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Research Article

Bufo spinosus in Tunisia: new data on occurrence, parasitism and tadpole morphology

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ABSTRACT – *Bufo spinosus* belongs to a successful Bufonidae lineage widespread in the western Palaearctic ecozone, reaching its southwestern limits in northern Africa. However in this latter region appears in fragmented populations, mainly restricted to mountain areas, and its biology is poorly known. Here we reviewed the scattered knowledge on *B. spinosus* in Tunisia, including new ecological and morphological data. We quantified the regional and local niche of the species in Tunisia and examined the interactions established with sympatric species, specifically focusing in predation and parasitism. Our data revealed that *B. spinosus* is mainly confined to the humid-subhumid broadleaved forests in the extreme north-western region, where it mainly breeds in streams and streams pools. In these montane habitats the common toad can occur in sympatry with *Amietophrynus mauritanicus* and *Bufotes boulengeri*, although *B. spinosus* is the most common species at higher elevations. In the area we found also two species of natricine snakes and a fresh-water crab, which could prey on the larvae and adults (*Natrix natrix* only) of the common toad. We also reported the first observation in the Maghreb of parasitism on *B. spinosus* by one species of Diptera (*Lucilia sericata*), and three Hirudinea species (*Batracobdella algira*, *Limnatis nilotica* and *Hirudo troctina*). Finally we described the morphology of African *B. spinosus* larvae based on Tunisian specimens.

INTRODUCTION

The common toad *Bufo bufo* complex is part of a group of amphibians with Palaearctic affinities (such as *Alytes maurus* and *Salamandra algira*; Escoriza et al., 2006; Márquez et al., 2011) which occur in relict populations in the mountain ranges of north-western Africa (from western Tunisia to the south of the Atlas chain in Morocco; Schleich et al., 1996). As a result of recent phylogeographical analysis of the *B. bufo* lineage (Litvinchuk et al., 2008; Recuero et al., 2012), all populations in the Maghreb, the Iberian Peninsula and parts of France, referred herein to *Bufo spinosus* (Recuero et al., 2012). The recognition at the species level of *B. spinosus* was based on morphological data, a deep genetic divergence and allozyme data analysis (Arntzen et al., 2013 a and b). The African and Iberian populations of *B. spinosus* showed a long isolated evolutionary history, as it is revealed by mtDNA and nDNA phylogenies (Recuero et al., 2012). Based on allozyme and mtDNA data, two subclades are recognized in North Africa (Recuero et al., 2012).

Bufo spinosus is likely a rare species in Tunisia, mainly confined to the mesic forests in the extreme north-western region (Ben Hassine & Nouira, 2012). This toad was recorded for the first time in Tunisia near Aïn Draham by Blanc in 1904 and was confirmed later by Gadeau de Kerville (1908) (Blanc, 1935). No additional sighting in Tunisia was reported until Schneider (1974), who recorded the species in the same region. Later, records at Aïn Draham were confirmed by Joger (2003), Brito et al. (2008), and Litvinchuk et al. (2008). Sicilia et al. (2009) reported the presence of *B. spinosus* at a single locality in an oak forest near the reservoir of Beni Mtir, at 631 m where an adult male specimen was found. This sighting was supported by additional records in Beni Mtir village by others authors (Ben Hassine & Nouira, 2012; Recuero et al., 2012; Bogaerts et al., 2013). Several new populations of *B.*

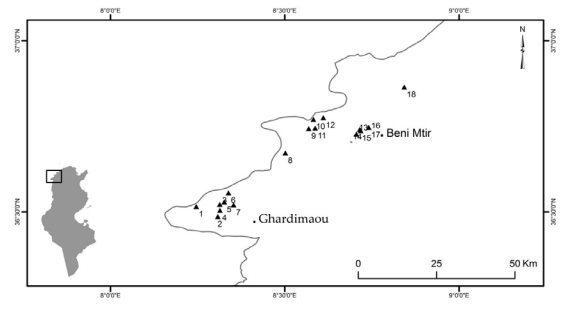

Figure 1. Distribution of *B. spinosus* in Tunisia.

spinosus at Feija National Park, Statir mountains and Aïn Soltane in Ghardimou (close to the Algerian border) were discovered between 2007 and 2009 (Ben Hassine & Nouira, 2012). However, many regions in the Khemir mountains (north-western Tunisia) are still not well explored and the distribution of *B. spinosus* in Tunisia may be underestimated.

In this study, we provided an up-to-date survey of the range of *B. spinosus* in Tunisia. First we examined its regional niche based on occurrence data and niche modeling. Then, we characterized the terrestrial habitats and breeding sites of the species by measuring several physical and chemical parameters of the aquatic habitats. We also report the first record of parasitism and predation on North African *B. spinosus* by one species of Diptera and three species of Hirudinea. Finally, we described for the first time the tadpole morphology of this species based on North African specimens.

MATERIAL AND METHODS

Study area and species

The study area comprised north-western Tunisia, region known as Kroumiria formed by the Mogods and Khemir mountains. As a part of a broader study on the ecology of amphibians (Ben Hassine, 2013), explorations in Kroumiria were carried out during two time periods; phase 1: 2007-2010 and phase 2: 2012-2013. Surveys were conducted from February to May, as previous investigation showed that during the winter-spring period there is an increased activity of all amphibian species in the Maghreb (Doumergue, 1901; Pasteur & Bons, 1959; Sicilia et al., 2009). *B. spinosus* was considered as present if any stage of its development could be identified (i.e., spawn, larvae, newly metamorphosed individuals or adults). Between 19:00 and 02:00 (local time), water bodies and their surroundings, roads and paths near potential breeding sites were explored to detect adults calling and migratory activity.

Competition, predation and parasitism are believed to be important elements structuring amphibian communities with implications for their population stability (Beebee, 1996; Raffel et al., 2010). In this sense, amphibians species occurring within the same localities of *B. spinosus* and potential predators of the common toad where recorded. All common toad specimens were examined *in situ* for ectoparasites and released in the same capture sites. The occurrence of every specimen was geo-referenced using a Garmin GPS navigator Dakota 100.

Regional niche analysis

The realized niche of *B. spinosus* was modeled using MaxEnt 3.3.3k (Phillips et al., 2006) in order to assess those environmental factors which better determine the distribution of this species in Tunisia and the possible existence of not prospected but suitable areas for this species. ENM was calibrated using 25% of the localities to test and 75% as training and including three bioclimatic variables, as follows: annual precipitation, mean temperature of warmest quarter (i.e., during a 3 months period) and mean temperature of coldest quarter, obtained from the WorldClim database (Hijmans et al., 2005). These variables describe the thermal extremes and environmental availability of water, which largely contributed to explain the distribution of other species of amphibians in temperate regions (Whittaker et al., 2007). The climate within the studied area was classified following Köppen-Geiger system (Peel et al., 2007) and the bioclimatic stages and climate variant classification of Emberger (1955).

Breeding and terrestrial habitat characterization

We characterized the breeding habitats where *B. spinosus* tadpole presence was detected. This characterization included variables describing the morphology of the water bodies (depth, cm and pond surface area, m²) and water physical and chemical parameters: temperature (ºC), dissolved oxygen (mg / L), pH, conductivity ($\mu S \cdot cm^{-1}$) and water flow ($m \cdot s^{-1}$). Average pond depth is the mean value of five successive

Figure 2. Different patterns in adults specimens of *B. spinosus* from Tunisia. A and B: Beni Mtir; C: Close to the fauna reserve of Dar Fatma; D-F: El Feija National Park. F: Photo Daniel Escoriza; A-E: Photos Jihène Ben Hassine.

measurements from the shore to the centre of the pond. Pond surface area was obtained by measuring the maximum length of the longitudinal axis and the length of the transversal axis, and assuming an elliptical shape for the water body. Chemical water parameters were measured using a Crison 524 conductivity meter (for conductivity), an EcoScan ph6 (for pH) and a Hach HQ10 Portable LDO meter (for dissolved oxygen). In the localities where the occurrence of *B. spinosus* was confirmed, local vegetation communities were described based on field observations and literature (Hoenisch et al., 1970; Stambouli-Essassi et al., 2007).

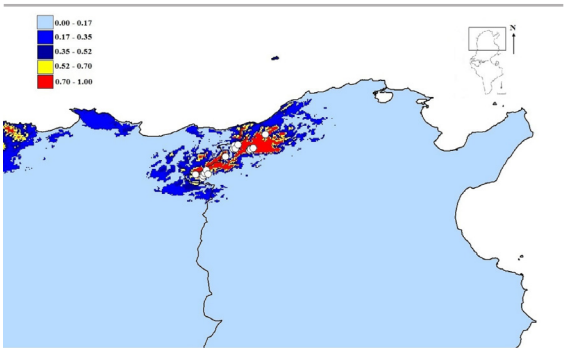

Figure 3. ENM obtained for *B. spinosus* based on three WorldClim layers (Annual precipitation; Mean temperature of warmest quarter; Mean temperature of coldest quarter). Suitability in predictive range from clear blue (unsuitable conditions) to red (highly suitable conditions). White circles represent the localities described on Table I.

Tadpole morphology

Tadpoles of *B. spinosus* were not previously described based on North African specimens since the description provided by Pasteur and Bons (1959) was based on European specimens, described by Boulenger (1898).

Different Gosner stages of *B. spinosus* were captured using a 250 µm mesh size net from two breeding sites: Beni Mtir and El Feija National Park. General tadpole morphology was examined under a stereomicroscope and Gosner stage between 27 and 39 were used for this study. The morphological description is based on traits which are not correlated to the tadpole length. Features of oral apparatus and labial Tooth Row Formula (LTRF) were described based on Altig and McDiarmid (1999).

RESULTS

***Bufo spinosus* distribution in Tunisia**

Our findings confirmed that the geographical distribution of *B. spinosus* in Tunisia is limited to the extreme north-west (Fig. 1 and 2; Table 1). We report for the first time occurrence of the common toad in the region between Hammam Bourguiba and Aïn Soltane (localities n° 8 to 12; Fig. 1 and 2). The area close to the fauna

Figure 4. Habitat of *B. spinosus* in Tunisia. A. Close to Dar Fatma (locality n°18); B. El Feija National Park (locality n°7); C. Beni Mtir (locality n°13); D. El Feija National Park (locality n°3). Photos Jihène Ben Hassine.

reserve of "Tourbière Dar Ftama" represents the easternmost known locality of the species range in Tunisia (locality n° 18; Fig. 1 and 2). The presence of *B. spinosus* in Beni Mtir (13 km south-eastern Aïn Draham) was confirmed. Thus the geographical range of *B. spinosus* in Tunsia is extended about 50 km to the south-west, to the region of Ghardimou, not far from the Algerian border (Fig. 1 and 2; Table 1). According to our sampling, *B. spinosus* achieves the maximum altitude for the species in Tunisia at El Ghorra (1062 m, locality N°5; Table 1).

The ENM had a high performance (AUC = 0.97) and revealed that potential suitable areas are mainly confined to Khemir mountains (Fig. 3). The variable annual precipitation had the highest contribution to the model (Table 2). In this sense the actual distribution of *B. spinosus* in Tunisia is strictly confined to the humid bioclimatic stage with temperate-to-cool winters climate variant characterised by high precipitation values (Table 2). It is likely that the annual precipitation is one of the factors which it acts constraining the presence of *B. spinosus* in Tunisia.

Bufo spinosus habitat

B. spinosus mainly inhabits humid montane forests (800-1100 m) composed by the deciduous oak *Quercus canariensis* associated with *Alnus glutinosa*. At lower altitudes (below 800 m), this species also occurs in mixed formations of *Q. canariensis*, *Q. suber* and *Q. coccifera* associated with a dense under storey of small trees and bushes such as *Arbutus unedo*, *Laurus nobilis*, *Erica arborea*, *Olea europea*, *Pistacia sp.*, *Myrtus sp.* and *Calycotome sp.* (Fig. 4 A and B).

The reproductive season of *B. spinosus* extends from the first week of March to the second week of April. The tadpoles of common toad were found in only three water bodies in the prospected area (Fig. 4 C and D; Table 2). Physical and chemical parameters of these breeding water bodies at Beni Mtir are summarized in Table 3, showing that *B. spinosus*

Locality number	Longitude°	Latitude °	Altitude (m)	*Amietophrynus mauritanicus*	*Bufotes boulengeri*	Bufonidae species richness
1	8.24	36.51	768	x	x	3
2	8.30	36.48	678	x		2
3	8.31	36.52	1039			1
4	8.31	36.50	758	x	x	3
5	8.32	36.52	1062			1
6	8.33	36.55	888	x		2
7	8.35	36.52	642	x	x	3
8	8.50	36.67	575			1
9	8.56	36.74	269	x		2
10	8.58	36.76	185			1
11	8.58	36.74	450			1
12	8.61	36.77	314	x		2
13	8.70	36.72	410	x		2
14	8.71	36.74	448			1
15	8.717	36.73	643	x	x	3
16	8.74	36.74	490		x	2
17	8.68	36.72	645			1
18	8.84	36.86	599	x	x	3

Table 1. *B. spinosus* distribution data in Tunisia and Bufonidae species occurring in sympatry.

breed in lotic (but with low flow waters) and lentic habitats, with very variable sizes and with relatively low values of water dissolved oxygen and conductivity, usually with no aquatic vegetation. Tadpole were found at the botton of the slow-moving section of rivers and streams at El Feija National Park and Beni Mtir (Fig. 4 C and D; Table 3).

Species interactions (predators and parasitism)

According to our findings, *B. spinosus* occurs in sympatry with five anuran species: *H. meridionalis*, *A. mauritanicus*, *B. boulengeri*, *Discoglossus pictus*, *Pelophylax saharicus*, and more rarely with *Pleurodeles nebulosus* (only at El Feija National Park and Aïn Soltane).

Several potential predators to eggs, tadpoles and adults of *B. spinosus* such as *N. natrix*, *N. maura* and the freshwater crab *Potamon algeriensis* were found in and around these breeding habitats (Fig. 5 C). We report the first records in North Africa of *B. spinosus* parasited by Diptera and Hirudinea species (Fig. 5 A and B). The Diptera was identified as *Lucilia sericata*. The eggs and the larvae of the common green bottle fly were found on the skin of injured common toads. Eggs were laid in wounds and hatched larvae started to feed on the host flesh (Fig. 5A). Some parasited specimens showed a lethargic behaviour.

Three species of predator and parasite

Climate variables	Mean	Minimum	Maximum	% contribution
AP	1013.6	704	1144	96.8
MTW	24.1	21.1	25.6	2.5
MTC	8.3	4.8	11.9	0.7

Table 2. Descriptive statistics of main predictor variables for *B. spinosus* localities in Tunisia. Abbreviations: AP: Annual precipitation (mm); MTW: Mean temperature of warmest quarter (°C); MTC: Mean temperature of coldest quarter (°C); % contribution: Relative contribution of the predictive variables to ENM (%).

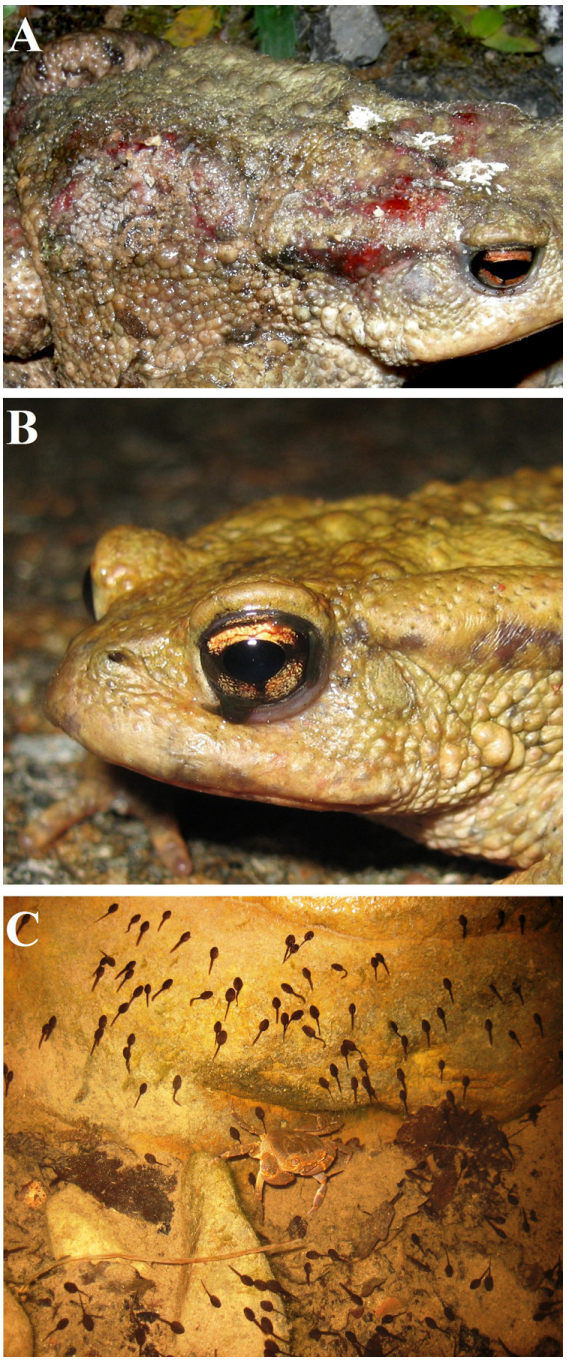

Figure 5. Parasitism and predation. Adult *B. spinosus* parasited by (A) *Lucilia sericata* (Meigen, 1826) and by (B) *Batracobdella algira* (Moquin-Tandon, 1846); C. *Potamon algeriensis*, an endemic crab predator of *B. spinosus* tadpoles (Beni Mtir, Tunisia). Photos Jihène Ben Hassine.

leeches were observed during our surveys. They were found around the eyes and on the limbs of the common toads. They were identified as *Batracobdella algira* (Moquin-Tandon, 1846) (Rhynchobdellida, Glossiphoniidae), *Limnatis nilotica* (Savigny, 1822) (Arhynchobdellida, Hirudinidae) and *Hirudo troctina* Johnson, 1816 (Arhynchobdellida, Hirudinidae) (Fig. 5 B).

Bufo spinosus tadpole morphology

A total of 210 tadpoles of *B. spinosus* from Feija National Park (n = 93) and Beni Mtir (n = 117) and between Gosner's stage between 27 and 39 were examined. These common toad larvae had a uniform dark coloration (brown to black), showing the tail a lighter colour and being broadly rounded at the end (Fig. 6). Tail fin ended at the muscular base of the tail, grey coloured and finely speckled with black (Fig. 6). Tail fins with or without black patches independently of ontogeny stages (Fig. 6 B, C and D). Eyes positioned dorsally on head (Fig. 6 B and C). Anus median. Spiraculum sinistral (Fig. 6 A).

In general, tadpoles remain small (up to 30 mm). Total length of the tadpole about once and a half the length of the tail. Tail length four times tail height. Dorsal tail fin and ventral tail fin are nearly equal in depth. Interorbital space nearly 1½ times as wide as the internarial space.

Oral apparatus features are shown in Figure 7. Oral apparatus is subterminally showing lateral emarginations with dorsal and ventral gaps. Labial teeth disposed in a single rows in each series. The labial tooth row formula (LTRF) is 2(2)/3[1].

Three uninterrupted lower dark tooth rows on the posterior labium (some specimens presented P1 interrupted by a very narow gap) while a pair of upper rows on the anterior labium. Second row of upper labium (A2) separated by a median gap. Only one tadpole of the total examined material showed exeptionaly uninterrupted second tooth rows in the upper labium (A2). We don't consider this specimen for the LTRF.

DISCUSSION

The data present in this paper together with previous studies (Schneider, 1974; Sicilia et al., 2009; Ben Hassine and Nouira, 2012) improve our knowledge about the range and ecology of *B. spinosus*, allow an up-to-date survey distribution, population's status, providing original data on parasitism and predation and tadpole morphology.

Regional niche

B. spinosus is a rare species in Tunisia with

Figure 6. *B. spinosus* tadpoles from Tunisia: Tadpole body morphology and coloration. A. Sinistral position of the spiraculum is highlighted by the arrow; B-D. Tadpoles (Gosner stages: 36-37 for B; 28 for C and D) showing tail fins grey finely speckled like powdered with black, with black patches (D) or without black patches (B and C) independently of ontogeny stages. Photos Jihène Ben Hassine.

assumed low population's densities in Maghreb (Schleich et al., 1996), being mainly restricted to the humid-subhumid oak forests of Kroumiria (Ben Hassine & Nouira, 2012). ENM indicated the existence of limited suitable habitats for *B. spinosus*, as expected for peripheral population in the edge of the genus range (Schleich et al., 1996).

The occurrence of *B. spinosus* in Tunisia appeared to be more limited by precipitations than temperatures and, is largely related to forested habitats, as described for other populations in southern Mediterranean areas (Romero & Real, 1996). In this sense its actual range comprised to the upper and lower wet humid climatic stages (Emberger, 1955) characterised by very high annual precipitation values (1500 to 2000 mm; Hoenisch et al., 1970; Stambouli-Essassi et al., 2007) and a short dry summer period (below three months, Hoenisch et al., 1970). In Tunisia *B. spinosus* occurs at lower altitude than reported from Algeria (~185-1062 in Tunisia vs 900-1200 m in Algeria) (Samraoui et al., 2012). These authors described the existence of clear altitudinal stratification between *A. mauritanicus* and *B. spinosus*. However, in Tunisia we failed to observe this pattern and there is almost a complete overlap in their altitudinal range in the Khemir mountains (Ben Hassine & Nouira, 2012).

Aquatic habitat selection, larval guild and morphology

Only three breeding habitats were identified during our survey. However, contrary to the described breeding habitats in north-eastern Algeria (one seasonal pond with *Ranunculus baudotii*; Samraoui et al., 2012) and in Morocco (two permanent and stagnant water bodies, relatively deep with aquatic vegetation; El Hamoumi et al., 2007), typical reproductive

	Type	Surface area (m^2)	Average depth (cm)	Max. depth (cm)	T (°C)	O2 (mg/L)	pH	Cond. ($\mu S \cdot cm^{-1}$)	Water flow (m/s)
Beni Mtir	Stream	340.21	26.2	37	15.1	5.92	7.6	234	0.04
	Stream pool	5.89	12.4	22	18	6.84	7.2	173.9	0
Feija	Stream pool	10	30	56	13	6.02	7.3	124	0

Table 3. Physical and chemical characteristics of the breeding sites of *B. spinosus* in Tunisia.

Figure 7. Oral apparatus morphology of *B. spinosus* tadpole from Tunisia. Photo Jihène Ben Hassine.

habitats in Tunisia were open streams and stream pools (with no aquatic vegetation), such was observed in other parts of the species range (Strijbosch, 1979; García-París et al., 2004; Malkmus, 2004). This toad is one of the few amphibians in Tunisia that can breed in lotic waters (Ben Hassine a&Nouira, 2012).

The morphological description of *B. spinosus* tadpole's native from Tunisia is nearly identical to that proposed by Boulenger (1898) and García-París et al. (2004), describing European common toad. However, these authors reported different tadpole's size along the species distribution in Europe. According to the description made by Boulenger (1896) based on tadpole of *B. bufo* from London, the interruption of second upper tooth series in this specie seems narrower than that observed in Tunisian *B. spinosus*, and the presence of black patches within the tail fin, of a narrow gap for P1 as well as it absence for A2 was also not described by Boulenger (1896; 1898).

Predation and parasitism

Predation and parasitism of *B. spinosus* populations by *Lucilia sericata*, *Batracobdella algira*, *Limnatis nilotica* and *Hirudo troctina* are described for the first time in North Africa. Infestation of amphibians by Diptera has been revised by Kraus (2007). While *L. sericata* was described previously to infest american bufonid species (Stewart & Foote, 1974), this is the first record of its occurrence as parasite of *B. spinosus*. This toad was known previously to be commonly parasited by *Lucilia bufonivora*. This diptera has lethal consequences in adult specimens, taking a heavy toll on many populations of amphibians in Europe (Strijbosch, 1980; Schleich et al., 1996; Gosá et al., 2009). We report the first case of *Limnatis nilotica* infesting amphibians in Maghreb. However, the parasitism by *Batracobdella algira* and *Hirudo troctina* on other Tunisian amphibians have been reported for *P. nebulosus* (Ben Hassine et al., 2013), *D. pictus* (Ben Hassine et al., 2011), *A. mauritanicus* and *P. saharicus* (Ben Ahmed et al., 2008; Ben Hassine et al., 2011).

Implications for species conservation

Kroumiria has the most humid conditions in Tunisia (Boughrara et al., 2007) and also shelter the highest amphibian diversity. In this relative small region appears all the populations of *B. spinosus* in Tunisia (Ben Hassine & Nouira, 2012). Kroumiria is suffering an intense deforestation (Zaimeche & Sutton, 1997; Rouchiche & Abid, 2003), and this fact together with the limited number of adequate breeding sites for *B. spinosus* could have a very negative effect on the relict populations of the common toad (Ben Hassine & Nouira, 2012).

These anthropogenic disturbances could possibly favor the penetration of more generalist species of toads, such as *A. mauritanicus* and *B. boulengeri*, more adapted to open habitats (Ben Hassine and Nouria, 2012). For this reason a complete understanding of the existence of negative interactions among Bufonid species may be necessary to assess the proximal causes that constrain the range of the common toad in Tunisia.

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HERPETOLOGICAL BULLETIN REPORT

January 2014

A total of 50 manuscripts were submitted to *Herpetological Bulletin* during 2013 with an additional 3 (not included in the table below) rejected without review as inappropriate for *Herpetological Bulletin*. This represents a steady stream of submissions but is down on submissions for 2012. There was also a slightly lower acceptance rate (68% versus 73%). We hope that the change to A4 for *Herpetological Bulletin*, which has better paper presentation and has generally been well received, will eventually increase submission rates and overall quality of manuscripts.

Following on from a review of herpetological activities at ARC, which appeared in issue 122, a review of herpetological activities at KRAG (Kent Reptile and Amphibian Group) appeared in issue 126. We will continue soliciting additional material of this kind for future issues.

The following people gave their time and expertise reviewing manuscripts for *Herpetological Bulletin* during 2014: Roger Avery; John Baker; Chris Barratt; Trevor Beebee; Daniel Bennett; Jon Coote; Carl Ernst; Chris Gleed-Owen; Stuart Graham; Rowland Griffin; James Hennessy; Rick Hodges; Adrian Hailey; Laurence Jarvis; Robert Jehle; Simon Maddock; Roger Meek; Christine Tilley; Wolfgang Wuster; John Wilkinson; Todd Lewis.

ROGER MEEK, ROGER AVERY

Editors

	Submitted	Accepted	Percent accepted
Full papers	17	12	70.1
Short Notes	6	5	83.3
Natural History Notes	27	17	62.9
Total	50	34	68.0