2/80.6		
	12					T_jv/fp	3/44.4/44.4			T_jv/fp	5/15.9/43.0		
	47						3/77.3/77.3				5/94.0/97.5		
	12					B_ad/fp	3/9.1/9.1			B_ad/fp	5/3.7/52.2		
	47						3/93.2/93.2				5/92.4/93.4		

*Legend: Amphibian species: R: R. arvalis, T: T. cristatus, P: P. fuscus, B: B. bombina; age classes: ad: adult; jv: juvenile; migration directions: fp: from ponds, tp: to ponds, n.a.: no application.

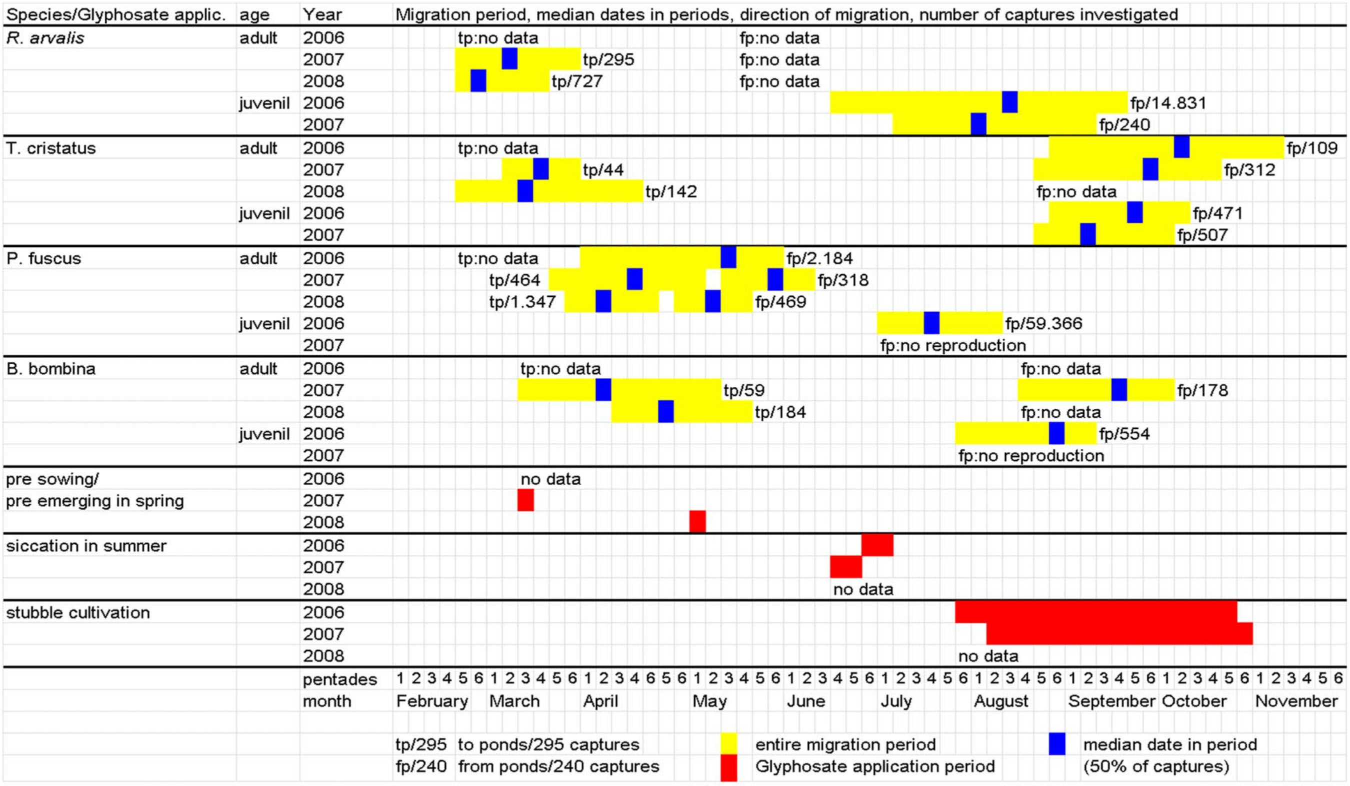

Figure 1 | Migration periods of amphibians, N of captures investigated and corresponding periods of Glyphosate application (from February to November).

adults of early species leaving ponds such as *P. fuscus* do not coincide with summer Glyphosate applications during their field passage. Adults of species leaving ponds late and juveniles in general coincide more often; depending on the time of application, considerable population proportions may be affected. Late Glyphosate applications from October onwards were only relevant to *T. cristatus* indicating the need of species specific knowledge and approaches in conservation.

Agriculture is continuously changing[17]. Along with new technologies, for instance no till and reduced soil cultivation, there are market-driven changes in crop shares such as for the renewable energy plants maize and winter rape, which have significantly increased in Germany and the EU. In Germany the area stocked with maize increased by 47% from 1.74 million ha in 2006 to 2.57 million ha in 2012[20]. This in turn contributed to modified Glyphosate application schemes and rates of farms in general. This is in line with a nearly 50% increase in its consumption in Germany from 2000 to 2010, with an increasing share of ai Glyphosate compared to other herbicide substances used from 19.7 to 30.3%[21]. In pond-rich agricultural landscapes these processes increase the exposure probability of amphibians. They are also likely to lead to a shift from late summer/autumn coincidences to higher spring coincidences. While in summer/late autumn mostly juveniles of all species and adults of late pond-leaving species are affected, the coincidence shift to spring may impact adults migrating across fields towards breeding ponds. Depending on the eco-toxicological impact of Glyphosate on these terrestrial stages the viability of reproductive individuals could decrease[22], potentially affecting overall population viability.

The modified herbicide technology also entails other side effects. For instance, until post-emergent Glyphosate spraying, more biomass is available as shelter and food for amphibians[23]. After spraying, however, biomass drops compared to selective herbicide application. Furthermore, spraying is usually done before weed seed development[24], reducing both the weed seed bank and potential feeding organisms in the long term. This can impact the food chain, including arthropod food for amphibians[25]. Finally, the overall Glyphosate exposure also depends on the proportion of each crop species grown, the crop- and growing stage-specific interception for water and pesticides[26], the species-specific behaviour during resting and walking, and the type of surfactant used[9]. The latter factors were not part of our study. Environmental risk assessments (ERA) of herbicides to amphibians do not yet take into account their terrestrial exposure[10]. Recent eco-toxicological experiments on herbicide impacts on terrestrial amphibians[18] as well as our results suggest that terrestrial amphibian life stages should be regularly assessed during the ERA. This would help reduce the amphibian population decline driven by agriculture.

There are still many knowledge gaps related to the ERA of amphibians. We recommend further research on the following issues: a) The real exposure of amphibian individuals depends on the specific landscape situation. In specific locations untreated plots could be preferred for migration which could reduce population exposure. What are the minimum requirements for the proportion and distribution of these features in intensively used and pond rich arable landscapes to keep the exposure below critical values? b) There are yet no findings on the toxicity of Glyphosate to terrestrial stages of amphibians under field conditions. We recommend experiments comparing amphibians' health status on treated and untreated plots. c) Our study dealt with amphibians migrating from and to ponds. Both the foraging duration and numbers of amphibians in crop fields may be higher. Hence, stubble applications during summer may coincide with much higher proportions of amphibian populations than assumed for the migration. Furthermore, there is yet little evidence on preferred crops for foraging. d) Glyphosate is an herbicide which is part of a complex weed controlling strategy. The extent of Glyphosate used can vary depending on the application schemes of other herbicides. This may entail different eco-toxicological effects.

Methods

To quantify the proportion of amphibian populations coinciding with Glyphosate application, we investigated amphibian activity using fence trapping. We installed 49 drift fences consisting of 26 open, 10-m-long cross-shaped fences and 23 enclosures. The cross-shaped fences were regularly distributed in a 400 × 400 m grid to record amphibian migration activity in fields. They were located between tramlines of field machinery. Enclosures encircled biotopes (wood lots, small water bodies) located at the field edge or completely within fields. The investigation area is shown in Figure S1 (supplementary information). Depending on the migration direction either inner or outer traps were analysed. The individuals we caught were registered and released in 10–15 meters at the opposite site of the fence. We determined the species' main migration periods to and from ponds, analysed the total and daily active number of captures, and calculated relative daily activity values of the population[6]. Our core study area encompassed 700 ha in a pond-rich arable landscape in the north-eastern plain of Germany, 50 km east of Berlin, with arable fields covering 85% of the area. Using an extended area of 2850 ha belonging to the surrounding seven farms, totalling up to 108 fields, we gained sufficient data on Glyphosate applications per single field and crop as recorded in work documentaries. Based on individual amphibian observations and literature studies, we assumed an average daily migration distance of 100 m per day, not distinguishing between species and age, and calculated three days for amphibians to cross the average field edge-to-pond distance of 300 m in our study area[6]. We investigated the migration of adults from and into ponds from 2006 to 2008. Juveniles were studied in 2006 and only partly in 2007 because of reduced reproduction. The number of captures per investigated period ranged from 44 to 59,366 (Figure 1).

The half-life of Glyphosate in soils (DT50) ranges from 2 to 197 days, with a typical field half-life of 47 days[22], whereas that of the POEA surfactant (Monsanto's MON 0818) has been conservatively estimated at 21–41 days[10]. The European Pesticide Properties DataBase[27] used by German Authorities provides DT50-values under field conditions ranging from 5–21 days and recommends 12 days as an average value. We therefore applied two DT50 (12 and 47 days) to determine the potential Glyphosate exposure of amphibians. Thus, amphibians migrating over a field during three days prior to and 12 resp. 47 days after application were assumed to temporally coincide with a single Glyphosate application. The proportions of the coinciding populations were calculated by summarising relative daily amphibian activities during these periods. We applied the non-parametric Kruskal-Wallis-Test for species differences in coincidence levels.

The investigations described here were approved by the Ministry of Environment, Health and Consumer Protection, State of Brandenburg, Germany.

1. Piha, H., Luoto, M. & Merila, J. Amphibian occurrence is influenced by current and historic landscape characteristics. *Ecol. Applic.* **17**, 2298–2309 (2007).
2. Collins, J. P. & Storfer, A. Global amphibian declines: sorting the hypotheses. *Divers. & Distr.* **9**, 89–98 (2003).
3. Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H. & Kuzmin, S. L. Quantitative evidence for global amphibian population declines. *Nature* **404**, 752–755 (2000).
4. Beebee, T. J. C. & Griffith, R. A. The amphibian decline crisis: A watershed for conservation biology? *Biol. Cons.* **125**, 271–285 (2005).
5. Ortiz, M. E., Marco, A., Saiz, N. & Lizana, M. Impact of ammonium nitrate on growth and survival of six European amphibians. *Environ. Contam. & Toxicol.* **47**, 234–239 (2004).
6. Berger, G., Graef, F. & Pfeffer, H. Temporal coincidence of migrating amphibians with mineral fertiliser applications on arable fields. *Agric., Ecosyst. & Environ.* **155**, 62–69 (2012).
7. Mann, R., Hyne, R. V., Choung, C. & Wilson, S. Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environ. Poll.* **157** (11), 2903–2917 (2009).
8. Ortiz-Santaliestra, M. E., Marco, A. & Lizana, M. Sensitivity and behavior of the Iberian Newt, *Triturus boscai*, under terrestrial exposure to ammonium nitrate. *Bull. Environ. Contam. Toxicol.* **75**, 662–669 (2005).
9. Moore, L. J. *et al.* Relative toxicity of the components of the original formulation of Roundup® to five North American anurans. *Ecotoxicology and Environmental Safety.* **78**, 128–133 (2012).
10. Giesy, J. P., Dobson, S. &Solomon, K. R. Ecotoxicological risk assessment for roundup herbicide. *Rev. Environ. Contam. Toxicol.* **167**, 35–120 (2000).
11. Paganelli, A., Gnazzo, V., Acosta, H., López, S. L. & Carrasco, A. E. Glyphosate-based herbicides produce terato-genic effects on vertebrates by impairing retinoic acid signaling. *Chem. Res. Toxicol.* **23** (10), 1586–1595 (2010).
12. Relyea, R. A. Growth and survival of five amphibian species exposed to combinations of pesticides. *Environ. Toxicol. & Chem.* **23**, 1737–1742 (2004).
13. Bruehl, C. A., Schmidt, T., Pieper, S. & Alscher, A. Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? *Sci. Rep.* 3:1135, DOI: 10.1038/srep01135. (2013).
14. Benbrook, C. M. Impacts of genetically engineered crops on pesticide use in the United States: The first thirteen years, http://www.organic-center.org/science.pest.php?action=view&report_id=159 (2009, date of access 18.07.2013).
15. Duke, S. O. & Powles, S. B. Glyphosate: A once-in-a-century herbicide. *Pest Man. Sc.* **64**, 319–325 (2008).
16. Griffin, J. L., Boudreaux, J. M. & Miller, D. K. Herbicides as harvest aids. *Weed Sc.* **58**, 355–358 (2010).
17. Steinmann, H. H., Dickeduisberg, M. & Theuvsen, L. Uses and benefits of glyphosate in German arable farming. *Crop Protection* **42**, 164–169 (2012).
18. Bruehl, C. A., Pieper, S. & Weber, B. Amphibians at risk? - Susceptibility of Terrestrial Amphibian Life Stages to Pesticides. *Environmental Toxicology and Chemistry*. **30**, 2465–2471 (2011).
19. Berger, G., Pfeffer, H. & Kalettka, T. (Eds). Amphibienschutz in kleingewässerreichen Ackerbaugebieten (Conservation of Amphibians in agricultural landscapes rich in small water bodies). Natur & Text, Rangsdorf, 384p (2011).
20. DMK. German Maize Committee. Area stocked with maize in Germany. http://www.maiskomitee.de/web/public/Fakten.aspx/Statistik/Deutschland/Gesamtflaechen_entwicklung (date of access 22.08.2012).
21. BVL, Sales of plant protection products in Germany. Statistics according to § 19 Plant Protection Act (PflSchG) for year 2010. (Federal Office for Consumer Protection and Food Safety Braunschweig, 24 p. 2011).
22. Miller, A., Gervais, J. A., Luukinen, B., Buhl, K. & Stone, D. Glyphosate Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/glyphotech.pdf (2010, date of access 17.07.2013).
23. Strandberg, B., Bruus Pedersen, M. & Elmegaard, N. Weed and arthropod populations in conventional and genetically modified herbicide tolerant fodder beet fields. *Agric., Ecosyst. & Environ.* **105**, 243–253 (2005).
24. Dewar, A M., Haylock, L. A., Bean, K. M. & May, M. J. Delayed control of weeds in Glyphosate-tolerant sugar beet and the consequences on aphid infestation and yield. *Pest Man. Sc.* **56**, 345–350 (2000).
25. Firbank, L. G. *et al.* Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops. *Biol. Lett.*, online 2005 doi: 10.1098/rsbl.2005.0390 (2005).
26. FOCUS, 2012. Generic guidance for Focus groundwater scenarios. Version 1.2, FOCUS Working Group. Interception, p.27. http://viso.ei.jrc.it/focus/sw/docs/Generic%20FOCUS_SWS_versioncontrol_1_2.pdf (date of access 20.08.2013).
27. PPDB, Pesticide Properties Data Base. http://sitem.herts.ac.uk/aeru/footprint/en/index.htm (date of access 17.07.2013), University of Hertfordshire.

Acknowledgements

Funding from the German Federal Environment Foundation (DBU) supported this work. Many technical staff conducted the field work under a wide range of weather conditions. We thank them for their particular contribution. Many thanks to the farmers that provided data on Glyphosate applications to crops. Thanks also to Uli Stachow, Joerg Hoffmann and Harald Kaechele for their comments on the manuscript. We also thank M. Stachowitsch for improving the English and two anonymous referees for their helpful comments.

Author contributions

G.B. planned and supervised the project and performed the data analysis including statistics. G.B. and F.G. wrote the manuscript. H.P. organized the field investigations and arranged all necessary permissions from authorities. All authors discussed the results and commented on the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Berger, G., Graef, F. & Pfeffer, H. Glyphosate applications on arable fields considerably coincide with migrating amphibians. *Sci. Rep.* **3**, 2622; DOI:10.1038/srep02622 (2013).