

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

Kat E Oliver , Xavier A Harrison

In this manuscript, the authors analyse the influence of temperature, in relation to climate change, land cover change and chemical pollution on the breeding success of the Common frog (*Rana temporaria*).

The objective of this work is interesting since it proposes the analysis of the breeding period from different perspectives, or in other words, analyzing the influence of different anthropic changes on the reproduction of the Common frog.

However, the manuscript has some important deficiencies that must be corrected in order to be accepted.

First, I find lacking a total absence of the data on which work has been done. I think in Suppl. Mat. or in some repository, tables with data on land use, phenology, years and water analysis must be included.

In addition, throughout the reading the manuscript at no time are we told what dates we are dealing with. Really are spring temperatures? I don't know. Nor can we see the level of change in the start dates of the breeding period, spawn, land uses or the values of the variables used. Basic data are missed.

Secondly, the presentation of the results is somewhat cryptic and difficult to understand. There is no factor score tables from any of the principal component analyses. Perhaps correspondence analysis could be more visual. Authors don't include any significance levels of the estimated correlations or models given.

Thirdly, there is a great deficiency in the bibliography consulted. There is a lot of work that needs to be included in the introduction and especially in the discussion.

Most studies use day of the year (DOY) as a variable to test how the start of breeding success varies in different years and to analyses its association with climatic variables. Only in Suppl. Mat. Use this variable but it isn't explained I methodology.

I believe that in its present form the manuscript cannot be accepted. It needs a very deep restructuring and a greater and clearer development of the analysis of the variables used. The complete lack of tables with raw data makes it impossible to verify how the analyses have been carried out. It is also not detailed how the models were developed and when the predictor variables were used.

Specific comments:

Line 35:

I think would be better than Wilcove et al. 1998 because of Wilcove is older reference and only refer to USA.

Perhaps:

Thuiller W, Lavergne S, Roquet C, Boulangeat I, Lafourcade B, Araujo MA. 2011. Consequences of climate change on the tree of life in Europe. *Nature* 470(7335):531–534 DOI 10.1038/nature09705.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Townsend Peterson A, Phillips OL, Williams SE. 2004. Extinction risk from climate change. *Nature* 427(6970):145–148 DOI 10.1038/nature02121.

Line 38:

This reference (Nagelkerken & Munday 2016) only talks about marine ecosystems Is there no reference that says the same in terrestrial ecosystems?

Lines 49-60

This paragraph could be improved. Most important and recent and specific references to the subject of the study are missing.

See:

Bison M, Yoccoz NG, Carlson BZ, Klein G, Laigle I, Van Reeth C, Delestrade A. 2021. Earlier snowmelt advances breeding phenology of the common frog (*Rana temporaria*) but increases the risk of frost exposure and wetland drying. *Frontiers in Ecology and Evolution* 9:645585 DOI 10.3389/fevo.2021.645585.

See

Montori & Amat 2023. page 2.

Montori A, Amat F. 2023. Surviving on the edge: present and future effects of climate warming on the common frog (*Rana temporaria*) population in the Montseny massif (NE Iberia). *PeerJ* 11:e14527 DOI 10.7717/peerj.14527 :

Changes in temperature or precipitation can potentially influence the timing of amphibian reproduction (**Blaustein et al., 2010**). An earlier onset of spring breeding behaviour correlated with a warming climate has been observed among various pond-breeding frogs and toads in Europe (**Beebee, 1995; Tryjanowski, Mariusz & Sparks, 2003; Scott, Pithart & Adamson, 2008**) and Asia (**Kusano & Inoue, 2008; Primack et al., 2009**). However, other species may exhibit either a trend towards a later onset of spring breeding or no trend at all (**Todd et al., 2010; Klaus & Loughheed, 2013**). **While & Uller** (2014) and **Ficetola & Maiorano** (2016) concluded that this group is strongly influenced by

climate change after carrying out a meta-analysis with worldwide phenological data of amphibians.

Rewrite please all the paragraph including more references about effects of warming on amphibians.

Lines 62-63

This sentences could be improved with

Amphibian declines have also been related to pollution by agrochemicals (Ortiz-Santaliestra et al. 2006, 2010; García-Muñoz et al. 2011; Egea-Serrano et al. 2012), since they are diffusely released to the environment and have the potential to contaminate a great number of sites inhabited by amphibian populations (Berger 1989; Sparling et al. 2001; Blaustein and Kiesecker 2002).

Line 67-68

See more recent paper about ammonium in one European species in

Garriga, Núria; Montori, A.; Llorente, G. A. (2017). Impact of ammonium nitrate and sodium nitrate on tadpoles of *Alytes obstetricans*. *Ecotoxicology*, 26(5), 667–674. doi:10.1007/s10646-017-1799-y

And:

More recent papers:

Another nitrogen form that also occurs in the aquatic environment is the ammonium ion (NH_4^+), which can remain in water at high concentrations that have shown to be toxic to amphibians (**Ortiz-Santaliestra et al. 2006; Griffis-Kyle and Ritchie 2007; Mann et al. 2009; García-Muñoz et al. 2011**). This compound affects the nervous and muscular systems and thus reduces tadpoles' activity (**McKenzie et al. 2009**).

Line 78

Need to refer more references about ammonium. The literature consulted is deficient.

Add

Garriga, Núria; Montori, A.; Llorente, G. A. (2017). Impact of ammonium nitrate and sodium nitrate on tadpoles of *Alytes obstetricans*. *Ecotoxicology*, 26(5), 667–674. doi:10.1007/s10646-017-1799-y for *A. obstetricans* (now *almogavarii*) and

Ortiz-Santaliestra ME, Marco A, Fernández MJ, Lizana M (2006) Influence of developmental stage on sensitivity to ammonium nitrate of aquatic stages of amphibians. *Environ Toxicol Chem* 25 (1):105–111

Ortiz-Santaliestra ME, Fernández-Benítez MJ, Marco A, Lizana M (2010) Influence of ammonium nitrate on larval anti-predatory responses of two

amphibian species. *Aquat Toxicol* 99:198–204.
doi:10.1016/j.aquatox.2010.04.020

Egea-Serrano A, Tejedo M, Torralva M (2009) Populational divergence in the impact of three nitrogenous compounds and their combination on larvae of the frog *Pelophylax perezi* (Seoane, 1885). *Chemosphere* 76:869–877.
doi:10.1016/j.chemosphere. 2009.05.017

Miaud C, Oromí N, Navarro S, Sanuy D (2011) Intra-specific variation in nitrate tolerance in tadpoles of the Natterjack toad. *Ecotoxicol* 20:1176–1183.
doi:10.1007/s10646-011-0662-9

Line 84:

Add:

Cayuela, H.; Valenzuela-Sánchez, A.; Teulier, L; Martínez-Solano, I.; Léna, J. P.; Merilä, J.; Muths, Erin; Shine, R.; Quay, L.; Denoël, M.; Clobert, J.; Schmidt, B.R. (2020). Determinants and Consequences of Dispersal in vertebrates with Complex Life Cycles: A Review of Pond-Breeding Amphibians. *The Quarterly Review of Biology*, 95(1), 1–36. <https://doi.org/10.1086/707862>

Sinsch, U. Structure and dynamic of a natterjack toad metapopulation (*Bufo calamita*). *Oecologia* 90, 489–499 (1992). <https://doi.org/10.1007/BF01875442>

Lines 103-104

It is a very old reference in terms of distribution. I would remove it and leave only Sillero et al. 2014. I would even look for a newer reference since a new species *Rana parvipalmata* has recently been described in the Iberian Peninsula.

Christophe Dufresnes, Alfredo G. Nicieza, Spartak N. Litvinchuk, Nicolas Rodrigues, Daniel L. Jeffries, Miguel Vences, Nicolas Perrin e Íñigo Martínez-Solano. 2020. Are glacial refugia hotspots of speciation and cytonuclear discordances? Answers from the genomic phylogeography of Spanish common frogs. *Mol Ecol*. 2020;00:1–15. <https://doi.org/10.1111/mec.15368>

Vences, M., Susanne Hauswaldt, J., Steinfartz, S., Rupp, O., Goesmann, A., Künzel, S., et al. 2013. Radically different phylogeographies and patterns of genetic variation in two European brown frogs, genus *Rana*. *Molecular Phylogenetics and Evolution* 68:657-670.
<https://doi.org/10.1016/j.ympev.2013.04.014>

Line 107:

Incomplete references. There are several published studies that indicate the advancement of phenology in *Rana temporaria*.

See:

Bison M, Yoccoz NG, Carlson BZ, Klein G, Laigle I, Van Reeth C, Delestrade A. 2021. Earlier snowmelt advances breeding phenology of the common frog

(*Rana temporaria*) but increases the risk of frost exposure and wetland drying. *Frontiers in Ecology and Evolution* 9:645585 DOI 10.3389/fevo.2021.645585.

Montori A, Amat F. 2023. Surviving on the edge: present and future effects of climate warming on the common frog (*Rana temporaria*) population in the Montseny massif (NE Iberia). *PeerJ* 11:e14527 DOI 10.7717/peerj.14527

While GM, Uller T. 2014. Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. *Ecography* 37(10):921–929 DOI 10.1111/ecog.00521.

Beebee TJC. 1995. Amphibian breeding and climate. *Nature* 374(6519):219–220 DOI 10.1038/374219a0.

Terhivuo J. 1988. Phenology of spawning for the Common Frog (*Rana temporaria* L.) in Finland from 1846 to 1986. *Annales Zoologici Fennici* 25:165–175.

Ficetola GF, Maiorano L. 2016. Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance. *Oecologia* 181(3):683–693 DOI 10.1007/s00442-016-3610-9.

Phillimore AB, Hadfield JD, Jones OR, Smithers RJ. 2010. Differences in spawning date between populations of common frog reveal local adaptation. *Proceedings of the National Academy of Sciences of the United States of America* 107(18):8292–8297 DOI 10.1073/pnas.0913792107.

and

Neveu, A. (2009). Incidence of climate on common frog breeding: long-term and short-term changes. *Acta Oecologica*, 35, 671-678.

Advancements

Montori & Amat 26 days/decade

While & Uller (2014) 28.1 days/decade

Neveu: between 1984 and 2007 could be calculated as p 26.6 Days

Nowhere in the text is it indicated the magnitude of the change in the start of the breeding period over the years.

Line 131:

Predictions or hypothesis?

Line 138:

Spawn mortality or egg mortality, or number of non-viable embryos, egg-mass dead?

Methods

Line 149:

*From 1st January every year. Add: from *** to *****

Line 154:

percentage of spawn found dead were recorded when each pond was sampled.

How has been estimated this percentage? Were whole gates considered dead or number of eggs in a sample? If all the eggs in a clutch were not dead, how was it calculated? Explain it please. When and how authors consider that egg-mass is dead?

Line 158:

Rowland et al., not Rowland.

Lines 180-181

Where? In the matrix? Explain more accurately, please.

Lines 181-185:

Authors must include the three tables as Suppl. Mat. (ECN by year, Reproductive data and phenologic data. It is very important that these data be available for future studies.

Julian days are DOY?

Lines 187-194:

Also these data in Suppl. Mat. Table

Line 198:

Table 2 don't shows land cover class each year.

Line 201-208:

Also, data has not been shown. In general data for analysis is not presented in the work. It is needed create data tables in Suppl. Mat or in public repository. In general, the work is too much cryptic explain methods, showing data and results.

Line 212:

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

I can't access it. It seems that not contain data.

Line 230-234:

Where are the results of models?

Results

Lines 253-255:

Please include one table with Factor coordinates of the variables,. Perhaps Suppl. Mat

Line 256-261:

What models? Where are the model data?? I don't understand how the authors create these two graphs (B and C for all figures). Explain it better please.

In figure 2 and subsequent authors indicate: (B) Significant negative relationship between PC1 and congregation date (C) Significant negative correlation between maximum temperature at site and congregation date.

Comentat [AMF1]: And other variables in figures 3,4...

However, no significance value is given. must be included in the results text.

Line 265-267:

and higher proportions of urban and arable area were 267 associated with earlier spawning (Fig. 3A,B). There was no support for an effect of PC2 (credible intervals crossed zero). The full model was a superior fit to a null model ($\Delta\text{LOO-IC } 40.3$). The model explained 87% of variation in spawn date [95% CI 77.3 = 92%].

Why PC2 and not PC1? The authors presents results in an unclear way. Without table results, signification levels (P), DoF, ... Why not a simple correlation between variables. On the other hand. The labels of the graph of PC are too much small and coloured with less contrasted colours.

Lines 275-276

For hatch date, we detected significant effects of PC2, where increases in freshwater area and higher minimum temperatures were associated with earlier hatching,

The same comments.

Suppl. Material

Figure S1. Factors associated with variation in the hatch date. More air frost days (A) resulted in later hatching. Higher built up area (B) and arable area (C) were associated with earlier hatching, whereas increased grassland area (D) was linked to later hatching

Figure S2. Factors associated with variation in the percentage of dead spawn. More air frost days (A) results in higher death. Higher built up area (B) and arable area (C) were associated with higher spawn survival, whereas increased grassland area (D) was linked to larger proportions of dead spawn.

Both figures present the bivariate relationships between the different variables analysed. These figures are much easier to understand and visualize the relationships between variables much better than those presented in the main text. I think they should be included there in the main text and move figures 2 to 5 to Supl. Mat. Moreover, the value of r^2 or the significance P are not shown and it is assumed that some are significant and others are not. The main text of the results must be rewritten including these figures and giving the values of r , r^2 and the significance P . Otherwise believing what they explain is an act of faith.

i.e: Lines 289-293: *Higher 290 cumulative days of air frost and higher grassland area were associated increased percentages dead spawn (Fig. S2), whilst increases in arable area, built-up area, and maximum temperature led to reductions in the percentage of dead spawn (Fig 5B; Fig S2). This model explained 45% of the variance in percentage of dead spawn (95% CI 22.5 . 60.8%).*

What model and significance.???

Discussion

Line 314:

There is a lot of literature missing that has not been consulted or included in this paragraph of the discussion. Moreover, Reading don't conclude the link of temperatures shifts with breeding phenology. He only analyses body condition and survival rates. I think that the first author linking phenology and temperatures was Beebee 1995.

Add and comment:

Bison et al 2021; Montori & Amat, 2023; Beebee, 1995; Corn, 2003; Ficetola & Maiorano, 2016; Kusano & Inoue, Parmesan 2006, 2007; Primarck et al 2009; Scott et al 2008; Sheridan et al 2018; Terhivuo, 1998; Todd, 2010; While & Uller, 2014.

Beebee TJC. 1995. Amphibian breeding and climate. *Nature* 374(6519):219–220 DOI 10.1038/374219a0.

Corn PS. 2003. Amphibian breeding and climate change: importance of snow in the mountains. *Conservation Biology* 17(2):622–625 DOI 10.1046/j.1523-1739.2003.02111.x., 2008

Kusano T, Inoue M. 2008. Long-term trends toward earlier breeding of Japanese amphibians. *Journal of Herpetology* 42(4):608–614 DOI 10.1670/08-002R1.1.

Montori A, Amat F. 2023. Surviving on the edge: present and future effects of climate warming on the common frog (*Rana temporaria*) population in the Montseny massif (NE Iberia). *PeerJ* 11:e14527 DOI 10.7717/peerj.14527

Parmesan C. 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology* 13(9):1860–1872 DOI 10.1111/j.1365-2486.2007.01404.x.

Primack RB, Ibáñez I, Higuchi H, Lee SD, Miller-Rushing AJ, Wilson AM, Silander JA. 2009. Spatial and interspecific variability in phenological responses to warming temperatures. *Biological Conservation* 142(11):2569–2577 DOI 10.1016/j.biocon.2009.06.003.

Sheridan JA, Caruso NM, Apodaca JJ, Rissler LJ. 2018. Shifts in frog size and phenology: testing predictions of climate change on a widespread anuran using data from prior to rapid climate warming. *Ecology and Evolution* 8(2):1316–1327 DOI 10.1002/ece3.3636.

Terhivuo J. 1988. Phenology of spawning for the Common Frog (*Rana temporaria* L.) in Finland from 1846 to 1986. *Annales Zoologici Fennici* 25:165–175.

Todd BD, Scott DE, Pechmann JHK, Gibbons JW. 2010. Climate change correlates with rapid delays and advancements in reproductive timing in an amphibian community. *Proceedings of the Royal Society of London B* 278(1715):2191–2197 DOI 10.1098/rspb.2010.1768.

Tryjanowski P, Mariusz R, Sparks T. 2003. Changes in spawning dates of Common Frogs and Common Toads in western Poland in 1978–2002. *Annales Zoologici Fennici* 40:459–464.

While GM, Uller T. 2014. Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. *Ecography* 37(10):921–929 DOI 10.1111/ecog.00521.

Line 322

Insert:

Phillimore AB, Hadfield JD, Jones OR, Smithers RJ. 2010. Differences in spawning date between populations of common frog reveal local adaptation. *Proceedings of the National Academy of Sciences of the United States of America* 107(18):8292–8297 DOI 10.1073/pnas.0913792107.

Line 325:

I think that it is not plasticity. The minimal temperatures (Frost days) and warmer winters determine that thermal conditions to start breeding period occurs earlier. See;

Enriquez-Urzelai U, Palacio AS, Merino NM, Sacco M, Nicleza AG. 2018. Hindered and constrained: limited potential for thermal adaptation in post-metamorphic and adult *Rana temporaria* along elevational gradients. *Journal of Evolutionary Biology*. Dryad, Dataset DOI 10.5061/dryad.2fp14fg.

Also:

Do you need to read these articles:

Enriquez-Urzelai U, Palacio AS, Merino NM, Sacco M, Nicieza AG. 2018. Hindered and constrained: limited potential for thermal adaptation in post-metamorphic and adult *Rana temporaria* along elevational gradients. *Journal of Evolutionary Biology*. Dryad, Dataset DOI 10.5061/dryad.2fp14fg.

Enriquez-Urzelai U, Bernardo N, Moreno-Rueda G, Montori A, Llorente G. 2019a. Are amphibians tracking their climatic niches in response to climate warming? A test with Iberian amphibians. *Climatic Change* 154(1–2):289–301 DOI 10.1007/s10584-019-02422-9.

Nicieza AG, Tingley R. 2019b. Integrating mechanistic and correlative niche models to unravel range-limiting processes in a temperate amphibian. *Global Change Biology* 25(8):1–15 DOI 10.1111/gcb.14673.

Enriquez-Urzelai U, Tingley R, Kearney MR, Sacco M, Palacio AS, Tejedo M, Nicieza AG. 2020. The roles of acclimation and behaviour in buffering climate change impacts along elevational gradients. *Journal of Animal Ecology* 89(7):1–13 DOI 10.1111/1365-2656.13222.

Line 367;

These two works determine the importance of riparian strips, not ditches. I don't have found where they speak about arable ditches. Please explain it me.

Line 390

The same comment.

Line 398:

(Bullock *et al.* 2011), the reference is incomplete in References.

Conclusions

Here we have demonstrated associations between climate, land use change and parameters of amphibian breeding success and phenology at the landscape scale.

I'm not sure. Probably yes, but Authors must be including signification levels.

Lines 434-435:

Our results are consistent with investigations at the scale of individual ponds that uncovered similar relationships between temperature and the timing of amphibian breeding and larval development

In what sense. If the results are concordant, the conclusions should specify what they agree on.

Lines 438-439

*Future work on R. temporaria in the UK should prioritise pond-scale approaches to **these questions**, which will permit measurement of microhabitats experienced by breeding adults.*

What questions? You are in conclusions.

Occlusions must be more concise.

References:

References are not in PeerJ format. Additionally, all DOIs are missing.