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Importance of Agricultural Areas for the Distribution of Protected Amphibian Species in Bulgaria

Georgi Popgeorgiev[1], Simeon Lukanov[2,*], Blagovesta Dimitrova[2] & Borislav Naumov[2]

[1] National Museum of Natural History, Bulgarian Academy of Sciences, blvd. Tzar Osvoboditel 1, 1000 Sofia, Bulgaria
[2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

Abstract: We mapped the distribution for the past 18 years (2004–2022) of five protected amphibian species across Bulgaria using a spatial resolution of 100 m^2 in CORINE Land Cover 2018 (CLC). Since the altitudinal distribution and habitat preferences of these species in the country make them potentially vulnerable to agricultural activities, the primary aim was to establish what part of the known records of each species falls within agricultural lands. Results indicated that all five studied species inhabit CLC types falling within the general type "Agricultural areas" (CLC Level 1: 2), with four species predominantly occurring in such regions. In total, three species (*Bombina bombina, Triturus cristatus* and *T. ivanbureschi*) had more than 50% of their distribution records in agricultural areas. In another species, *Triturus dobrogicus*, the percentage was above 40%. The lowest number (under 36%) was for *B. variegata*. For *T. dobrogicus*, nearly 32% of all records were concentrated in the CLC Level 3 category "Non-irrigated arable lands". This data suggests that a large percentage of the populations of these protected species across Bulgaria are potentially exposed to agricultural practices such as pesticide use, or crop land aggregation and highlight the need for regional approach for species protection.

Key words: conservation, CORINE Land Cover, occurrence records, *Bombina, Triturus*

Introduction

Numerous ecosystem functions are being threatened globally by the continuous intensification of conventional agriculture (Tscharntke et al. 2012, Chagnon et al. 2014, Barral et al. 2020). Agricultural practices often result in the destruction and fragmentation of habitats, which leads to a decrease in species richness (Scales & Marsden 2008) and a shift in species composition (Newbold et al. 2016). They also pose a threat to biodiversity through the deposition of chemicals, which affect not only the pests that they are aimed at, but also non-target organisms that inhabit these lands (Thanomsit et al. 2020).

Amphibians and reptiles are the most threatened group of vertebrates in the world (Gibbons et al. 2000), with pesticide-related pollution recognised as one of the major factors contributing to population declines worldwide (Todd et al. 2010, Agostini et al. 2020). Agricultural areas often include or are in close proximity to essential habitats for herpetofauna, which explains the key role that they play in the observed population declines. During migration to and from spawning waters, or simply due to agricultural runoff, amphibians

*Corresponding author: simeon_lukanov@abv.bg

and reptiles encounter chemicals that have potentially devastating consequences for their survival (Jayawardena et al. 2011), reproduction (Adams et al. 2021), hatching success and development (Agostini et al. 2020), and behaviour (Guerkan et al. 2016, Acquaroni et al. 2020). Moreover, due to their physiological constrains, amphibians possess a low tolerance for habitat alterations, limited dispersal capacity and territoriality, These animals will likely suffer greater damages following land use change and agricultural intensification (Berger et al. 2018, Guiller et al. 2020). With the increased transformation of land into agricultural fields, species with low vagility can suffer even greater damages if the territory they inhabit goes through a rapid change and they do not have the capacity to escape from it.

Only few studies have investigated the distribution of amphibians and reptiles in different parts of agricultural landscapes such as woodland patches and hedgerows, and fewer still have looked into the impact of management regimes on herpetofauna biodiversity in woody crops (Carpio et al. 2016). This study focuses on the five amphibian species in Bulgaria, which have the highest protection status in the national and EU legislation (all included in Annexes II and III of Biological Diversity Act of Bulgaria, and in Annex II and Annex IV of Council Directive 92/43/EEC): the Great crested newt, *Triturus cristatus* (Laurenti, 1768), the Danube crested newt, *Triturus dobrogicus* (Kiritzescu, 1903), the Buresch's crested newt, *Triturus ivanbureschi* Arntzen and Wielstra, 2013, the Fire-bellied toad, *Bombina bombina* (Linnaeus, 1761) and the Yellow-bellied toad, *Bombina variegata* (Linnaeus, 1758). On the basis of the altitudinal distribution and habitat preferences of these species, it can be presumed that they are potentially vulnerable to the agricultural activities. We hypothesized that a substantial part of the national range of the five species falls within agricultural lands. In this regard, our aim was to establish what part of the known records of each species falls within such sites.

Materials and Methods

Study species

Triturus cristatus is the most widespread species from its genus, occurring from the British Isles and France in the west, to the Urals in the east and from the middle parts of Norway and Sweden in the north, to Bulgaria in the south (Litvinchuk & Borkin 2009, Wielstra et al. 2014). In Bulgaria, it occurs only in the north-western part of the country and its typical aquatic habitats are stagnant ponds of small area with shallow depth and lack of fish (Naumov et al. 2023).

Triturus dobrogicus is distributed from the Danube River basin floodplains in central Europe to the delta of the Dnipro River in the East (Wielstra et al. 2014). The species spends more time in the water than its congeners and has the longest aquatic phase (Arntzen & Wallis 1999). Bulgaria is at the southernmost part of its range and the species has only been established up to a few kilometers from the Danube (Naumov & Biserkov 2013, Popgeorgiev et al. 2019) and as an unconfirmed exception in the northern Black Sea coast (Gherghel & Iftime 2009). It is usually found in the shallow parts of stagnant water bodies or slow-flowing canals overgrown with aquatic vegetation (Stojanov et al. 2011).

Triturus ivanbureschi is distributed in the central and eastern parts of the Balkan Peninsula, as well as in the northwestern part of Asia Minor (Wielstra et al. 2013, 2014). The species occurs across most of the territory of Bulgaria, from the sea level up to ca. 1700 m elevation; however, it is largely absent from the immediate riverine area of the Danube, as well as from the north-western part of the country (Stojanov et al. 2011). It is the largest, most robust of the *Triturus* species in Bulgaria, and with the shortest aquatic phase (Arntzen & Wallis 1999). It breeds in various types of stagnant water bodies, such as lakes, swamps, temporary puddles, etc. (Stojanov et al. 2011).

Bombina bombina occurs across Central and Eastern Europe, to the south-eastern foothills of the Ural Mountains in the East, and to the north-westernmost part of Asia Minor (Sillero et al. 2014, Speybroeck et al. 2016). In Bulgaria, it is distributed in the lower parts of the country (below 400 m a.s.l.): the Danubian Plain, the Thracian Lowland, and sporadically the Black Sea coast. It inhabits a wide variety of water types such as natural and artificial lakes, swamps, rivers, canals, temporary puddles, etc. (Stojanov et al. 2011).

Bombina variegata is distributed across most of Europe, from the Atlantic coast of France to the Carpathians in the East, and from Northern Germany to Southern Italy and Greece (Sillero et al. 2014). In Bulgaria, it is widespread in the hilly and mountain parts of the country up to about 1500 m a.s.l. (as exception to 2100 m), but absent across the Thracian Lowland, the Black Sea coast and most of the Danubian Plain. It can inhabit a variety of terrestrial habitats and water body types: mountain streams and rivers, canals, lakes, marshes, temporary excavations, flooded ruts on dirt roads, etc. (Stojanov et al. 2011).

Study area

The present study covers the entire territory of Bulgaria (ca 111 000 km^2). On relatively small area, the country has highly diverse relief, ranging from extensive plains and lowlands to subalpine and alpine areas, as the altitude varies between 0–2925 m. The total area used for agricultural purposes (excluding land abandoned as a result of shifting cultivation) comprises approximately 45.2% of the territory of the country, most of which is concentrated in the lowlands below 1000 m a.s.l. (Ministry of agriculture 2022).

Data analyses

We complied distributional data for the target species for the past eighteen years (2004–2022) from observations provided by users of the data collection system with integrated Android application SmartBirds Pro (https://smartbirds.org), the Global Information Facility (GBIF: www.gbif.org) and personal unpublished observations by the authors. To prevent inconsistencies arising from variations in species nomenclature across the databases, we adopted the taxonomic framework outlined by Speybroeck et al. (2020). The standard geographic coordinate system WGS84 was used and in order to streamline the databases, we eliminated all extraneous details, retaining only the essential information such as species names, coordinates, locality, year and the data source. Our data refinement process involved eliminating entries that lacked specific species identification or precise geographical coordinates. Additionally, any data with coordinates less accurate than 100 m were excluded. We discarded duplicate records, defined as entries sharing identical species names, longitudes and latitudes. The final dataset consisted of 4231 occurrence points (see Supplementary material).

The ArcGIS v.10.3 software (ESRI, Redlands, CA, USA) was used for manipulation and analysis of the occurrence points dataset. The European CORINE Land Cover 2018 (CLC version v.2020_20u1) from the European Environment Agency (EEA 2018), with a spatial resolution of 100 m^2 was used as a base land-cover map. Using the “Intersect” function of the ArcGIS (with a 100 m buffer to account for the spatial resolution of CLC) we checked the occurrence points against land cover types, for both for level 1 (large-scale) and level 3 (small-scale) of CLC.

The species diversity of individual land cover types (CLC Level 3), falling within the general type “Agricultural areas” (CLC Level 1: 2.), was analysed via Rényi’s index family (diversity profiles), which is considered one of the most useful methods for ordering communities according to their diversity. Similarity between species in terms of number of records in individual land cover types within “Agricultural areas” was inferred using a classical cluster analysis (UPGM algorithm) based on the Morisita’s index, which is accepted to be least dependent on sample size. All analyses were performed with the computer program PAST 4.07 (Hammer et al. 2001).

Results

After applying the 100 m buffer, a total of 2968 occurrence points for the five study species were distributed across the CLC types. The distribution of the number of registrations by land cover types from CLC Level 1 is presented on Fig. 1. For four of the species, the land cover type with the most numerous registrations is “Agricultural areas” (three species had more than half of their occurrence records located in that type), and only for registrations of *B. variegata* the number was highest in the “Forest and semi natural areas” type.

Regarding land cover types from CLC Level 3 (Table 1), it has to be noted that out of the total 28 types which contain occurrence points, only four have records of all five studied species, and two of these types (CLC211 and CLC243) fall under “Agricultural areas”. In total, three species (*B. bombina*, *T. cristatus* and *T. ivanbureschi*) have more than 50% of their distribution records in agricultural areas, while the lowest number is for *B. variegata* (Table 1).

The comparison of the individual land cover types within the specific “Agricultural areas” (Fig. 2) shows the highest diversity of the studied five species in CLC211 and the lowest in CLC213, while the profiles of the remaining types intersect, i.e., cannot be sorted according to species diversity.

The comparison between the five species according to the number of their registrations only in the agricultural territories (Fig. 3) shows the formation of two main branches, one of which contains only *Triturus dobrogicus*, and the second – the other species, with the highest level of similarity between *T. cristatus* and *B. bombina*, and lowest between *T. dobrogicus* and *B. variegata*.

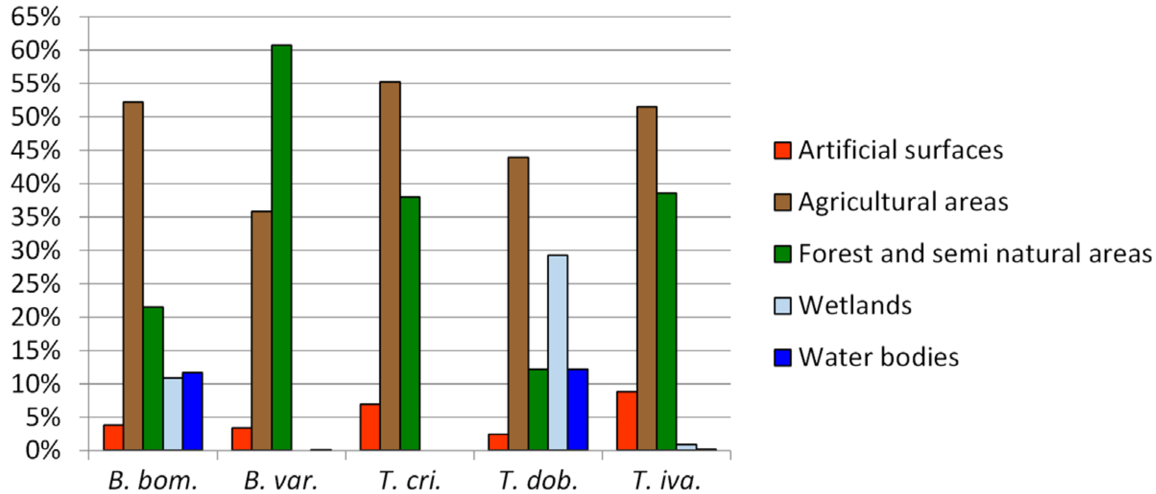

Fig. 1. Percent distribution of occurrence points for the five study species across the CLC Level 1 types; species names are abbreviated.

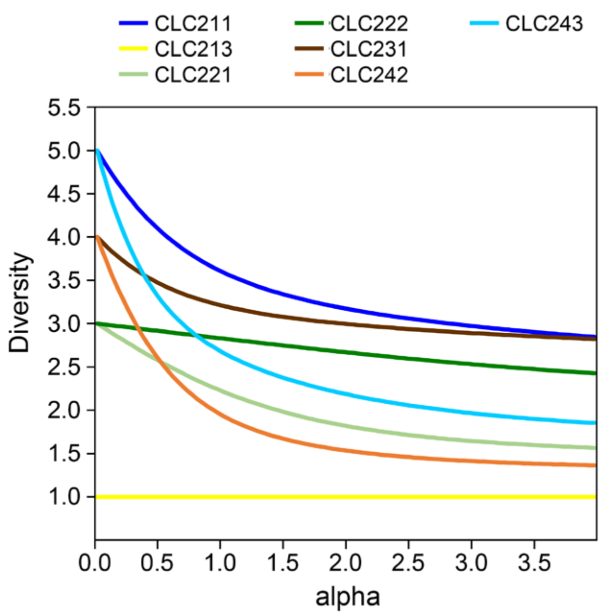

Fig. 2. Diversity profiles (based on the number of occurrence points for the five study species) of the CLC Level 3 types, which refer to "Agricultural areas"; for the code abbreviations see Table 1.

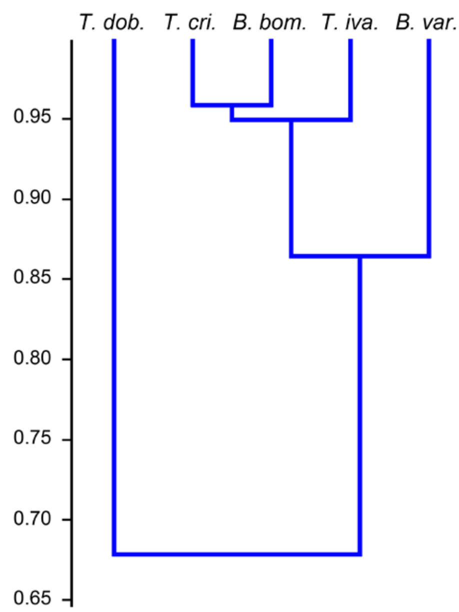

Fig. 3. Similarity between the five study species in the "Agricultural areas" (based on the Morisita Index); species names are abbreviated.

Discussion

From the CLC Level 1 analysis it can clearly be seen that all five studied species have populations that inhabit "Agricultural areas", with four of the five species being predominantly found in such areas. This data suggests that a large percentage of the populations of these protected species across Bulgaria are potentially exposed to agricultural practices such as pesticide use and crop land aggregation, and are therefore threatened by chemical pollution and habitat destruction. Negative effects of pesticides on amphibians have been extensively studied and the most commonly used pesticides are often shown to have severe consequences (Adams et al. 2021, Agostini et al. 2020, Guerkan et al. 2016).

Looking specifically at the five studied species in the context of agricultural fields, certain aspects

Table 1. Percent distribution of occurrence points for the five study species across the CLC Level 3 types; species names are abbreviated and the sample size (number of occurrence points) is given in brackets.

CLC Level 3	*B. bombina* **(577)**	*B. variegata* **(1878)**	*T. cristatus* **(29)**	*T. dobrogicus* **(41)**	*T. ivanbureschi* **(443)**
112 Discontinuous urban fabric	0.87	1.86	3.45	2.44	3.84
121 Industrial or commercial units	0.69	0.16	-	-	1.13
122 Road and rail networks and associated land	-	0.37	3.45	-	0.23
131 Mineral extraction sites	1.04	0.16	-	-	0.90
132 Dump sites	-	0.11	-	-	
133 Construction sites	-	0.53	-	-	
141 Green urban areas	-	-	-	-	0.68
142 Sport and leisure facilities	1.21	0.16	-	-	2.03
Codes 112-142	**3.81**	**3.35**	**6.90**	**2.44**	**8.80**
211 Non-irrigated arable lands	19.06	3.78	17.24	31.71	12.42
213 Rice fields	2.60	-	-	-	
221 Vineyards	0.17	0.05	-	-	1.13
222 Fruit trees and berry plantations	0.17	0.11	-	-	0.23
231 Pastures	6.24	2.50	10.34	-	5.87
242 Complex cultivation patterns	0.87	5.48	6.90	-	4.51
243 Land principally occupied by agriculture, with significant areas of natural vegetation	23.05	23.86	20.69	12.20	27.31
Codes 211-243	**52.17**	**35.78**	**55.17**	**43.90**	**51.47**
311 Broad leaved forest	14.73	22.58	-	9.76	14.00
312 Coniferous forest	0.17	2.88	-	-	1.35
313 Mixed forest	-	7.67	-	-	2.48
321 Natural grassland	0.87	8.73	24.14	2.44	10.38
324 Transitional woodland-shrub	5.37	17.36	10.34	-	9.03
331 Beaches, dunes, sands	0.35	0.05	-	-	0.90
332 Bare rock	-	0.53	-	-	0.23
333 Sparsely vegetated areas	-	0.96	3.45	-	0.23
Codes 331-333	**21.49**	**60.76**	**37.93**	**12.20**	**38.60**
411 Inland marshes	9.88	-	-	29.27	0.45
421 Salt marshes	0.17	-	-	-	0.23
422 Salines	0.87	-	-	-	0.23
Codes 411-422	**10.92**	**-**	**-**	**29.27**	**0.90**
511 Water courses	5.72	0.11	-	-	-
512 Water bodies	5.89	-	-	12.20	0.23
Codes 511-512	**11.61**	**0.11**	**-**	**12.20**	**0.23**

of their biology could make them specifically susceptible to the negative effects of agriculture. The most evident characteristic shared among all amphibians is their semi-permeable skin, because of which they are considered bioindicators of environmental contamination (Vitt & Caldwell 2009). This, combined with their complex life cycle both on land and in aquatic habitats, means that they are prone to the adverse effects of pollutants in the air, soil and water. In regards to the species in this study, the available literature clearly demonstrates the negative effects of dermal exposure to pesticides. Zaffroni et al. (1979) have shown that following dermal exposure to fungicide, *T. cristatus* individuals suffer changes to their peripheral blood cells. For *B. bombina*, laboratory experiments using dermal exposure of embryos and larvae to herbicide have resulted in abnormal behavior, improper gut coiling, delayed development, reduced growth rate and numerous malformations (Sayim 2010). Similar results have been found following dermal exposure to insecticides on both tadpoles and embryos of *B. variegata* (Greulich & Pflugmacher 2004). Dermal intake of toxins through the soil has also been recorded for *B. bombina* that live in close proximity to maize crops (Lenhardt et al. 2015).

In addition to their semi-permeable skin, the five species investigated in this paper can be characterized with low vagility, which is also an aspect of their biology that makes them more prone to exposure, since they are unable to distance themselves from environmental threats. A study by Kovar et al (2009) during March and April recorded that the maximum movement of *B. bombina* individuals through agricultural areas was 167 meters and their maximum distance traveled between habitats ranged between 160 and 230 meters. For *T. cristatus* distances away from the breeding pond were recorded using radio trackers between June and July, discovering that 68% of the tracked newts stayed within 20 m of the pond edge (Jehle & Arntzen 2000). Not only is pesticide exposure a threat in itself, but also the recovery of populations with low vagility, such as the great crested newt, is very slow due to the limited dispersal ability of such species (Ockleford et al. 2018).

The timing of application of pesticides is also important, as the breeding season of amphibians often coincides with it, suggesting that during their most active period, amphibians are exposed to the highest concentrations of pesticides. During this time, adults are in their aquatic phase, while deposited eggs and hatched larvae are found in the same aquatic habitats, indicating that individuals of all life stages are exposed to waterborne pollutants. The importance of timing of application is demonstrated by Jones et al. (2010), showing that application earlier in the season has a higher effect on tadpole mortality in comparison to later application, indicating that species may be more vulnerable at earlier stages of their development.

Habitat preference also plays an important role for these five species and the likelihood and intensity of their exposure to pesticides. In the case of the newts, for example, their preference for temporary ponds, such as farm ponds, may increase their chance to encounter pollutants in the water or suffer other damage due to cultivation practices. Temporarily flooded agricultural areas, such as rice fields, are also important summer habitats for amphibians, as they are constantly filled with water during the summer months, and some amphibians migrate to them when the natural ponds in their surroundings dry up. Migration from breeding ponds into wet spots in agricultural fields is particularly frequent after metamorphosis of juveniles (Berger et al. 2003). This increases their chance to encounter pollutants in the water and, in this study, we found that 2.6% of all *B. bombina* observations fall within such rice fields.

The analysis shows that *T. dobrogicus* and *B. bombina* are most widely represented in aquatic habitats (CLC types: “Wetlands” and “Water bodies”), which is in accordance with the accepted view of these species as closely related to water and to larger water bodies. The fact that the analysis does not immediately demonstrate the importance of water for the other three species can be explained by the fact that they are usually found in small water bodies (temporary ponds, mountain streams, roadside ditches, etc.) (Stojanov et al. 2011, Naumov et al. 2023), and thus remain “invisible” due to the relatively low resolution of the CLC.

The scale, at which a study is conducted, is of high importance in these types of investigations. A recent study done by Bancila et al. (2023) performed a similar analysis, looking at amphibians and reptiles on a European scale, with three different resolutions – at 50 km², 10 km² and using GPS coordinates. They discovered that there was a lack of consistency in terms of regions with high number of species (hotspots) between the coarse and fine scales when looking at the geographic location of species peaks (highest abundance of species at a location), as well as crop extent. Species hotspots were much more evident and easily detected at the larger scale of 50 km², and were in general agreement with the known distribution and geographic patterns of species and populations. At the finer spatial resolution of 10 km² such hotspots were less obvious, mainly accredited to the lack of homogeneous distribution data. Thus, it is important to bear in mind the scale-dependent spatial patterns of species richness and crop extent. In this study, the spatial resolution of 100 m² can be considered a more fine-scale investigation and this can explain why only four out of the total 28 CLC types have records of all five studied species.

Additionally, at larger resolution levels, the probability of false absences decreases because of the higher accumulation of data with the increase of the grid cells, suggesting higher probability of such types of errors when working on a finer scale (Bancila et al. 2023). Our study relies on presence-only data, which may present a problem, as these types of data have several shortcomings, among which is their lack of true absences, errors in coordinate records, species identification and geographical biases. It should also be noted that sampling bias may actually affect false negatives for species presence in agricultural areas, as these habitats are often not targets of investigation and field research. Therefore, there is a potential for species to be even more frequently inhabiting such areas, than the observed results indicate.

In terms of the locations and field types investigated, Bancila et al. (2023) grouped the agricultural areas from the CLC into five crop categories, which were as follows: 1) irrigated crops [codes 212, 213 and 242], 2) dry crops [codes 211 and 241], 3) woody crops [codes 221, 222 and 223], 4) pastures [code 231] and 5) agroforestry crops [codes 243 and 244]. Of those, four codes do not appear in our study because they are areas that are not present on the territory of Bulgaria or animals have not been discovered in these habitats. Those are 212 (permanently irrigated land), 241 (annual crops associated with permanent crops), 223 (olive groves) and 244 (agroforestry areas). When we take this into account, three of the five groupings become less relevant for our study because they are represented only by one code. Nevertheless, if we use this same grouping for our study species, the overall outlook does not change significantly and “agroforestry crops” would still hold the most records, followed by “dry crops”. Our results for agroforestry confirm the observation of Bancila et al. (2023) that species presence in this group was significantly more likely than would be expected by chance in 75% of their records at GPS coordinates resolution. Interestingly, they also found that “woody crops” positively affected the highest number of species at GPS coordinates resolution, which is probably due to their large species dataset, as vineyards and orchards are generally an important habitat for many amphibian and, especially, reptile species. However, for the five protected amphibian species in our study, this was the least important group, which highlights the need for regional approach for species protection based on their preferences and conservation status (e.g., conservation measures aimed at “woody crops” will almost completely miss all five amphibian species, protected in Bulgaria under the Habitat directive). While Bancila et al. (2023) indicated that “dry crops” negatively affected amphibian species richness, in our study this was the second most important agricultural area after “agroforestry crops” in terms of number of records for all species (except for *B. variegata*, which was also the only species with less than 40% of records in agricultural lands). This might be explained by the already mentioned habitat preferences of both *B. variegata* and *T. dobrogicus* (with 43.90% of records in agricultural lands), which are often associated with larger water bodies.

Crop heterogeneity is also an important factor in the establishment of biodiversity (Fahrig et al. 2011), as species richness increases with a more diverse habitat. This is reflected in our results, as the highest number of records for all species except *T. dobrogicus* is found in agricultural lands with significant areas of natural vegetation (code 243). If only one cover type is present in a given territory, then there will only be species that are adapted to it, but with the addition of a second cover type within the same territory, animals that inhabit each cover type separately, but also those that require a mixture of cover types will be present. This is particularly important for amphibians, as they need both aquatic and terrestrial habitats at different life stages, and are therefore more likely to occur in landscapes with a mosaic of the two (Pope et al. 2000). This is also confirmed by Bancila et al. (2023) in their analysis at both the 50 km² and 10 km² spatial resolution, showing that species richness increases significantly with crop heterogeneity.

In conclusion, it can be argued that agricultural territories are no less important than natural habitats for conservation-relevant amphibian species, which determines the need for their deeper study in order to clarify existing and potential conservation problems.

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Supplementary material. Primary data of records used for assessment of the importance of agricultural areas for distribution of protected amphibian species in Bulgaria Excel Spreadsheet:
https://www.acta-zoologica-bulgarica.eu/2024/002709_suppl.xlsx

References

Adams E., Leeb C. & Brühl C. A. 2021. Pesticide exposure affects reproductive capacity of common toads (*Bufo bufo*) in a viticultural landscape. Ecotoxicology 30: 213–223.

Agostini M. A., Roesler I., Bonetto C., Ronco A. E. & Bilenca D. 2020. Pesticides in the real world: The consequences of GMO-based intensive agriculture on Native Amphibians. Biological Conservation 241: 108355. doi:10.1016/j.biocon.2019.108355.

Acquaroni M., Svartz G. & Pérez C. 2022. Acute, chronic and neurotoxic effects of dimethoate pesticide on rhinella arenarum throughout the development. Journal of Environmental Science and Health Part B 57(2): 142–152. doi:10.1080/03601234.2022.2034459.

Arntzen J. & Wallis G. 1999. Geographic variation and taxonomy of crested newts (*Triturus cristatus* superspecies): morphological and mitochondrial DNA data. Contributions to Zoology 68(3): 181–203.

Bancila R. I., Lattuada M. & Sillero N. 2023. Distribution of amphibians and reptiles in agricultural landscape across Europe. Landscape Ecology 38(3): 861–874. doi:10.1007/s10980-022-01583-w.

Barral M. P., Villarino S., Levers C., Baumann M., Kuemmerle T. & Mastrangelo M. 2020. Widespread and major losses in multiple ecosystem services as a result of agricultural expansion in the Argentine Chaco. Journal of Applied Ecology 57(12): 2485–2498. doi:10.1111/1365-2664.13740.

Berger G., Pfeffer H., Kachele H., Andreas S. & Hoffmann J. 2003. Nature protection in agricultural landscapes by setting aside unproductive areas and ecotones within arable fields ("Infield Nature Protection Spots"). Journal for Nature Conservation 11(3): 221–233.

Berger G., Graef F., Pallut B., Hoffmann J., Brühl C. A. & Wagner N. A. 2018. How does changing pesticide usage over time affect migrating amphibians: a case study on the use of glyphosate-based herbicides in German agriculture over 20 years. Frontiers in Environmental Sciences 6:6. doi: 10.3389/fenvs.2018.00006

Carpio A. J., Oteros J., Tortosa F. S. & Guerrero-Casado J. 2016. Land use and biodiversity patterns of the herpetofauna: the role of olive groves. Acta Oecologica 70: 103–111.

Chagnon M., Kreutzweiser D., Mitchell E. A., Morrissey C. A., Noome D. A. & van der Sluijs P. J. 2014. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environmental Science and Pollution Research 22(1): 119–134. doi:10.1007/s11356-014-3277-x.

Fahrig L., Baudry J., Brotons L., Burel G., Crist T. O., Fuller R. J., Sirami C., Siriwardena G. M. & Martin J. M. 2010. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecology Letters 14(2): 101–112. doi:10.1111/j.1461-0248.2010.01559.x.

Gibbons J. W., Scott D. E., Ryan T. E., Buhlmann K. A., Tuberville T. D., Metts B. S., Greene J. L., Mills T., Leiden Y., Poppy S. & Winne C. T. 2000. The global decline of reptiles, déjà vu amphibians: reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. Bioscience 50: 653–666.

Gherghel I. & Iftime A. 2009. On the presence of the Danube crested newt, *Triturus dobrogicus*, at Durankulak Lake, Bulgaria. North-Western Journal of Zoology 5(1): 209–213.

Greulich K. & Pflugmacher S. 2004. Uptake and effects on detoxication enzymes of Cypermethrin in embryos and tadpoles of Amphibians. Archives of Environmental Contamination and Toxicology 47(4): 489–495. doi:10.1007/s00244-004-2302-3.

Guerkan M., Serbest S. & Hayretdağ S. S. 2016. Acute toxicity of the synthetic pyrethroid alpha-cypermethrin on the tadpoles of variable green toad, *Bufotes variabilis* (Amphibia:Anura). Ege Journal of Fisheries and Aquatic Sciences 33(4): 367–371. doi:10.12714/egejfas.2016.33.4.10.

Guiller G., Legentilhomme J., Boissinot A., Blouin-Demers G., Barbraud C. & Lourdais O. 2022. Response of farmland reptiles to agricultural intensification: Collapse of the common adder *Vipera berus* and the western green lizard *Lacerta bilineata* in a hedgerow landscape. Animal Conservation 25(6): 849–864. doi:10.1111/acv.12790.

Hammer Ø., Harper D. A. T. & Ryan P. D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 1–9.

Jayawardena U. A., Navaratne A. N., Amerasinghe P. H. & Rajakaruna R. S. 2011. Acute and chronic toxicity of four commonly used agricultural pesticides on the Asian common toad, *Bufo melanostictus* Schneider. Journal of the National Science Foundation of Sri Lanka 39(3): 267–276.

Jehle R. & Arntzen J. 2000. Post-breeding migrations of newts (*Triturus cristatus* and *T. marmoratus*) with contrasting ecological requirements. Journal of Zoology 251(3): 297–306.

Jones D. K., Hammond J. I. & Relyea R. A. 2010. Roundup and amphibians: the importance of concentration, application time, and stratification. Environmental Toxicology Chemistry 29(9): 2016–2025. doi: 10.1002/etc.240. PMID: 20821659.

Kovar R., Brabec M., Vita R. & Bocek R. 2009. Spring migration distances of some Central European amphibian species. Amphibia Reptilia 30(3): 367–378.

Lenhardt P. P., Brühl C. A. & Berger G. 2015. Temporal coincidence of amphibian migration and pesticide applications on arable fields in spring. Basic & Applied Ecology 16: 54–63.

Litvinchuk S. & Borkin L. 2009. Evolution, systematics and distribution of Crested newts (*Triturus cristatus* complex) in Russia and adjacent countries. Evropeisky Dom Press, St. Petersburg, 592 pp. (In Russian, English summary)

Ministry of Agriculture, Department "Agrostatistics" Results and analyses, No. 419 – October 2022

Naumov B. & Biserkov V. 2013. On the distribution and subspecies affiliation of *Triturus dobrogicus* (Amphibia: Salamandridae) in Bulgaria. Acta Zoologica Bulgarica 65(3): 307–313.

Naumov B., Lukanov S., Vacheva E. & Slavchev M. 2023. Distribution and ecology of *Triturus cristatus* (Laurenti, 1768) (Amphibia: Salamandridae) in Bulgaria. Acta Zoologica Bulgarica 75(1): 113–122.

Newbold T., Hudson L. N., Phillips H. R. P., Hill S. L. L., Contu S., Lysenko I., Blandon A., Butchart S. H. M., Booth H. L., Day J., de Palma A., Harrison M. L. K., Kirkpatrick L., Pynegar E., Robinson A., Simpson J., Mace G. M., Scharlemann J. P. & Purvis A. 2014. A global model of the response of tropical and sub-tropical forest biodiversity to anthropogenic pressures. Proceedings of the Royal Society of London. Series B 281: 20141371.

Ockleford C., Adriaanse P., Berny P., Brock T., Duquesne S., Grilli S., Hernandez-Jerez A. F., Bennekou S. H., Klein M., Kuhl T., Laskowski R., Machera K., Pelkonen O., Pieper S., Stemmer M., Sundh I., Teodorovic I., Tiktak A., Topping C. J., Wolterink G., Aldrich A., Berg C., Ortiz-Santaliestra M., Weir S., Streissl F. & Smith R. H. 2018. Scientific opinion on the state of the science on pesticide risk assessment for amphibians and reptiles. EFSA Journal 16(2): 5125 doi: 10.2903/j.efsa.2018.5125

Pope S.E., Fahrig L. & Merriam H.G. 2000. Landscape complementation and metapopulation effects on leopard frog populations. Ecology 81: 2498–2508.

Popgeorgiev G., Naumov B., Kornilev Y., Vergilov V., Slavchev M., Lukanov S., Dyugmedzhiev A., Stoyanov A., Dobrev D. & Tzankov N. 2019. Diversity and distribution of amphibians and reptiles in the Bulgarian part of the Lower Danube. In: Shurulinkov P., Hubenov Z., Beshkov S. & Popgeorgiev G. (Eds): Biodiversity of the Bulgarian–Romanian section of the Lower Danube. New York: Nova Science Publishers, pp. 283–314.

Sayim F. 2010. Toxicity of trifluralin on the embryos and larvae of the red-bellied toad, *Bombina bombina*. Turkish Journal of Zoology 34(4): Article 6. doi: 10.3906/zoo-0811-21

Scales B. R. & Marsden S. J. 2008. Biodiversity in small-scale tropical agroforests: a review of species richness and abundance shifts and the factors influencing them. Environmental Conservation 35: 160–172.

Sillero N., Campos J., Bonardi A., Corti A., Creemers R., Crochet P., Crnobrnja-Isailović J., Denoël M., Ficetola G. F., Gonçalves J., Kuzmin S., Lymberakis P., de Pous P., Rodríguez A., Sindaco R., Speybroeck J., Toxopeus B., Vieites D. R. & Vences M. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe. Amphibia–Reptilia 35(1): 1–31. doi: 10.1163/15685381-00002935

Speybroeck J., Beukema W., Bok B. & van der Voort J. 2016. Field guide to the amphibians and reptiles of Britain and Europe. London: Bloomsbury. 423 p.

Speybroeck J., Beukema W., Dufresnes C., Fritz U., Jablonski D., Lymberakis P., Martínez-Solano I., Razzetti E., Vamberger M., Vences M., V*örös* J. & Crochet P. 2020. Species list of the European herpetofauna – 2020 update by the Taxonomic Committee of the Societas Europaea Herpetologica. Amphibia–Reptilia 41(2): 139–189.

Stojanov A., Tzankov N. & Naumov B. 2011. Die Amphibien und Reptilien Bulgariens. Frankfurt am Main: Chimaira. 588 p.

Thanomsit C., Saowakoon S., Wattanakornsiri A., Nanuam J., Prasatkaew W., Nanthanawat P., Mongkolvai P. & Chalorcharoenying W. 2020. The glyphosate (Roundup): fate in aquatic environment. Naresuan University Journal: Science and Technology 21(1): 65–81.

Todd B. D., Willson J. D., Gibbons J. W. 2010. The global status of reptiles and causes of their decline. In: Sparling D. W., Linder G., Bishop C. A. & Krest S. (Eds): Ecotoxicology of amphibians and reptiles, 2nd edn. Boca Raton: CRC Press, pp. 47–67.

Tscharntke T., Clough Y., Wanger T. C., Jackson L., Motzke I., Perfecto I., Vandermeer J. & Whitbread A. 2012. Global food security, biodiversity conservation and the future of agricultural intensification. Biological Conservation 151: 53–59.

Vitt L. J. & Caldwell J. P. 2009. Spacing, movements, and orientation. In: Vitt L. J. & Caldwell J. P. (Eds): Herpetology: an introductory biology of amphibians and reptiles. San Diego: Academic Press, pp. 217–238.

Wielstra B., Litvinchuk S., Naumov B., Tzankov N. & Arntzen J. 2013. A revised taxonomy of crested newts in the *Triturus karelinii* group (Amphibia: Caudata: Salamandridae), with the description of a new species. Zootaxa 3682(3): 441–453.

Wielstra B., Sillero N., Vörös J. & Arntzen J. 2014. The distribution of the crested and marbled newt species (Amphibia: Salamandridae: Triturus) – an addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia–Reptilia 35: 376–381.

Zaffaroni N., Zavanella T. & Arias E. 1979. Peripheral blood cells in the crested newt after long-term exposure to the fungicide manganese ethylenebisdithiocarbamate (Maneb). Bulletin of environmental contamination and toxicology 23: 587–591.

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