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**Front Cover:** Adult female big-eyed viper (*Trimeresurus macrops*) captured and observed later in the wild as part of spatial ecology, behaviour, and habitat selection study of green pit vipers at the Sakaerat Biosphere Reserve in Thailand. See article on page 19 for movement and spatial ecology research of *T. macrops* and *T. albolabris*. Photograph © Curt H. Barnes.

# Pre- and post winter hibernation ecology of the eastern box turtle, *Terrapene carolina carolina*

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**ABSTRACT** - Thermal ecology of the eastern box turtle (*Terrapene carolina carolina*) was studied during the cooler months of the year (September to April) at the Mason Neck National Wildlife Refuge, Fairfax County, Virginia, USA. The research enlisted individual male and female turtles tracked by radio telemetry. Observations were made on behaviour and thermoregulation in relation to their effect as the turtles entered into hibernacula, moved during the winter, and emerged in the spring. We found extensive above ground movements were common through mid- to late December and ground movement from hibernacula to others were more frequent than expected. Turtles emerged in late March and early April and, depending on the spring warm-up, the turtles remained close to hibernacula before undertaking characteristic spring movement and activity. The observed thermal characteristics of microhabitats appeared to affect, and could predict, varied behaviours and movements. While turtles in geographic areas where temperatures fall below freezing enter hibernacula during the winter, our study found this to be a generalisation. The temperature profiles of specific microhabitats suggest a relationship between amount, type and degree of activity. In northern Virginia, we found turtles to be significantly more active than expected at temperatures that would otherwise suggest less movement. They entered hibernacula later, moved (relocated) dependent on environmental ambient temperatures, and seemed to be subject to freezing during the winter and emerged earlier.

## INTRODUCTION

There are numerous studies on turtles of the genus *Terrapene*, but field research focused on their natural winter ecology and behaviour is generally lacking. Box turtles are a cold and freeze adapted (tolerant) reptile with observations indicating that they may remain active during winter time (see review in Ernst & Lovich, 2009). The focus of this study is to expand on current knowledge of the winter ecology and behaviour of *Terrapene carolina carolina*, the eastern box turtle, in northern Virginia during the cooler months of the year.

## MATERIALS AND METHODS

Observations of box turtles were made on average every three days, with greater frequency during late autumn and late winter/early spring, at a 30-ha site on an Atlantic coastal plain peninsula of the Potomac River at the Mason Neck National Wildlife Refuge, Fairfax County, VA (38° 67' N, 77° 10' W; ~25-35 m elevation). The site was restricted and closed to the general public. The peninsula's vegetation is composed primarily of mixed deciduous upland forest. The length of the peninsula is bisected by a gravel road. The study area included five different habitats: (A), An old farmstead consisting of mixed hardwoods, grass plots and a parking area near the terminal point of the peninsula; (B), a ~3-ha field undergoing succession with the transecting gravel road to the west and surrounded

on the other three sides by woods; (C), a ~2-ha freshwater pond fed by a brook to the west, surrounded by woods on two sides, the gravel road to the east, and a brook flowing eastward to a tidal marshland; (D), a ~5-ha tidal-freshwater marsh along the Potomac River to the south; and (E), an extensive mixed second and third growth woods separating the other four habitats.

Turtle observations were made from September to mid-April of 1993-94 and 1994-95. Ten turtles (5 male / 5 female) were selected for the study each year. Sex was determined by visual inspection of the turtle using several sexually dimorphic traits; carapace shape, plastron shape, hind foot claw shape, tail length, tail width, position of vent on tail and iris colour (Ernst & Lovich, 2009). Turtles were fitted with radio transmitters (AVM, Type 2B, weighing 25 g) to track their movements. The transmitters were affixed to the carapace with PC-7 epoxy resin between the 3rd and 4th pleural scutes so as not to interfere with movement or the opening and closing of the plastron. The antennae were threaded through the marginal scutes, held in place by adhesive silicon sealant at the cervical scute. The antennae sat erect approximately 4 cm above the turtle but could fold backward against the carapace if individuals went under debris or entered a shallow form (pallet) or deeper hibernaculum.

Temperatures at both form and hibernaculum were recorded with Taylor Minimum/Maximum thermometers accurate to 0.1 °C that were placed on the ground surface and below the leaf litter adjacent to the form or hibernaculum.

Other temperature measurements were made with an Omega 22H electronic thermometer accurate to 0.1 °C equipped with constantin-tipped thermocouples. This was used to record air temperature (AT, 5 cm above the form/hibernaculum), surface temperature (ST, atop the leaf litter), and litter temperature (LT, below the leaf litter at the ground surface). It was also used to measure temperatures at the shallowest and deepest points of the hibernaculum based on the anterior and posterior positions of the turtle. Turtles were briefly removed from the hibernaculum to measure cloacal temperatures (CT) with a Schultheis cloacal thermometer accurate to 0.1 °C. Hibernaculum depth was measured at its shallowest (HDS) and deepest (HDD) points as determined by the anterior and posterior positions of the turtle. A 30 cm ruler, graduated in mm, was extended downward from directly above the hibernaculum to measure the distance between HDS/HDD and the ground surface.

## RESULTS

Before hibernacula entry, extensive above ground movements at Mason Neck were observed as late as 30 December (1994). The majority of observations were of turtle forms, but because individuals were tracked by radio telemetry, locations could be marked and straight line distances and direction between vacated and occupied forms determined. The mean monthly distances travelled by all turtles between observations, and mean monthly CT, AT, ST, LT and form temperatures, are presented in Table 1. A Spearman Rank Correlation test (Spearman's Rho) showed that all comparative temperatures were significantly correlated ( $p < 0.05$ ) and linear in their relationship. Thus, the overall influence of month on temperature and movement was analysed using a multivariate analysis of variance (MANOVA) with month as the independent variable and CT, AT, ST, LT and distance moved as dependent variables. The results indicated that, in addition to the trend noted above, monthly temperatures and distance moved were significantly different (Wilks' Lambda  $F_{15,456} = 19.382$ ,  $p < 0.0001$ ).

When box turtles finally entered hibernacula at Mason Neck, they favoured wooded areas with ample litter/detritus cover, downed trees, brush and logs, thick bushes (especially *Smilax* sp.) and rotting tree stumps. Of sixteen turtles that were tracked into hibernacula during this study, twelve (75%) selected this type of microhabitat. Two selected hibernacula on the ecotone between woods and an old field; one selected an open, less dense, wooded area; and another selected a depression that was either moist or water-filled. No hibernaculum site fidelity or multiple occupants of hibernacula were observed at Mason Neck, but multiple occupancy by *T. c. carolina* has been observed in Pennsylvania by Ernst (unpublished). Eight individuals included in the first year of this study, but not in the second year, could not be found using the probing method reported by Carpenter (1955) after searching a 314 m<sup>2</sup> area around their previous overwintering sites.

Of the sixteen turtles that entered hibernacula, five relocated. Eight did not move until they emerged in the late winter/early spring. The remaining three were removed

**Table 1.** Mean and range of monthly displacement (m) by *T. c. carolina* and mean monthly temperatures (°C)

	September	October	November	December
Mean displacement (m) (n)	50.61 (27)	34.28 (47)	23.95 (53)	12.68 (48)
Range	0 - 192	1.5 - 120	0.50 - 140	0.30 - 70
<b>Mean Temperatures</b>				
Air	18.48	13.64	11.70	6.83
Range	12.4 - 27.0	6.5 - 22.6	1.3 - 25.1	-3.8 - 13.20
Surface	18.30	13.76	11.57	7.24
Range	12.3 - 26.2	6.9 - 21.1	1.6 - 26.9	0 - 15.4
Litter	18.63	13.91	11.07	6.97
Range	13.8 - 24.8	9.0 - 19.7	5.4 - 21.1	1.5 - 14.3
Cloacal	17.99	13.47	11.57	7.45
Range	12.2 - 24.8	8.4 - 20.4	3.6 - 26.6	2.4 - 11.6
Form - Top	N.A.	N.A.	10.13	7.69
Range			6.4 - 14.5	3.4 - 12.0
Form - Bottom	N.A.	N.A.	10.22	7.49
Range			5.8 - 14.5	3.7 - 12.6

**Table 2.** Turtles relocating to different hibernacula during the hibernation period-initial dates of entry into hibernacula, dates of relocation, time elapsed between relocations, displacement differences between hibernacula, and final emergence date

Turtle ID	Date entered into Hibernaculum	Date Relocated	Elapsed Days (n)	Displacement (m)	Date Emerged
1044	10 December	13 March	93	15.0	4 April
1073	7 December	6 March	88	10.2	11 April
		15 March	97	22.0	
		19 March	101	18.5	
		3 April	116	0.45	
332A	18 December	6 March	77	12.3	13 March
4000	20 December	26 February	68	3.1	26 March
557	18 December	13 March	85	18.0	25 March

**Table 3.** Mean monthly hibernaculum depth (mm) at shallowest (HDS) and deepest (HDD) points

Month	HDS	Range	HDD	Range
December	62.18 ± 7.18	7 - 100	83.57 ± 6.25	25 - 150
January	67.62 ± 7.73	15 - 97	105.08 ± 7.57	36 - 187.5
February	74.82 ± 5.99	45 - 100	89.55 ± 7.58	36 - 187.5
March	61.49 ± 6.43	20 - 105	69.08 ± 4.56	20 - 140
April	38.31 ± 9.23	20 - 60	59.12 ± 5.96	30 - 80

from the research site before they emerged, because radio transmitter batteries had failed. Of the five that relocated, four relocated once and one moved three times. These relocating turtles all travelled similar distances and did not choose different hibernacula. Table 2 shows the dates of relocation and distance moved. The mean time elapsed between entry into a hibernaculum and the first relocation was 82.2 days and mean straight-line distance moved was 11.72 m (3.1 to 18 m).

Internally, hibernacula were slightly enlarged cavities, excavated by horizontal and/or vertical movement. On several occasions, up to 3 cm of lateral clearance was found between an individual and the side of the hibernaculum. This seemed to be related to soil temperatures because during colder periods (i.e., when temperatures near the surface were close to freezing) individuals were found snugly ensconced. A reduction in contact with air that might be slightly colder than the surrounding soil probably elicited such a change. Additionally, on several occasions, the top of the carapace was noted to be at or near the ground level, covered only by leaf litter or a thin layer of coarsely decomposed organic matter below leaf litter.

Tunnels excavated by turtles were found in hibernacula on 102 of 294 observations (34.7%) but not noted consistently or with any regularity. When a tunnel was present, the direction the turtle faced was mainly inward away from the opening surface of the hibernaculum (88 observations; 86.3%) rather than outward (14 observations; 13.7%). Above the hibernaculum, the depth of the leaf litter cover varied by individual location. The mean litter depth for all hibernacula was  $75.53 \pm 2.41$  mm (30-120 mm). The mean difference between minimum and maximum litter/surface temperatures was calculated as minimum litter temperature  $3.10 \pm 0.39$  °C warmer than minimum surface temperature and maximum litter temperature  $5.11 \pm 0.64$  °C colder than maximum surface temperature.

Hibernacula were confined to the top soil horizon which extends downward to a depth greater than 100 mm at Mason Neck. At no time did hibernacula extend into the A Soil horizon (mineral layer). The mean maximum depth of hibernacula at Mason Neck was determined to be  $85.47 \pm 3.57$  mm (20-187.5 mm) but monthly changes in hibernacula depth were observed (Table 3).

The mean carapace height of turtles in this study was  $60.97 \pm 2.34$  mm, so at various times over the course of the winter some part of the carapace was either at or slightly below the ground surface and covered only by leaf litter. Minimum litter temperatures in both January and February fell to -7.0 °C and -6.0 °C, respectively, which indicates that on a number of occasions part of the carapace was subject to freezing, and several times ice was observed on the posterior and apex of the carapace and/or leaf litter was frozen to the carapace, indicating that at least part of the turtle's surface was frozen. The mean duration of hibernation was  $103.46 \pm 10.31$  days (77 - 135 days). *T. carolina* can tolerate ice penetration throughout the body cavity and ice contents that can reach equilibrium values of more than 50% of total body water (Storey and Storey, 2004).

DeGregorio et al. (2017) reported that South Carolina *T. carolina* did not emerge from dormancy until the 5-day mean surface temperatures measured at hibernacula reached about 5 °C. Following emergence, our turtles entered an inactive period, here defined as movement and behaviour only undertaken within a 10 m radius of the hibernaculum (an area of 314 m<sup>2</sup>). In the turtles for which post-emergence data were recorded, emergence occurred between 6 March and 11 April (Table 4) and the number of days spent undertaking activity near the hibernacula varied considerably. During this time, the mean straight-line

**Table 4.** Dates of entry into and emergence from hibernacula

Turtle ID	Date of Entry	Date of Emergence	Length of Hibernation
♀			
107	26 November	11 April	135 days
332A	18 December	13 March	85 days
1081	30 December	18 March	77 days
136	18 December	6 March	78 days
303	28 November	26 March	117 days
4000	20 December	26 March	96 days
♂			
356	10 December	25 March	104 days
92	29 November	5 April	126 days
1044	10 December	4 April	114 days
1073	7 December	3 April	116 days
54	26 November	23 March	116 days
557	18 December	25 March	97 days
59	27 December	21 March	83 days

**Table 5.** Dates of emergence from, and movement away from, hibernaculum

Date Emerged	Turtle ID	Date Moved Away From Hibernaculum Site	Number of Days Close to Hibernaculum
4 April	1044	14 April	10
11 April	107	13 April	2
3 April	1073	4 April	1
6 March	136	12 April	37
13 March	332	7 April	25
25 March	557	11 April	15
25 March	350	5 April	13

**Table 6.** Numbers of *T. c. carolina* and frequency of their behaviours in sick and healthy individuals

	Basking	Thermal Regulating Under Leaf Litter	Form	Walking	Total
Healthy	20	21	41	1	83
%	(18.18)	(19.09)	(37.27)	(0.91)	(75.45)
Sick	16	9	1	1	27
%	(14.55)	(8.18)	(0.9)	(0.91)	(24.65)
Total	36	30	42	2	110
%	(32.73)	(27.27)	(38.18)	(1.82)	(100.00)

distance moved between observations (excluding no movement or zero distance measurements) was  $0.81 \text{ m} \pm 0.39 \text{ m}$  ( $n = 24$ ). Observed behaviours were walking (1), occupying form (23), basking (13) and behavioural thermal regulating under leaf litter (22).

Behavioural thermal regulation was a common activity after emergence, particularly under leaf litter, which has been unreported. This was characterised by an individual being located under a sparse covering of dead leaves (less than 10 mm in thickness), in full sunlight but with its head and/or limbs extended. Furthermore, it is considered herein

a modified form of thermal regulation because CT under leaf litter (*mean* = 15.69 °C) was significantly higher than CT for form (*mean* = 10.25 °C) (Unpaired t-test, *t* = 3.389, *df* = 40, *p* = 0.0016, *n* = 42), but was not significantly different from CT for traditional basking in sunlight (*mean* = 18.67) (Unpaired t-test, *t* = 1.654, *df* = 32, *p* = 0.1080, *n* = 34).

In all instances, either type of thermal regulation was within 64 mm of the form and on few occasions were individuals directly atop leaf litter or bare ground and exposed to full sun. Instead, in most observations turtles were encountered basking in the sun with the anterior portion of the carapace hidden under leaf litter, or in a tunnel of leaf litter, and the posterior end exposed to the sun. Physical evidence showed that individuals had backed out of their forms (which during this time of the year were often slight depressions covered only by leaf litter) to achieve this position. It was also noted, although less regularly, that on occasion turtles turned around in forms and basked with their heads facing outward.

Table 5 indicates that those individuals that emerged early in March spent more time near their hibernaculum than those that emerged later in April, and all individuals became active between 4-14 April. Once *T. c. carolina* were active at Mason Neck, straight-line distances travelled averaged  $27.8 \pm 6.80$  m (0.2 - 87 m; *n* = 43) between observations. Behaviours noted were basking (23), walking (11), form (19) and thermal regulating under leaf litter (8).

No turtles were found dead within hibernacula, but three turtles were found to have respiratory infections as evidenced by a yellow discharge emanating from the nares (Boucher & Ernst, 2004). The behaviours of sick and healthy turtles, along with the number of observations are shown in Table 6. A Chi-square test of independence on these data indicated that behaviour was not independent of health ( $\chi^2 = 20.019$ , *df* = 2, *p* = 0.0002). Basking was observed much more often in sick turtles.

## DISCUSSION

In the autumn, activity varied among individuals. Numerous observations (see review by Ernst & Lovich, 2009) indicate that *T. carolina* are often active during autumn, regardless of geographic location (Oklahoma: Carpenter, 1957; Ohio: Claussen et al., 1991; South Carolina: Congdon et al., 1989; Gatten, 1987; Tennessee: Dolbeer, 1971; New York: Madden, 1975), and turtles observed at Mason Neck were no exception. Their activity, however, was observed extending into late autumn and early winter, which has previously been rarely recorded. Hibernation by *T. carolina* has been reported to begin between mid-October and mid-November in Ohio (Claussen et al., 1991), by mid-November in Oklahoma (Carpenter, 1957), by late November in Illinois (Cahn, 1933), Indiana (Currylow et al., 2013), eastern Tennessee (Dolbeer, 1971), and from late October to late November in Maryland (Savva et al., 2010). The majority of box turtles studied at Mason Neck entered hibernacula in mid- to late December. The later hibernation dates in this study could possibly be explained

by thermal protection caused by the study site's proximity to the warmer Potomac River (land heats and cools faster than water). Biases in some of the previous studies may have been caused by keeping box turtles in enclosures, or by annual variations of entry into hibernacula caused by fluctuating temperature and the duration and severity of environmental conditions (Allard, 1935; Brisbin, 1972; Cahn, 1937; Carpenter, 1957; Claussen et al., 1991; Dolbeer, 1971; Doroff & Keith, 1990; Penn & Potthast, 1940; Stickel, 1950).

Box turtles relocation of hibernacula at Mason Neck did not appear to be nearly as common as reported by Carpenter (1957), who reported that the average length of time between entry into a hibernaculum and relocations for *T. c. triunguis* in Oklahoma was 63.4 days and mean distance moved was 49.4 m (< 1 to 286.5 m). The apparent longer period between relocation reported for the Oklahoma box turtles may indicate a significant difference in their thermal ecology compared to those in northern Virginia. Carpenter (1957) also observed that *T. c. triunguis* were in their hibernacula by mid-November and this indicates, using the 63.4 day mean time (1-149 days) between relocation, that individuals moved hibernacula sometime between mid-to late January and early February. Hibernacula entered this early, once the extreme low temperatures of winter set in, might not offer enough protection and individuals may possibly be forced to relocate. Thus, the stimuli for relocation could be that a hibernaculum was too cold. At Mason Neck, because *T. c. carolina* enter hibernacula later in the year, they appear to select more sheltered locations which exhibit thermal stability and protection. This assumption would explain why fewer individuals were observed relocating hibernacula than expected. Table 5 shows that relocations occurred between 26 February and 13 March, on average 23.4 days before spring emergence. Relocation so late in the winter and so close to emergence suggests that individuals were actually emerging but found ATs still too cold and then returned to hibernacula until ATs moderated.

The mean maximum depth of hibernacula for *T. c. carolina* at Mason Neck agrees with the range of 20-100 mm reported for *Terrepene* in Oklahoma, Ohio, Missouri, New York and Tennessee (Carpenter, 1957; Claussen et al., 1991; Dolbeer, 1971; Grobman, 1990; Madden, 1975). It is substantially deeper than the 0-50 mm reported by Congdon et al. (1989), but this was noted in a South Carolina population subject to less severe winter temperatures. Currylow et al. (2013) reported an average depth of 100 mm in Indiana *T. carolina*, but reached 300 mm.

Hibernation at Mason Neck is shorter than at other locations. Studies on the duration of hibernation in *Terrapene carolina* report that it ranges from 141 to 216 days depending on taxon, geographic location and weather conditions (Claussen et al., 1991, in Ohio *T. c. carolina* - 142 days; Currylow et al., 2013, in Indiana *T. c. carolina* - 140 days [including emergence]; Madden, 1975, in New York *T. c. carolina* - 141 days; Stickel, 1950, in Maryland *T. c. carolina* - 168 days; Schwartz & Schwartz, 1974, in Missouri *T. c. triunguis* - 177 days; Doroff & Keith, 1990, in Wisconsin *T. ornata* - 216 days; Legler, 1960, in Kansas

*T. ornata* - 165 days). The dates of emergence at Mason Neck range between March and April and agree with the time of emergence reported elsewhere for *T. c. carolina* (Claussen et al., 1991; Currylow et al., 2013; Grobman, 1990; Madden, 1975; Stickel, 1950), so the short period of hibernation is a function of the late entry into hibernacula at Mason Neck.

Since basking was observed significantly more often in sick turtles than healthy turtles, they appeared to be simulating a pyrogenic response (sensu Monagas & Gatten, 1983).

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# Amphibians of Algeria: New data on the occurrence and natural history

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**ABSTRACT** - Algeria is a country from the Maghreb with a little known batrachofauna. In order to improve knowledge of the distribution of amphibians in this country we carried out several surveys in northern Algeria between 2010 and 2017. Maps with original data on the distribution ranges and niche model for every species have been made for the first time. This includes original data on breeding phenology, breeding habitat and terrestrial habitat features of the observed species. Our data indicated that several species could be more widespread in Algeria than previously suggested. The apparent discontinuity of their ranges and the supposed rarity of some species such as *Pleurodeles nebulosus*, *Salamandra algira algira* and *Bufo spinosus* is likely due to a lack of previous survey effort. Some species, such as *Discoglossus pictus*, *Pelophylax saharicus*, *Hyla meridionalis* and *Sclerophrys mauritanica* are widely distributed and abundant in the studied region. Our results confirmed the presence of several species in historical sites, but also the presence of numerous new populations. Some historical records of *P. nebulosus*, *S. algira algira* and *H. meridionalis* were not confirmed. This could be due to a possible recent extinction of marginal populations, but also to errors in the classical literature, since some of these localities are likely to be outside the limits of the environmental tolerance of these species.

## INTRODUCTION

Algeria has a key location as a biogeographical contact area between the Maghreb and Europe, where several amphibians' species are endemic or show a restricted distribution. However, Algeria is one of the less studied countries on the Maghreb in terms of herpetology (Pasteur & Bons, 1959; Bons & Geniez, 1996; Schleich et al., 1996; Ben Hassine & Nouira, 2009; 2012; Ben Hassine et al., 2013; Beukema et al., 2013). Preliminary inventories of the Algerian herpetofauna appeared during the late 18th century and mainly comprised compilations of collected specimens, providing zoological studies, species descriptions and anecdotic data on their natural history (Poiret, 1789; Rozet, 1833; Gervais, 1835; 1836; 1844; Boulenger, 1891; Doumergue, 1901; Llabador, 1947). However, at the present time the available data on the status and the ecology of Algerian amphibians are still scarce, except for some groups such as Salamandridae [see Escoriza & Ben Hassine (2015); Ben Hassine et al. (2016 a, b); Escoriza et al. (2016)] and some regions such as Oranie (Doumergue, 1901) and Numidia (Samraoui et al., 2012).

There is increasing concern about habitat loss in Algeria, particularly affecting critical habitats for amphibians, for example forest and wetlands (Zaimeche, 1994 a, b; Samraoui et al., 2011; Samraoui et al., 2012). In this context, increasing our knowledge of amphibian ecology is crucially needed to implement successful conservation strategies (Stuart et al., 2008). In this paper the scattered and sparse information on Algerian amphibians is summarised, reviewed along with additional new data to improve our understanding of their ecology and distribution. This will improve the database

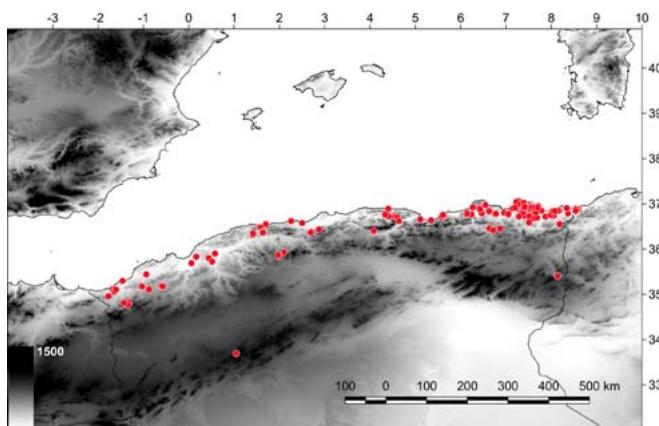
on the distributional records in Algeria. Additionally, we have produced distributional maps and analysed how the climatic factors could influence the potential distribution of the studied species using Maxent models. Our data were then compared with those available in literature. Finally we describe new data on the breeding phenology and aquatic and terrestrial habitat features.

## MATERIAL AND METHODS

### Study area and sampling

The study area was the north of Algeria, encompassing the region between the Tunisian and the Moroccan borders (Fig. 1). The region shows an important climatic contrast between the coastal regions, with a relatively humid Mediterranean climate (*Csa* type, Köppen classification; Peel et al., 2007); particularly in the extreme north-east, with rainfall values of 923 mm  $y^{-1}$ , at El Collo Massif), and the inland regions that become progressively more arid. At Batna, 170 km from the coast of El Collo Massif, rainfall values drop to 329 mm  $y^{-1}$ , typical values of steppic climates (*Bsk* type; Peel et al., 2007).

Several surveys were conducted as a part of a broader study on the ecology and phylogeny of the north-African amphibians (Escoriza et al., 2014; 2016; Escoriza & Ben Hassine, 2015; Ben Hassine et al., 2016 a, b). These surveys were carried out over an eight year period (2010–2017), mainly from February to August (with punctual prospections between October and December). Previous surveys showed that this period covered most of the breeding activity of Maghrebian amphibians (Doumergue, 1901; Schleich et al., 1996; Ben Hassine & Nouira, 2012; Escoriza & Ben



**Figure 1.** Topographical overview map of the study area indicating the amphibians observations sites in Algeria included in Appendix 1. Darker colours indicate higher altitude.

Hassine, 2014). Sampling was performed in aquatic and surrounding terrestrial habitats and included visual and acoustic detection and dip-netting, the latter with the purpose to assess the presence of eggs and larvae (Wilkinson, 2015). In addition, we also included some records using road-kills, when identification was possible. The coordinates of each locality were collected in situ with a Global Positioning System (Garmin Dakota 100; Garmin Ltd., Olathe, KS, USA).

### Breeding habitat characterisation

We surveyed temporary ponds, permanent ponds, springs and stream pools using dip netting. Habitat characterisation comprised two assemblies of variables, one describing the characteristics of the water bodies: average depth (cm) and surface area ( $m^2$ ), and other water physical and chemical parameters: temperature ( $^{\circ}C$ ), dissolved oxygen ( $mg\cdot L^{-1}$ ), pH, and conductivity ( $\mu S\cdot cm^{-1}$ ). Average depth was taken as the mean value of five successive measurements from the shore to the centre. The surface area was estimated using a Garmin Dakota 100. Turbidity was assessed using a scale ranging from 1 (transparent waters/10 Nephelometric Turbidity Units; NTU) to 3 (very turbid waters/300 NTU) based on light extinction estimated at Secchi disk depth. The percent of emergent vegetation covering the pond surface was estimated from photographs. The physical and chemical parameters of the aquatic habitats were measured between 12:00 hrs and 15:00 hrs (local time) to maintain homogeneity in the measurements.

### Ecological niche models

We were also interested in evaluating the potential distribution of the target species, based on proxies of their fundamental niches (Pearson et al., 2007). These models were performed using four variables, one topographic (altitude) and three climatic (mean annual precipitation, temperature seasonality and mean annual temperature; Hijmans et al., 2005), which are relevant to explain the occurrence of amphibians in the region (Ben Hassine et al., 2016 b). The ecological niche models were performed with Maxent 3.4.1 (Philips et al., 2017), using the default settings and 25% of the localities as random tests. In order

to provide robustness to the models, we also included presence records of the same species of amphibians in Tunisia from Ben Hassine & Nouira (2012). The projections showed the minimum training presence threshold and 10th percentile training presence thresholds (Pearson et al., 2007).

## RESULTS AND DISCUSSION

We compiled 306 new distribution records from 162 localities of eight amphibian species from a total of 12 known species occurring in Algeria according to Schleich et al. (1996) (Appendix 1, Figs. 1 and 2). These include 87 breeding sites (Tables 1 and 2; Fig. 3) belonging to two species of urodela [*Pleurodeles nebulosus* (Guichenot, 1850) and *Salamandra algira algira* Bedriaga, 1883] and six anurans [*Discoglossus pictus* Otth, 1837, *Pelophylax saharicus* (Boulenger, 1913), *Hyla meridionalis* Boettger, 1874, *Sclerophrys mauritanica* (Schlegel, 1841), *Bufo spinosus* Daudin, 1803 and *Bufo boulengeri* (Lataste, 1879)]. Specific richness per site varies between 1 species (43% of the sampled localities) and up to 5 species (at one site) (Appendix 1).

Mateo et al. (2013) reported on a finding of *Barbarophryne brongersmai* (Hoogmoed, 1972) caught by Jesús Peña in 1990 in four sites in the Saharan Atlas of north-western Algeria. Because of its cryptic nature and the punctual distribution, this species was not found during our prospections. *Hoplobatrachus occipitalis* (Günther, 1858) and *Sclerophrys xeros* (Tandy, Tandy, Keith & Duff-MacKay, 1976) are present in southern Algeria in the mountains of the Tassili and the Hoggar (Cox et al., 2006) and were not treated in this paper. *Alytes maurus* Pasteur & Bons, 1962 is allegedly cited to occur in western Algeria (Mateo et al., 2013). However data on the ecology, distribution, larvae description and genetic assessment of the micro endemic *Pleurodeles poireti* (Gervais, 1835) could be consulted in recent published papers by Escoriza & Ben Hassine (2015), Ben Hassine et al. (2016 b), Escoriza et al. (2016) and Escoriza & Ben Hassine (2017a).

### Ecological niche models

The projections of the ecological model were robust (AUC, mean = 0.93, range 0.87-0.97; Table 3) and showed that amphibians responded divergently to the environmental gradient. The occurrence of *S. algira algira* and *B. spinosus*, both Palaearctic relicts, was highly influenced by precipitation, whereas the influence of this variable was lower in other species as *B. boulengeri* and *P. saharicus* (Table 3). These models also suggested that the real distribution of these species could be largely underestimated.

### Species accounts

#### North African fire salamander

##### *Salamandra algira algira* Bedriaga, 1883

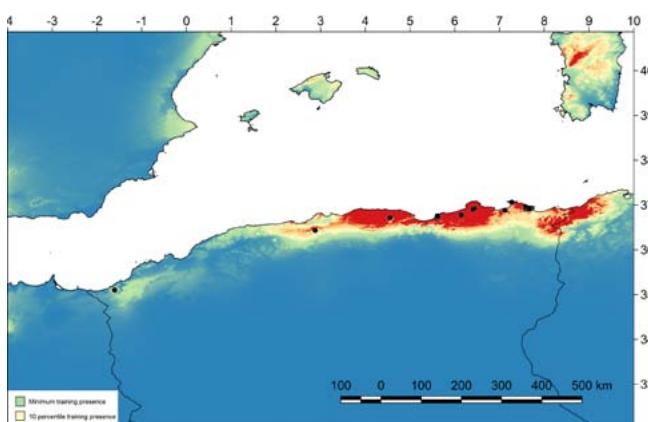
After the initial Bedriaga's description (1883) of the North African fire salamander based on specimens from Edough Peninsula (north-eastern Algeria), data on the distribution and ecology of the Algerian populations of *S. algira*



**Figure 2.** Adults specimens of the eight studied amphibians species from Algeria. **A-** and **B-** Two different patterns of *S. algira algira* from the population from Seraidi (Province of Annaba); **C-** *P. nebulosus* (Province of Ain Defla). **D-** *D. pictus* (Province of Skikda); **E-** *S. mauritanica* (Province of Skikda); **F-** *S. mauritanica* (Province of Tlemcen); **G-** *B. spinosus* (Kabylia); **H-** *B. boulengeri* (Province of Annaba); **I-** *H. meridionalis* (Province of Annaba); **J-** *H. meridionalis* (Province of Skikda); **K-** *P. saharicus* (Province of Skikda); **L-** *P. saharicus* (Province of Tlemcen). Photos A - K: Jihène Ben Hassine; Photo L: Daniel Escoriza.



**Figure 3.** Breeding habitats of amphibians in northern Algeria. **A-** Breeding habitat of *S. algira algira* in Kabylia; **B-** Breeding habitat of *S. algira algira*, *D. pictus*, *H. meridionalis* and *P. poireti* in Edough Peninsula; **C-** Breeding habitat of *B. boulengeri* in Tacheta Zoughagha; **D-** Breeding habitat of *P. nebulosus* and *D. pictus* in Chlef; **E-** Breeding habitat of *P. nebulosus* in Theniet el Had (rock pool); **F-** Breeding habitat of *P. nebulosus*, *H. meridionalis*, *D. pictus*, *S. mauritanica* and *P. saharicus* in Theniet El Had (Cedar forest); **G-** Breeding habitat of *P. nebulosus*, *H. meridionalis*, *P. saharicus* and *S. mauritanica* in Bouchtata; **H-** Breeding habitat of *H. meridionalis* and *S. mauritanica* in Hounaine; **I-** Breeding habitat of *S. mauritanica* and *D. pictus* in Edough Peninsula. Photo C: Daniel Escoriza; Photos A - B; D - I Jihène Ben Hassine.



**Figure 4.** Niche model and distribution records of *S. algira algira* in Algeria. Warmer colours indicate higher climate suitability. Red: 10th percentile training presence thresholds; Yellow: minimum training presence thresholds; Blue: Null predicted suitability.

*algira* remain scarce (Samraoui et al., 2012; Escoriza & Ben Hassine, 2014). Recently, Ben Hassine et al. (2016 a) provided new data on the patterns of genetic, ecological variation, distribution records and coloration patterns of the Algerian populations.

Classified as Vulnerable by IUCN, *S. algira algira*, (as part of *S. algira*; IUCN, 2017) is endemic to Algeria, and present in a continuous range between Edough Peninsula and Blida Atlas (Escoriza & Ben Hassine, 2014; Ben Hassine et al., 2016 a; Fig. 4). This species also appears in a small region of the north-western part of Algeria, close to the Moroccan border where it was previously signaled in the 19th century (Guichenot, 1850; Doumergue, 1901). Here we confirmed its presence in this western part of Algeria in the region of Beni Menir (Fig. 4) where we found larvae in 2011. In the extreme eastern part of the species distribution, we confirmed the presence of *S. algira algira* in Bône (actually “Annaba”) (Ben Hassine et al., 2016 a). Moreover we extended its known range with the discovery of several new populations in Edough Peninsula and its surroundings between Cap de Fer, Aïn Barbar and Oued l’Aneb (Fig. 4). The North African fire salamander was recently discovered at El Collo Massif in Skikda (Escoriza & Ben Hassine, 2014). Barkat (2014) reported its presence in Sétif (at Djebel Ouled Massoud). However, the occurrence of this species in some historical sites, such as Constantine, Laarba (Guichenot, 1850) and Alger (Boulenger, 1882; 1891) remain to be confirmed. In the wet forests of Edough Peninsula, Kabylies and Blida Atlas, this salamander has a continuous distribution area including a large part of the coastal mountainous areas [Edough, Collo, Jijel, Béjaia, Tikjda, Tizi n’Kouilal, Iboudraren Darna, Tala-Guilef, Aghribi, Tiz-Ouzou, Djurdjura, Haïser, Afkadou, Fort National (actually “Larbaa-Nath-Irathen”)]. It was previously reported in some of these localities (Guichenot, 1850; Boulenger, 1891; Doumergue, 1901; Seurat, 1930; Bons, 1972; Bouali & Oneimi, 2005; Ben Hassine et al., 2016a).

The niche model on Fig. 4 shows a proxy of the fundamental niche of *S. algira algira* in Algeria. The model indicates that the distribution in Algeria is possibly

well known and structured in two geographical nuclei, separated by the Oran semi-arid depression. Following this model, its presence in El Kala, the Tunisian border and in the north-east of Tunisia could be possible, although we could not confirm this (Ben Hassine & Nouira, 2012).

Recently, the combination of colour pattern of *S. algira algira* was described by Ben Hassine et al. (2016a). However, intra population variation is quite common on the Algerian populations where specimens from the same localities may show different patterns of coloration across its distribution. For example, *S. algira algira* from Seraidi (Edough Peninsula, north-eastern Algeria) may have red discoloration in different parts of the body (parotids, head, tail, members...). Irregularly shaped tiny whitish specks may also be present on the gular, members, toes, and ventral regions. White rounded spots located asymmetrically on both sides of the body may be present or totally absent (the number varies between 0 and 12 white spots). These spots may be mixed or not with pronounced red coloration (Fig. 2 A and B). To the lateral white spots could be also associated lateral yellow spots. These patterns of coloration appear all over *S. algira algira* distribution in Algeria.

Our data and those of previous publications indicate that this salamander may be a common species in Edough Peninsula and Kabylies. *Salamandra algira algira* was found at altitudes between 20 m and 1280 m above sea level, inhabit in meso-thermic broad-leaved forests (composed by *Quercus canariensis* and *Quercus suber*). This species occurs in habitats mostly located under the humid to sub-humid ombroclimates with mean annual precipitation = 913.27 mm/year and mean annual temperatures = 15.62 °C (Table 1).

This subspecies breeds in lotic and lentic habitats such as temporary ponds, stream pools, rock pools, springs, and man-made fountains (Fig. 3A, B). They are small aquatic habitats, with an average surface area of 24.59 m<sup>2</sup>, which is clear with a low-cover of emergent vegetation (mean = 21.45%; Table 2). As indicated previously (Escoriza & Ben Hassine, 2015), the larvae are mainly found in association with lower water temperatures than other amphibians of Algeria, possibly because the former mainly breed during the autumn and winter, and other species are late winter-spring breeders (Table 2). During our surveys, we found larvae between November and late April. Pellegrin (1927a) and Seurat (1930) found larvae of *S. algira algira* in the Blida Atlas between March and April at 1300 m of altitude. However larvae could be found until summer in some localities (e.g., August in Edough Peninsula, personal observation). We observed cannibalism in *S. algira algira* larvae that could also prey on *D. pictus* tadpoles. *Salamandra algira algira* larvae can co-occur with *H. meridionalis*, *P. saharicus* and *D. pictus* larvae.

#### Algerian ribbed newt

##### *Pleurodeles nebulosus* (Guichenot, 1850)

Listed as a vulnerable species (IUCN, 2017), *P. nebulosus*, is endemic to northern Algeria and Tunisia (Fig. 2 C). The species occurs over a continuous range from Bizerte (Tunisia) to north-western Algeria with some relict Tunisian populations in Cap Bon (Ben Hassine et al.,

**Table 1.** Summary of the prospected localities in Algeria. n: number of sampled breeding habitats; N: Total number of the localities where a species was recorded; Altitude: Mean and range values of altitude (m); P: Mean and range of annual value of precipitation (mm/year); T: mean and range of annual value of temperature in the year (°C); P.n.: *P. nebulosus*; S.a.a.: *S. algira algira*; D.p.: *D. pictus*; S.m.: *S. mauritanica*; B.s.: *B. spinosus*; B.bl.: *B. boulengeri*; H.m.: *H. meridionalis*; P.s.: *P. saharicus*; \* See Table 3 and Appendix 1 for additional details.

	<i>P.n.</i>	<i>S.a.a.</i>	<i>D.p.</i>	<i>S.m.</i>	<i>B.s.</i>	<i>B.bl.</i>	<i>H.m.</i>	<i>P.s.</i>
n*	10	24	56	5	0	1	29	28
N*	16	33	99	18	2	4	43	62
Altitude (m)	555.06	495.36	274.36	321.94	882.5	656.25	232.93	369.5
(mm/y)	26–1378	20–1281	0–1378	0–1378	501–1264	11–1340	4–1378	0–1378
P (°C)	787.62	913.27	740.19	643.72	957.5	518	753.83	743.83
T (°C)	527–1027	445–1414	339–1365	146–955	888–1027	273–736	444–961	273–1027
(°C)	15.93	15.62	17.08	16.80	14.25	16.22	17.33	16.73
	12.2–18.5	11.2–18	12.2–19.1	12.2–18.4	12.5–16	14.2–18.3	11.9–18.9	12.2–19.1

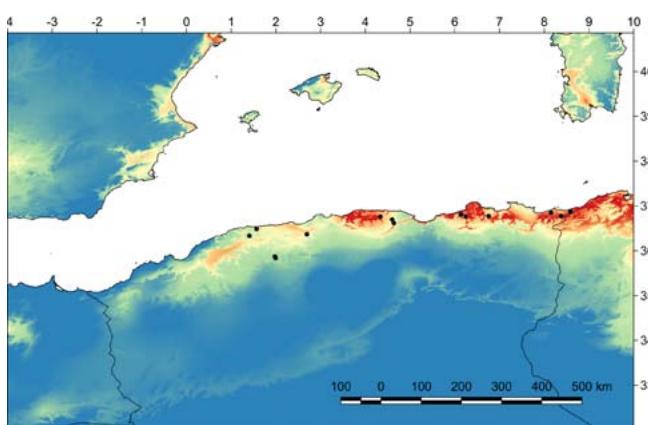
**Table 2.** Breeding habitat characteristics (mean and range) of amphibians in Algeria. n: number of sampled ponds; AD: Water body average depth (cm); BS: Water body surface area ( $m^2$ ); T: Water temperature en °C; O<sub>2</sub>: Dissolved oxygen in water (mg·L<sup>-1</sup>); pH: Water pH; Cond: Water conductivity ( $\mu S \cdot cm^{-1}$ ); Turb: Water turbidity; EV: Emergent vegetation cover (%).

	<i>Pleurodeles nebulosus</i>	<i>Salamandra algira algira</i>	<i>Discoglossus pictus</i>	<i>Sclerophrys mauritanica</i>	<i>Bufo boulengeri</i>	<i>Hyla meridionalis</i>	<i>Pelophylax saharicus</i>
n	10	24	56	5	1	29	28
AD (cm)	38.36	20.02	31.05	45.49	21.6	37.86	43.87
	19.4–66	3.2–91	7–95.2	21.2–83.8		6.1–93	8.4–117.4
BS ( $m^2$ )	1761.06	24.59	1735.52	1925.65	24	1418.94	421.32
	117.59–9112.8	0.18–361	1.35–26702.75	10.5–5089.9		1.26–5775.9	0.28–4328.2
T (°C)	19.21	12.1	14.49	16.44	16.5	14.82	14.9
	13.5–28.7	6.1–16	8.2–23.8	13.4–21		6.1–28.7	8.4–28.7
O <sub>2</sub> (mg·L <sup>-1</sup> )	6.69	7.51	7.27	8.54	-	7.11	8.64
	3.58–8.82	4.3–11.2	3.29–11.7	7.54–9.62		3.29–11.7	3.6–11.7
pH	8.11	7.37	7.67	7.68	8.3	7.69	7.92
	7.4–8.9	5.9–9.05	5.7–9.2	6.7–8.8		5.7–8.59	6.7–9.05
Cond ( $\mu S \cdot cm^{-1}$ )	727.35	441.66	623.39	634.24	449	409.51	380.4
	114.5–3550	108.2–1611	94.2–3550	94.2–1479		94.2–1972	94.2–1025
Turb	1.7	1.12	1.78	1.4	3	1.55	1.35
	1–3	1–2	1–3	1–3		1–3	1–3
EV (%)	64	21.45	54.64	31.2	0	56.76	32.17
	5–90	0–100	0–99	1–70		0–95	0–90

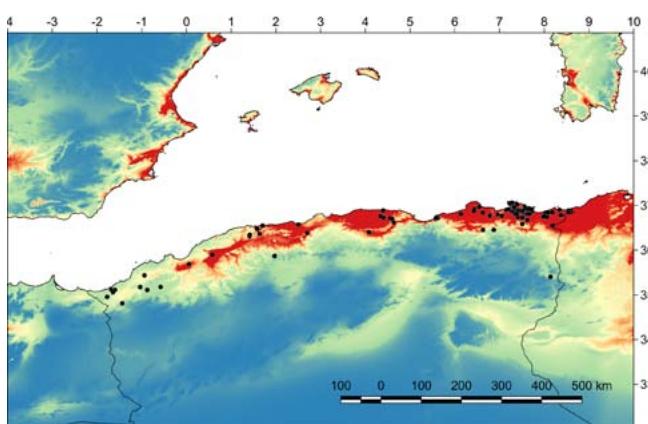
2013). In Algeria, *P. nebulosus* mostly occurs in the circum-coastal regions (between Tunisian border and Blida Atlas) penetrating weakly in the interior (about 80–100 km; Fig. 5).

In this study we confirmed the presence of *P. nebulosus* in the Blida Atlas and the Algérois, where it was already reported at Laarba, Tamazguida, Mouzaïa and Blida (Boulenger, 1891; Pellegrin, 1927 a; Pasteur & Bons, 1959; Matz, 2007; Escoriza et al., 2016). In north-western and north central Algeria, we confirmed its presence in the provinces of Chlef, Tissemsilt, Médéa, Tiz-Ouzou and Béjaia (Strauch, 1862; Lataste, 1881; Boulenger, 1891; Olivier, 1894; Doumergue, 1901; Pellegrin, 1927 a; Gervais, 1936; Dahmana et al., 2006; Escoriza et al., 2016; Ferrer et al., 2016) and reported its presence for the first time at Aïn Defla. We confirmed its presence in Skikda where it was previously reported by Escoriza & Ben Hassine (2014), Jijel (Escoriza & Ben Hassine, 2014), and El-Taref (Samraoui et al., 2012; Escoriza & Ben Hassine, 2014).

Recently Samraoui et al. (2012) signaled *P. nebulosus* at Souk Ahras. The species can be locally common in the Kabyles where it occurs in several localities between Tiz-Ouzou (Friha and Afkadou) and Jijel. However, some historical records are still not confirmed. The presence of the species should be confirmed in Alger, Laarba, Mouzaïa (Gervais, 1839; Guichenot, 1850; Lallemand, 1867; Boulenger, 1882; Matz, 2007), Guyotville (Aïn Benian, Boulenger, 1891; Pellegrin, 1927a), Bab Ezzouar (Matz, 2007), carrière de la porte Bab-El Oued (Strauch, 1862) and Oued Sebdou (Lallemand, 1867; Olivier, 1894). In the eastern part of the country, *P. nebulosus* was reported in Constantine (Lataste, 1881; Seurat, 1930; Salvador, 1996). The westernmost records of the species are located in Mascara at Sidi Daho from where two specimens were collected in February 1911 by Dr. Collozi (Doumergue, 1911 a, b), and Oran “intérieur des terres” (Guichenot, 1850). The southernmost historical records are in Biskra



**Figure 5.** Niche model and distribution records of *P. nebulosus* in Algeria. Warmer colours indicate higher climate suitability.



**Figure 6.** Niche model and distribution records of *D. pictus* in Algeria. Warmer colours indicate higher climate suitability.

(Böettger, 1885; Boulenger, 1891; Pellegrin, 1926; Seurat, 1930; Le Berre, 1989) and south of Chott Echeroui (at el Kreider, Kolar, 1955). The latter record is a desert region (salty water Chott) possibly unsuitable for *P. nebulosus*. For this reason we considered that this record could be doubtful. The niche model also supported this statement (Fig. 5).

A highly terrestrial newt *P. nebulosus* becomes aquatic during the reproductive period (Fig. 2C). It has been found in Algeria in open weakly structured agricultural and steppic landscapes, similar to the Tunisian populations (Ben Hassine & Nouira, 2012; Ben Hassine et al., 2013). However, it also occurs in mountainous evergreen oaks and cedar forests reaching 1378 m of altitude in central Algeria. The distribution of *P. nebulosus* comprises humid to semi-arid areas with annual precipitation ranging between 527–1027 mm/year (Table 1, Appendix 1).

The broad variation in the parameters of the breeding habitats highlight the ecological plasticity of *P. nebulosus* similar to those observed in other *Pleurodeles* species in Maghreb (Ben Hassine & Escoriza, 2014a; Escoriza & Ben Hassine, 2015; Ben Hassine et al., 2016). Reproduction takes place in moderate to large dimensions temporary ponds (117.59–9112.80 m<sup>2</sup>), where usually the water is turbid, with a low to dense layer of emergent vegetation (5–90%) (Table 2; Fig. 3 D–G). The mating season depends

on altitude and temperature and it begins with the onset of the seasonal rains. Similarly to that previously described for the Tunisian populations (Ben Hassine & Nouira, 2012; Ben Hassine et al., 2013), larvae of *P. nebulosus* in Algeria could be found between December and late May (until June in Kabylies and Médéa, personal observation). The larvae of this newt co-exist with some anuran species that breed in ponds with moderate to large hydroperiods such as *H. meridionalis* (31%), and more generalist species such as *D. pictus* (50%) and *S. mauritanica* (16%). Syntopy between *S. algira algira* and *P. nebulosus* in the breeding habitats was not observed. However the occurrence of this syntopy is highly possible as it was previously observed for *P. poireti* and *S. algira algira* in Edough peninsula (Fig. 3 B; Escoriza and Ben Hassine, 2017b).

### Mediterranean painted frog

#### *Discoglossus pictus* Otth 1837

The painted frog is listed as Least Concern (IUCN, 2017). Our surveys showed that the distribution of *D. pictus* in northern Algeria is likely to be continuous from the Tunisian border westwards to the border of Morocco (Figs. 2 D and 6). The painted frog is a very common species in Algeria, where it was found in 60% of the prospected localities (Fig. 6; Appendix 1).

We found *D. pictus* in Seraïdi, Bouzizi, Tebessa, Constantine, El Collo Massif, Béjaia, Azagza, Theniet El Had and Tlemcen and confirmed its presence in Annaba, El-Taref, Lac Fetzara, the Guerbes-Senhadja wetlands complex and El Kala, where it was previously mentioned by Samraoui et al. (2012), Jijel (Escoriza et al., 2014), Skikda (Warner, 1894; 1892), Kabylies [Akfadou and El Milia (Zangari et al., 2006), Yakouren, Tiwidiwine, Iguerssafen, Tala-Guilef, Freha, Aghribs (Bouali & Oneimi, 2005) and Tizi-Ouzou (Warner, 1914)], and Chlef (Brunet et al., 2009; Ferrer et al., 2016). The presence of *D. pictus* is also confirmed in the northern part of central Algeria, where it was previously reported in Alger (Strauch, 1862; Boulenger, 1882; Taibi et al., 2009), Médéa, and Chréa National Park (Warner, 1894, 1892). In western Algeria, we confirmed its presence between Oran and Tlemcen. In this area, it was collected previously in Oran, Kristel, Saint-Cloud, Saint-Lucien, le Tlèlat, Misserghin, Bou-Sfer, le Sig, Aïn-Témouchent, Arsal, Tafna Valee close to Sebdou, Daya, Saïda, Marnia, Tlemcen (Olivier, 1894; Doumergue, 1901; Zangari et al., 2006), Souk El Ténine (Sura, 1983) and Tiaret (Brunet et al., 2009; Ferrer et al., 2016). This species was mentioned at Tazoult (Warner, 1894; 1892), Sétif (Salvador, 1996; Barkat, 2014), Biskra (Salvador, 1996) and Batna (Boulenger, 1891; Olivier, 1894; Salvador, 1996). According to our data, *D. pictus* occurs in northern Algeria between the sea level and 1378 m of altitude. Following Seurat (1930), it can reach up to 1700 m. The southern distribution limits of *D. pictus* in Algeria are not known. The niche model of *D. pictus* represents the realised distribution of the species in northern Algeria and shows the potential wide range of suitable habitats for the species (Table 3; Fig. 6).

The painted frog is common in the northern part of the country, inhabiting cultivated and semi-arid steppes,

though it also occupies humid mountainous forests areas (Fig. 6). This species tolerates a broad variation of rainfall (339–1365 mm/year) and temperature (12.2–19.1 °C; Table 1). *Discoglossus pictus* shows a wide ecological plasticity in terms of breeding habitat selection (Table 2). The opportunism displayed in its foraging behaviour (Ben Hassine & Nouira, 2009), and its extensive reproductive season between autumn to late spring time (Ben Hassine & Nouira, 2012, Escoriza et al., 2014), are possibly important factors in *D. pictus* being one of the most common amphibians in northern Algeria.

The painted frog is an explosive generalist breeder, using rain puddles, rock pools, seasonal streams and ephemeral ponds (Fig. 3 B, D, F and I). However, it also breeds in rivers, large semi-permanent and permanent ponds. The surface area of these water bodies range between 1.35 m<sup>2</sup> and 26702.75 m<sup>2</sup> (Table 2). The breeding activity of *D. pictus* is highly depending on rainfall in humid areas, being continuous almost over the year in some artificial oases of southern Tunisia (Ben Hassine & Nouira, 2012). Tadpoles in different Gosner's stages could be found almost all year round in Algeria. Doumergue (1901) observed mating activity from February to late June in Oran. He stated that *D. pictus* tadpole needs about two months to achieve metamorphosis. These tadpoles could be found in syntopy with *H. meridionalis* tadpoles (28%) and *S. algira algira* larvae (10%).

### Berber toad

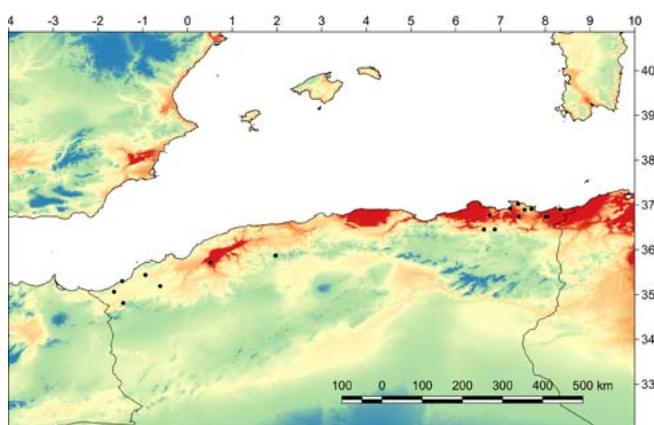
#### *Sclerophrys mauritanica* (Schlegel, 1841)

The Berber toad *S. mauritanica* is listed as Least Concern (IUCN, 2017) and found throughout northern Algeria in an apparently continuous distribution between the Tunisian and the Moroccan borders (Figs. 2 E-F, 7). We discovered *S. mauritanica* in Constantine, Relizane and Tlemcen and confirmed its occurrence in El-Kala and Annaba (Samraoui et al., 2012), Oran (Doumergue, 1901) and Chlef (Brunet et al., 2009; Ferrer et al., 2016). Several records were reported recently from different areas in Algeria: Kabylies [at Bouira (Harris & Perera, 2009); Jijel (Boumezbeur & Ameur, 2002); Sidi-Khlifa and Oued Diss in Freha (Bouali & Oneimi, 2005); Azzafoun, Aghrabs, Azagza, Freha, Iboudrarene and Draâ-El-Mizan (Targa, 2013)], Tiaret (Brunet et al., 2009; Ferrer et al., 2016), Batna (Harris & Perera, 2009), Sétif (Barkat, 2014) and Timerganine (Samraoui et al., 2012). Historical records indicate its occurrence over all northern Algeria (Lallemand, 1867; Boulenger, 1891), being common around Alger, Blida (Gervais, 1836; Strauch, 1862; Lallemand, 1867; Boulenger, 1882) and Annaba (Gervais, 1836).

The presence of this toad was reported at Biskra and Sâada (Olivier, 1894), Oran, Sania, Sig, Pérrégaux, La Macta, Terny, Saïda and Géryville (Doumergue, 1901; Seurat, 1930), Ghazouet (Llabador, 1947) and Kherrata (Sura, 1983). Boulenger (1891) stated that *S. mauritanica* was collected by Lataste at Biskra, Bou-Saada and Laghouat and that it could be found as far southwards to the margins of the Sahara. According to Salvador (1996), this species occurs in the Saharan Atlas and western Hamadas. Schleich et al. (1996) considered that *S. mauritanica* is

the only toad species that could be found in the Tassili Mountains, where it is a relict species but records of its presence in south-eastern Algeria (Angel & Lhote, 1938; Joger, 1981; Schleich et al., 1996) correspond in fact to a Sahelian species, the Savanna toad, *Sclerophrys xeros* (Mateo et al., 2013; Philippe Geniez, pers. com.). The exact southern limits of *S. mauritanica* in Algeria are not known.

The Berber toad inhabits all types of landscapes ranging from 0 to 1378 m above sea level. All the mountainous ranges of northern Algeria could be suitable habitat (Fig. 7; Table 3). Its ecological range included humid Mediterranean forests (cedar and oaks), agricultural lands and semi-arid steppes. It occurs in sites where mean annual precipitation varies between 146 and 955 mm/year and mean annual temperatures of 18.4°C (Table 1).



**Figure 7.** Niche model and distribution records of *S. mauritanica* in Algeria. Warmer colours indicate higher climate suitability.

Doumergue (1901) commented that the breeding season in Oran depends on the onset of the rains and that *S. mauritanica* could start breeding from early April or mid May. However, isolated reproductive episodes may occur over summer; tadpole metamorphosis occurs within 45 days (Doumergue, 1901). We observed tadpoles at different Gosner's stages between March and April in northern Algeria and recently metamorphosed toads were observed during early April in Tlemcen. The Berber toad breeds in small to large water bodies, including temporary and semi-permanent to permanent ponds and streams and rivers (mean depth = 45.49 cm; area range: 10.5–5089.9 m<sup>2</sup>; Table 2; Fig. 3 F, G, H and I). These habitats may or may not have a layer of emergent aquatic vegetation (1–70 %). Tadpoles of *S. mauritanica* could be found in syntopy with tadpoles of *D. pictus*, *H. meridionalis*, *P. saharicus* and *P. nebulosus* larvae.

### Mediterranean common toad

#### *Bufo spinosus* Daudin, 1803

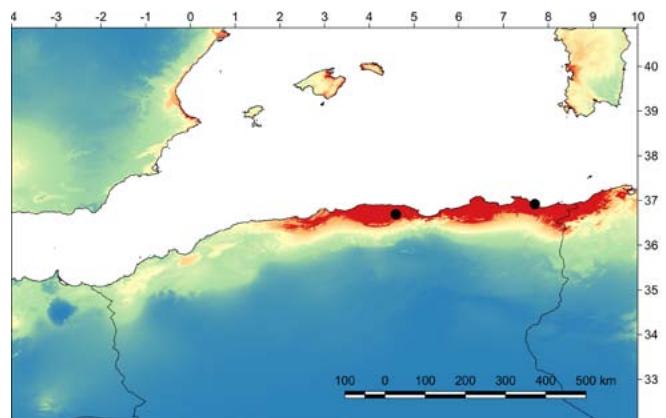
Classified as Least Concern by IUCN (as part of *Bufo bufo* (Linnaeus, 1758), IUCN, 2017) *B. spinosus* appears to be confined to the mountainous areas of northern Algeria (Figs. 2G, 8). The historical records of *B. spinosus* in Algeria are scarce and previous publications considered it

**Table 3.** Variables contribution to the ecological niche models (only those variables that contributed more than 10%)

	AUC	Variables	Contribution
<i>P. nebulosus</i>	0.931	Altitude	39.1
		Precipitation	36.9
		Seasonality	20.3
<i>S.algira algira</i>	0.969	Precipitation	85.0
		Altitude	50.6
		Precipitation	19.5
		Seasonality	18.7
<i>B. boulengeri</i>	0.913	Temperature	11.2
		Altitude	50.6
		Precipitation	19.5
		Seasonality	18.7
<i>B. spinosus</i>	0.943	Temperature	11.2
		Precipitation	64.8
		Altitude	15.0
		Temperature	11.7
<i>D. pictus</i>	0.928	Altitude	29.7
		Precipitation	27.9
		Seasonality	24.3
		Temperature	18.1
<i>H. meridionalis</i>	0.947	Precipitation	48.9
		Altitude	33.7
		Seasonality	11.3
<i>P. saharicus</i>	0.905	Altitude	49.3
		Precipitation	25.0
		Seasonality	16.8
<i>S. mauritanica</i>	0.866	Precipitation	52.2
		Altitude	30.1
		Seasonality	10.5

rare (Lallemand, 1867; Boulenger, 1891; Seurat, 1930). We confirmed its presence in the Kabylies where it was recently observed by Targa (2013) in several localities (Azzafoun, Aghribs, Azagza, Freha, Iboudrarene and Draâ-El-Mizan). We confirmed also the record of Hagenmüller (1882) in Edough region (Annaba, northeastern Algeria), although our record was in Seraïdi (Fig. 8).

Recently, Samraoui et al. (2012) reported its presence at El Ghorra Mountains where it was reported from the Tunisian side of this mountainous range (Ben Hassine & Nouira, 2012; Ben Hassine & Escoriza, 2014b). *Bufo spinosus* was mentioned in Alger and Blida Atlas (Guichenot, 1850; Lataste, 1881; Strauch, 1862; Salvador, 1996; Schleich et al., 1996), Jijel (Raouag, 1997), El Kala and El Taref (Salvador, 1996) and Skikda (Schleich, 1996). Isolated occurrences have been reported from Oran (Salvador, 1996) and Tlemcen (Böttger, 1880-1881; Salvador, 1996). However, its presence at the latter localities in the north-western part of Algeria require confirmation, as there are suitable habitats for the species in the region (Fig. 8), when taking into account its occurrence in semi-arid areas in other parts of its range (Bons & Geniez, 1996; Garcia-Paris et al., 2004).



**Figure 8.** Niche model and distribution records of *B. spinosus* in Algeria. Warmer colours indicate higher climate suitability.

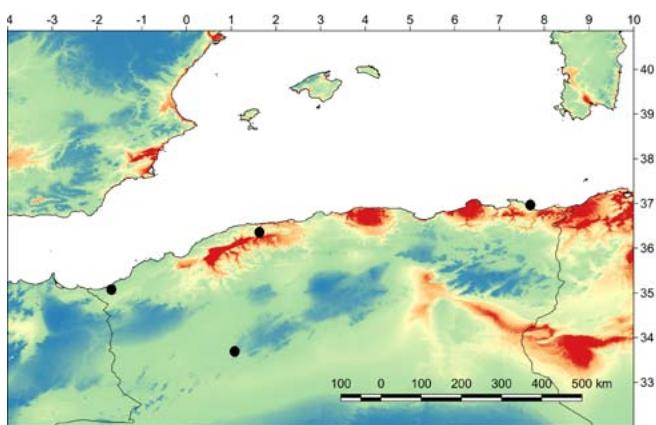
The niche model predicted the presence of continuous suitable conditions from the Tunisian border to the Blida Atlas, and in a small region in the north-western part of the country (Fig. 8; Table 3). The common toad has been found at elevations of 501 and 1264 m above sea level (Table 1). This toad inhabits broad-leaved forests composed by evergreen and deciduous oaks (*Quercus canariensis*, *Quercus suber* and *Quercus ilex*), similarly to the Tunisian populations (Ben Hassine et al., 2012; Ben Hassine & Escoriza, 2014b). However in Algeria, *B. spinosus* may be found at higher altitudes, occurring in *Cedrus atlantica* formations. It could be the most common Bufonidae in the Kabylies given the presence of highly suitable habitats in the region. The few observations could be due to its discreet behaviour and nocturnal activity. However more surveys would be necessary to better define the limits of its distribution.

#### African green toad

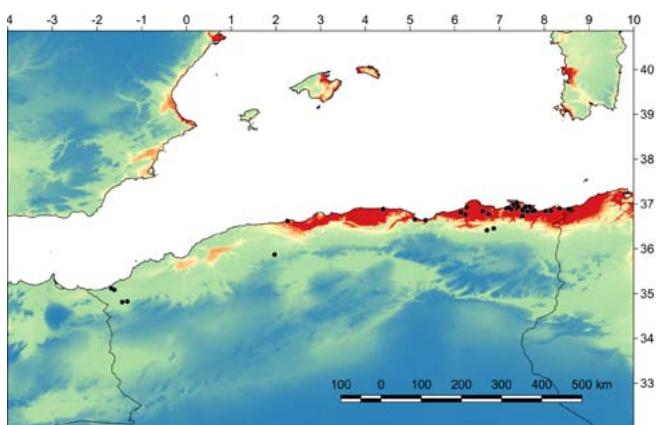
##### *Bufo boulengeri* (Lataste, 1879)

Listed as Least Concern (IUCN, 2017), *B. boulengeri*, could be considered one of the most widely distributed and ubiquitous amphibians in North Africa (Schleich et al., 1996; Joger, 2003). Historical records indicated *B. boulengeri* was found all over Algeria, being common around “l’Oranie” (Strauch, 1862; Lallemand, 1867; Boulenger, 1891; Olivier, 1894; Doumergue, 1901) an area from where we found it in Tlemcen (Figs. 2 H and 9). We also found it at el Bayadh and Aïn Defla and confirmed its occurrence in Djebel Edough at Jnène el Bey, where it was previously mentioned by Boulenger (1891). Samraoui et al. (2012) also recorded this toad in Skikda and Timerganine. Recently, it has been recorded at Tizi-Ouzou (Targa, 2013), Tiaret (Brunet et al., 2009; Ferrer et al., 2016), Souf, Taïbet and Touggourt (Mouane, 2010), Ghardaïa (Stöck et al., 2006) and Ouargla (Mebarki, 2012).

The North African green toad was confirmed present at: Mouzaïa and Alger (Strauch, 1862), Méchuria and Kreider (Doumergue, 1901), Hauts plateaux (Aïn Oussara: Seurat, 1930), Aurès, Saharan Atlas, western Hamadas (Seurat, 1930) and the Grand Erg occidental (Salvador, 1996), Musaya on the Algerian Atlas, plateau Sersou, El Guerah and Tilremt (Boulenger, 1891). According



**Figure 9.** Niche model and distribution records of *B. boulengeri* in Algeria. Warmer colours indicate higher climate suitability.



**Figure 10.** Niche model and distribution records of *H. meridionalis* in Algeria. Warmer colours indicate higher climate suitability.

to Seurat (1930) and Salvador (1996), in the Hoggar Mountains, *B. boulengeri* lives at elevations of 1400–2500 m above sea level close to permanent streams. In Algeria, the southernmost records of this species are in the Hoggar Mountains, Mzab and the Ahaggar Gueltas Mtajer and Tamekrest (Pellegrin, 1927b, 1934; Joger, 1981; Samraoui et al., 2012). In northern Algeria, *B. boulengeri* occurs at elevations between 11 and 1340 m above sea level (Table 1). This toad inhabits mainly open landscapes in northern Algeria but was found from littoral ponds and marshes up to the humid Oro-Mediterranean forests (Fig. 9). In northern Algeria, the localities are characterised by mean annual rainfall values of 273–736 mm/year and mean annual temperatures 14.2–18.3 °C (Table 1). This species breeds in ephemeral ponds especially in the arid areas of Algeria (Table 2; Fig. 3 C). According to Doumergue (1901), in Oran *B. boulengeri* appears some weeks earlier than *S. mauritanica* and mates between February and March. Salvador (1996) stated that after rain, they breed in any season in the Hoggar Mountains, when amplexus was observed in April, June, and August and tadpoles found in September. We observed tadpoles of *B. boulengeri* from early March to April. African green toad tadpoles may occur with those of *H. meridionalis* and *D. pictus*. The three Bufonidae species (*S. mauritanica*, *B. spinosus* and

*B. boulengeri*) are sympatric in the meso-thermal forests of Algeria (Figs. 7–9; Appendix 1), in a similar pattern to that observed in Tunisia and the Atlas of Morocco (Schleich et al., 1996; Ben Hassine & Nouira, 2012; Ben Hassine & Escoriza, 2014b) but no syntopy between two of the three toad species was observed (Figs. 7–9). The reproductive strategies of the African green toad (Le Berre, 1989; Guillou et al., 2004) may provide an adaptative advantage in arid environment and could partly explain its presence in the oases and in the Hoggar Mountains.

### Mediterranean tree frog

#### *Hyla meridionalis* Boettger, 1874

*Hyla meridionalis* is listed as least Concern (IUCN, 2017). We discovered its presence in Annaba city, Seraïdi, El Collo Massif, Ain Defla, and Béjaïa (Figs. 2 I–J, 10) and confirmed previous records in El-Taref, Skikda (Samraoui et al., 2012), Constantine (Salvador, 1996), Kabylies [Jijel (Raouag, 1997), Yakouren, Aghribs, Tigzirt, Tiwidiwine, Iguerssafen and Boudouaou (Strauch, 1862; Bouali & Oneimi, 2005)], Theniet el Had, Tlemcen (Anderson, 1892; Seurat, 1930) (Fig. 10). Recently, it was reported from Tiaret and Chlef (Brunet et al., 2009; Ferrer et al., 2016) and Sétif (Barkat, 2014). Presence in Alger (Guichenot, 1850; Strauch, 1862; Lallement, 1867; Seurat, 1930), La Séria, Bou-Sfer (Boulenger, 1891; Olivier, 1894; Doumergue, 1901; Seurat, 1930) and in Guelma at Hammam-Meskoutine (Seurat, 1930) remains to be confirmed although highly probable. The record of *H. meridionalis* in south-western Algeria (Méchria province) reported by Salvador (1996) could be considered far beyond the limits of suitable habitat of the species as confirmed by the niche model on Figure 10 and needs to be verified.

According to the niche model a large part of the northern region of Algeria could be suitable habitat (Fig. 10; Table 3). In the north-eastern and north-central parts of Algeria, *H. meridionalis* is a relatively common amphibian at elevations between 4 m and 1378 m above sea level (Table 1; Appendix 1). In western Algeria, the Mediterranean tree frog could be more localised and is present in Mediterranean bush land, close to temporary ponds. It occurs at sites with high level of annual rainfall (444–961 mm/year) and temperate annual temperatures (11.9–18.9 °C; Table 1). According to our findings, *H. meridionalis* is a generalist breeder breeding in permanent, semi-permanent or temporary ponds and streams. The surface area of the water bodies ranges between 1.26–5775.9 m<sup>2</sup> with or without aquatic emergent vegetation cover (0–95%) (Table 2; Fig. 3 B, F, G and H).

According to our observations, different Gosner's tadpoles and eggs clutch of this species could be found from February to late April (during May and June at high altitude). According to Doumergue (1901), in Oran the mating occurs mainly by the end of March and tadpoles metamorphose about 3 months after. Tadpoles of the Mediterranean tree frog co-occur with mainly those of *D. pictus* (93% of the localities).

### North African frog

#### *Pelophylax saharicus* (Boulenger, 1913)

Listed as Least Concern (IUCN, 2017), *P. saharicus*, was considered by Olivier (1894) and Doumergue (1901) as common in Algeria, occurring in all oases, and being especially abundant in the south of Biskra, Oued Djeddi, Saada, and Ouargla. We found *P. saharicus* in Rechgoune Beach, Tlemcen National Park, Theniet El Had, Collo Massif, Béjaia, and others localities in central Algeria (Figs 2 K-L, 11) and confirmed presence in Constantine (Boulenger, 1891), El-Taref, Annaba and Skikda (Samraoui et al., 2012), Jijel (Raouag, 1997), Géryville (actually “El Bayadh”) and Oran (Doumergue, 1901), Chlef (Ferrer et al., 2016). Bouali & Oneimi (2005) reported its presence in the Kabylies at several localities (Tizi-Ouzou, Yakouren, Azeffoun, Freha, Tiwidiwine, Iguerssafen, Tigzirt, Aghrib and Yakouren). However, Brunet et al. (2009) and Ferrer et al. (2016) reported its presence in Tiaret. Recently Barkat (2014) recorded it in Sétif. Its occurrence was confirmed recently at Souf, Taibet and Touggourt (Mouane, 2010) and Ouargla (Boulenger, 1891; Mebarki, 2012). The North-African frog was reported from the oasis of Tidikelt (Boulenger, 1891), Sebdou, Bedeau (actually ‘Ras el Ma’), Sidi-Chaib south of Daya, le Kreider, Djebel Ksel, Stitten, lake of El Goléa (actually ‘El Ménia’) and Igli (Doumergue, 1901). Sura (1983) reported the presence of the species at Oued Berd, Souk El Ténine, Grarem, and Monts du Hodna. In the northern margins of the Sahara, their presence is limited to oases (Salvador, 1996). According to Doumergue (1901) and Seurat (1930), *P. saharicus* inhabits the Hoggar Mountains and Ifédlil in the Tassili N’Ajjer (confirmed by Philippe Geniez, pers. com., who observed it in 2009 in the latter massif).

This frog occurs in humid to arid climates (mean annual precipitation: 273-1027 mm/year; mean annual temperature: 12.2-19.1 °C; Fig. 11, Table 1). It breeds mainly in large water bodies (0.28-4328.2 m<sup>2</sup>) ranging from ponds (temporary, semi-permanent or permanent), rivers, springs, rocks pools and reservoirs with or without aquatic emergent vegetation (0-90%) (Table 2; Fig. 3 F and G). *Pelophylax saharicus* is associated to deep water bodies (reaching 117.4 cm of depth). Reproduction occurs later in the season than other amphibians in the region (Schleicher et al., 1996). Doumergue (1901) reported its presence in Oran with mating in June and the presence of

tadpoles between July (at La Séria) and late September (at Sebdou). In the studied area, including in Oran, tadpoles at different Gosner’s stages were found between March and April. This species occurs mainly with *H. meridionalis*, *D. pictus* and *S. algira algira* (Appendix 1).

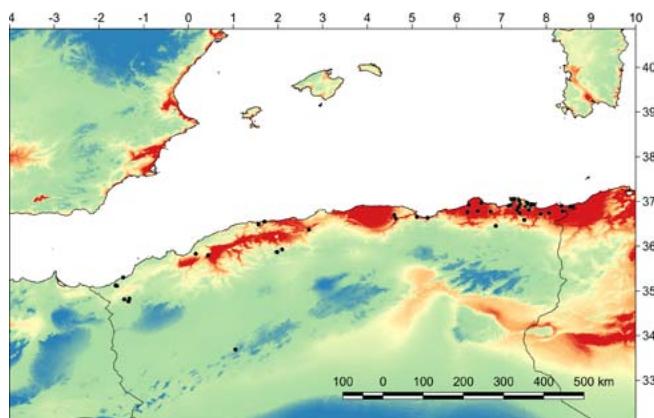
## CONCLUSION

This study summarises available bibliographic data related to the Algerian batrachofauna, along with new data on the localities of different species, their terrestrial and breeding habitats characteristics and ecological status. Further research is necessary to complete this study, focusing particularly in southern Algeria.

Our data indicated that several species could be more widespread in Algeria than historical records suggested. The apparent discontinuity of their ranges and the supposed rarity of some species such as *P. poireti* and *S. algira algira* are likely due to previous low survey effort (Escoriza & Ben Hassine, 2014; Ben Hassine et al., 2016 a, b; Escoriza et al., 2016). Some historical records were not confirmed and/or could be doubtful. This could be partly explained by the former subdivision of northern Algeria until 1918 when the region was divided in three territorial areas: in the center Alger (Tizi-Ouzou, Alger, Médea, Chlef; 54,861 km<sup>2</sup>), in the west Oran (Mostaganem, Oran, Tlemcen and Tiaret; 67,262 km<sup>2</sup>) and in the east Constantine (Annaba, Béjaia, Constantine, Batna, Sétif, Tébessa and Biskra; 87,578 km<sup>2</sup>). In this sense, the record of *S. algira algira* in Constantine (Guichenot, 1850) could be attributable to the former administrative subdivision of the country.

The presence of amphibians in northern Algeria likely depends on climate gradients and on the spatial heterogeneity permitting coexistence and cohabitation of several species in sympatry or even in syntopy (Figs. 4-11, Table 1, Appendix 1). However, the habitats in northern Algeria are under intense anthropogenic pressures with the impacts affecting both forest and wetlands. The habitat destruction is particularly notable in the coastal regions. The scarce records of amphibians in western Algeria, particularly between Oran and Alger could be explained by abiotic conditions but also, anthropic degradation of the forest environments (associated to a fragmentation of natural habitats frequented by amphibians) and particularly a rarefaction of suitable sites for reproduction. Temporary ponds, that are typical breeding habitats for most Algerian amphibians, frequently suffer from water usage for irrigation (personal observation) and pollution, especially close to the cities. Many amphibian communities can be found close to urban surroundings (Ben Hassine et al., 2016 b; Escoriza et al., 2016). Associated with the expansion of urban structures, are alteration and drainage of many wetlands, overgrazing, forest fires, the spread of alien species, such as the eastern mosquito fish (*Gambusia holbrooki*) that may directly impact on reproduction success of many amphibians and are potential threat to amphibians in Algeria.

Six Algerian amphibians of the total number of twelve species have been listed as protected species under Algerian law and several listed under both global and national IUCN



**Figure 11.** Niche model and distribution records of *P. saharicus* in Algeria. Warmer colours indicate higher climate suitability.

regulations (Stuart, 2008; Jora, 2012). Most of these occur in national parks and protected areas of Algeria (such as Chréa National Park, Tlemcen National Park, Theniet el Had National Park, Djurdjura National Park, Gouraya National Park..). However, the actual function and status of national parks does not provide sufficient protection to ensure future persistence of most amphibian species.

## ACKNOWLEDGEMENTS

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# Movement and home range of green pit vipers (*Trimeresurus* spp.) in a rural landscape in north-east Thailand

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**ABSTRACT** - Rural communities and agricultural landscapes serve as important areas for biodiversity, yet study of snakes in these fragmented environments is severely lacking. Hospital records indicate that green pit vipers (*Trimeresurus* spp.) inflict the highest number of venomous snakebites of any snake group in the Nakhon Ratchasima, Pak Thong Chai, and Wang Nam Khieo rural regions of north-east Thailand, causing debilitating injuries and, subsequently, negative perceptions of these species. We utilised radio telemetry to assess male and female *Trimeresurus albolabris* (N = 1, N = 1) and *T. macrops* (N = 2, N = 7) movements and home ranges in rural portions of the Sakaerat Biosphere Reserve in north-east Thailand from October 2015 through January 2017. Green pit vipers of both species were tracked for a mean of  $97.6 \pm 15$  days (median = 88, range 35-190). They moved a mean distance of  $26.3 \pm 3.32$  meters between locations (median = 25.11, range 13.2-50.4) and exhibited mean minimum convex polygon home ranges of  $0.14 \pm 0.043$  hectares (median = 0.095, range 0.006-0.423). Big-eyed green pit vipers (*T. macrops*) differed in movement patterns and home range size by sex and fecundity, although not statistically so. Understanding green pit viper space use will aid in future conservation and snakebite mitigation efforts for this interesting but severely understudied group.

## INTRODUCTION

Conversion of natural habitats to agriculture is among the major threats to biodiversity (Foley et al., 2005). Previous studies of terrestrial vertebrate fauna in fragmented habitats have primarily focused on birds and mammals, with far fewer studies of reptiles and amphibians conducted (MacNally & Brown, 2001). Snakes play an important role in natural systems (predators and prey) and can serve as crucial bioindicators for environmental health (Campbell & Campbell, 2001). Lack of information pertaining to reptile and amphibian natural history in human modified landscapes limits conservation efforts (Bury, 2006).

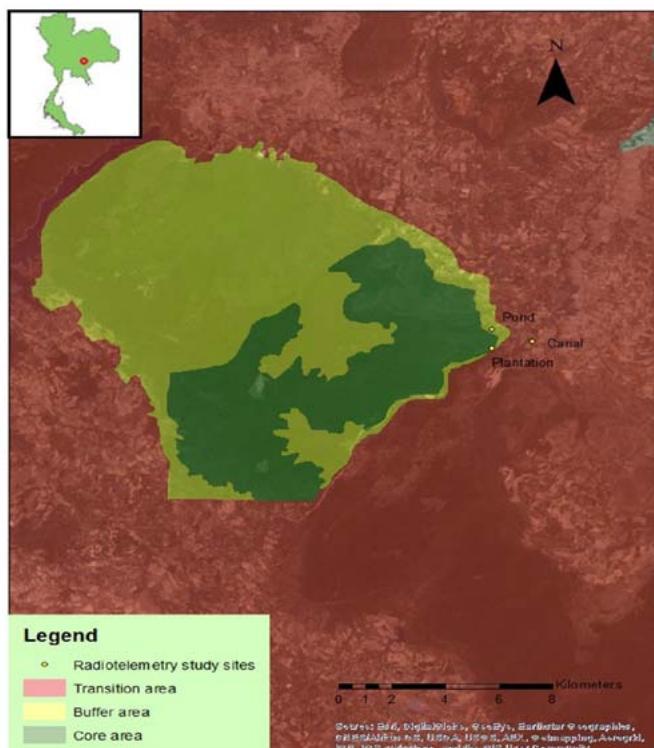
South-east Asia is a complex biodiversity hotspot which faces many anthropogenic threats (Hughes, 2017). However, this region remains under-represented in studies of faunal response to habitat loss and human modified landscapes (Trimble & Aarde, 2012). More than 142 species of amphibians and 218 species of reptiles have been described from Thailand (IUCN, 2014), yet this country has the smallest area of remnant forest cover in south-east Asia.

Green pit vipers (*Trimeresurus* spp.) are a diverse and complex vertebrate predator group in the Asian tropics (Orlov et al., 2002). At least 8 species are present in Thailand (Chanhome et al., 2011), with new species having been described as recently as 2011 (*T. phuketensis*, Sumontha et al., 2011). One species of Thai green pit viper, *T. kanburiensis*, is listed as Endangered due to its limited distribution and illegal harvest for the pet trade (IUCN, 2012). Four other species have been labeled Data Deficient (IUCN, 2016). Taxonomic confusion and nomenclature

inconsistency is particularly prevalent within the *Trimeresurus* group, making assessment and conservation of these snakes difficult (David et al., 2001; David et al., 2011).

A snakebite is a devastating environmental and occupational health hazard prevalent in rural developing countries, particularly in the tropics (Warrell, 2010). White-lipped (*T. albolabris*) and big-eyed (*T. macrops*) green pit vipers previously accounted for 40% of total venomous snake bites throughout Thailand (Viravan et al., 1992) and 95 % for the metropolitan city, Bangkok (Meemano et al., 1987; Mahasandana & Jintakune, 1990). Green pit vipers are regularly encountered in our study area and account for the highest number of snakebites of any venomous snake group present in the region (compilation of local hospital records, unpublished data). Limited spatial study has been previously been conducted with green pit vipers in the core area (most protected zone) of our study area (Strine, 2015), herein, we present the first investigation of green pit viper (*Trimeresurus* spp.) natural history in non-natural habitats. Our study bridges the current knowledge gap for green pit viper ecology in rural habitats, which are places of high conservation priority and human safety concern for this group of snakes.

We describe the spatial ecology of 2 green pit viper species in 3 study sites in agricultural areas of Sakaerat Biosphere Reserve. We report preliminary (1) movement, (2) home range, and (3) home range overlap patterns of *T. albolabris* and *T. macrops* to supplement future habitat selection, thermoregulation, prey selection, and spatial study of green pit vipers in rural habitats.



**Figure 1.** Map of study area with the core, buffer, and transition areas of the Sakaerat Biosphere Reserve delineated. Insert shows map of Thailand with study locality represented by red circle.

## MATERIALS AND METHODS

The study area, Sakaerat Biosphere Reserve (SBR), is located in Nakhon Ratchasima Province, Thailand ( $14.44\text{--}14.55^{\circ}\text{N}$ ,  $101.88\text{--}101.95^{\circ}\text{E}$ ). Approximately 80 km $^2$  of SBR is designated as a core area and set aside to preserve and maintain species diversity, genetic variation, and landscapes and ecosystems. The buffer and transition areas surround the core area and consist primarily of agricultural areas and settlements. Together they comprise approximately 360 km $^2$ .

We obtained green pit vipers for radio telemetry by opportunistic searches and concurrent site occupancy surveys at three study sites (designated plantation, canal, and pond) within the transition and buffer areas of SBR (Fig. 1). All three sites contained ephemeral water sources. The plantation and canal sites contained creeks and were downstream from a local dam and pond. The pond site contained a small pond (0.1 ha). The plantation site bordered the core, buffer, and transition areas of the Sakaerat Biosphere Reserve and was less than 50 m south of a major highway (Highway 304). It was characterised by a rubber (*Hevea brasiliensis*) plantation with heterogeneously disturbed forest (HDF) and bamboo patches. The canal site was located in the transition zone of SBR and was characterised by a small creek (< 20 m at widest point) directly adjacent to multiple small households and a variety of agricultural types and practices including cassava, corn, Eucalyptus tree (*Eucalyptus camaldulensis*), and coconut at various stages of cultivation. The pond site was approximately 100 m from a small Buddhist temple and

surrounded by a patchwork of cassava fields interspersed with HDF. This site was primarily in the transition area, but was also bordered by dipterocarp forest in the core area of the biosphere reserve. Dry dipterocarp forest is a forest type endemic to south-east Asia and characterised by thick *Vietnamosasa pusilla* grass ground cover and dipterocarp trees including *Shorea siamensis* and *Shorea obtusa* (Lamotte et al., 1998). Dry Dipterocarp forest is fire adapted with man-made or natural fires occurring in the dry season (March and April), and effectively clearing ground cover.

We captured vipers during visual searches and surveys at night, and recorded morphometrics (snout-vent length-SVL and body mass) the following day using the acrylic tube method and isoflurane anaesthesia as described by Wilkinson (2014). We further assessed body condition for *T. macrops* using a scaled mass index (SMI, Peig and Greene, 2009) with previous *T. macrops* captured at the Sakaerat Biosphere Reserve as a comparison population (Strine et al., 2015), and checked for gravidity by presence of vitellogenic follicles with light palpation while under anaesthesia. We then surgically implanted radio transmitters (1.8 g, Holohil BD2-THX) into the coelomic cavities of 11 vipers (7 female and 2 male *T. macrops*, of which 5 females were gravid and 2 were not; 1 female and 1 male *T. albolabris*) following (Reinert & Cundall, 1982; Hardy & Greene, 2000). All vipers were returned to their location of capture within 72 hours of capture.

When radio tracking snakes we attempted to locate each snake once per day and once per night. The day being the usual inactive period (when they were most likely to be sheltering or resting) and the night being the active period (more frequently observed foraging and moving). Relocations of 5 m or greater from the last observation were recorded as a “move”. Distances between moves were calculated in the field with handheld GPS units (Garmin GPSMap64s and eTrex10) and confirmed later using ArcGIS 10.1. We calculated number of moves, mean distance moved, and mean daily displacement (MDD, number of days tracked divided by distance moved).

We used the adehabitat package in program R (version 3.1.2) to estimate home range, using fixed kernel (50 and 99 %) and minimum convex polygon (MCP, 100 %) methods. Accuracy of both methods has been questioned (Row & Blouin-Demers, 2006), but were utilised in this work for comparison to previous studies. The least-squares cross validation method was used to select the smoothing factor for core (50 %) and activity (99 %) area utilisation distributions (Tiebout & Cary, 1987). Home range asymptotes using the MCP method were estimated for individual vipers using the hrBootstrap function in the sp package in program R.

We assessed statistical assumptions of normality and heterogeneity for all variables using Shapiro-Wilk and Levene tests. We implemented a GLMM (generalised linear mixed-effect model) with Wald test (R packages lme4 and car) to determine if days or number of fixes were better predictor covariates for home range size using MCP as a response variable and species as a random effect. Our data displayed a Poisson distribution; days and

fixes were discrete variables, MCP was continuous. We compared movement patterns and home range size of *T. macrops* using two sample independent t- tests, activity area by males and females was log transformed to obtain a normal distribution. Number of moves and SVL of gravid and non- gravid females could not be adequately transformed (obtaining a normal distribution) and we used non- parametric Wilcoxon rank sum tests (using the wilcox.test function in R) with the understanding that sample size is a severe limiting factor. Male and female *T. macrops* were compared, as were gravid and non- gravid female *T. macrops*. We also present descriptive spatial data for the single female and male *T. albolarbris*. Means are reported for morphometric and space use data with standard error and medians.

Spatial overlap was calculated for concurrently tracked vipers using utilisation distribution overlap index (UDOI, Fieberg & Kochanny, 2005) with the R package KernSmooth for fixed kernels and the intersect function in ArcGIS 10.1 for MCP overlap analysis. Overlap is presented as area (ha and %) for MCP and as the UDOI for kernels. Values from the UDOI range from < 1 which suggests less overlap relative to uniform space use, 1 if both home ranges are uniformly distributed and have 100 % overlap, and values > 1 indicate higher than normal overlap relative to uniform space use. We also present results from 2 relatively infrequently used indices for comparative purposes; volume of intersection index (VI, Seidel, 1992; Kernohan et al., 2001) and Bhattacharyya's affinity (BA, Bhattacharyya, 1943), both of which range from 0 (no overlap) to 1 (identical home ranges). Advantages and biases for the different overlap indices are discussed in Fieberg & Kochanny (2005).

## RESULTS

Green pit vipers, *T. albolarbris* (TRAL; N = 1 male, 1 female) and *T. macrops* (TRMA; N = 2 male, 7 female) were radio tracked for a mean of  $97.6 \pm 14$  days (median = 88) and  $66.0 \pm 12.3$  fixes (median = 67.0, Tables 1-3, Fig. 2). Results of tests for assumptions for statistical analyses for *T. macrops* varied in normality and homogeneity (Table 4). Male (N = 2) and female (N = 7) *T. macrops* were tracked for a similar number of fixes ( $t = 0.28$ , df = 7, P = 0.79) and days ( $t = -0.30$ , df = 7, P = 0.77). Gravid (N = 5) and non- gravid (N = 2) female *T. macrops* were tracked for a similar number of fixes ( $t = -0.83$ , df = 5, P = 0.44) and for a similar number of days ( $t = -0.32$ , df = 5, P = 0.76). Number of days and fixes for all vipers exhibited similar effects on MCP size ( $\beta = -0.005 \pm 0.003$ ,  $t = -0.152$ , P = 0.879 and  $\beta = 0.0008 \pm 0.004$ ,  $t = 0.231$ , P = 0.817; respectively, with intercept  $\beta = 0.1262 \pm 0.114$ ,  $t = 1.104$ ); i.e. unless an asymptote is reached number of fixes and to a lesser extent days tracked should be positively correlated with MCP size. Tracked male *T. macrops* were significantly smaller than females based on SVL ( $t = 3.26$ , df = 7, P = 0.01) and almost significantly by body mass ( $t = 2.26$ , df = 7, P = 0.058). Gravid *T. macrops* were not significantly longer or heavier than non-gravid females (SVL; W = 2, P = 0.33; body mass;  $t = -0.91$ , df = 5, P = 0.40).

Vipers (both species collectively) relocated a mean of  $8.18 \pm 1$  times (median = 9), moved  $26.3 \pm 3.32$  m (median = 25.11) straight line distance per relocation, and displayed mean MDD straight line distance of  $0.32 \pm 0.06$  m/day (median = 0.30). Number of moves was not significantly different between males and female *T. macrops* ( $t = -0.07$ , df = 7, P = 0.94); distance moved was not significantly different ( $t = -1.01$ , df = 7, P = 0.34); and MDD was not significantly different ( $t = -0.56$ , df = 1.04, P = 0.67).

**Table 1.** Summary of *T. albolarbris* (TRAL) radiotracked, tracking site (plantation- PL, & pond- PO), tracking start and end date, sex, snout- vent length (SVL, mm), mass (g), standardised mass index (SMI), number of days tracked (days), number of fixes recorded (fixes), number of moves (moves), mean distance between moves (distance, m), mean daily displacement (MDD, m/day), minimum convex polygon (MCP, 100 %, ha), and 50 and 99 % fixed kernels (50 and 99 FK, ha, respectively).

Viper ID	Site	Start	End	Sex	Morphometrics			Tracking		Movement			Home range		
					SVL	Mass	SMI	Days	Fixes	Moves	Distance	MDD	MCP	FK50	FK99
TRAL013	PO	10/01/15	03/13/16	Female	625	113.0	-	163	127	21	28.75	0.18	0.357	0.214	1.354
TRAL016	PL	11/19/15	12/24/15	Male	501	40.2	-	35	10	3	25.12	0.72	0.062	0.084	0.554

**Table 2.** Summary of male *T. macrops* (TRMA) radiotracked, tracking site (canal- CA, plantation- PL), tracking start and end date, snout- vent length (SVL, mm), mass (g), standardised mass index (SMI, using Strine et al., 2015 as comparison population), number of days tracked (days), number of fixes recorded (fixes), number of moves (moves), mean distance between moves (distance, m), mean daily displacement (MDD, m/day), minimum convex polygon (MCP, 100 %, ha), and 50 and 99 % fixed kernels (50 and 99 FK, ha, respectively) with total mean and standard error (SE).

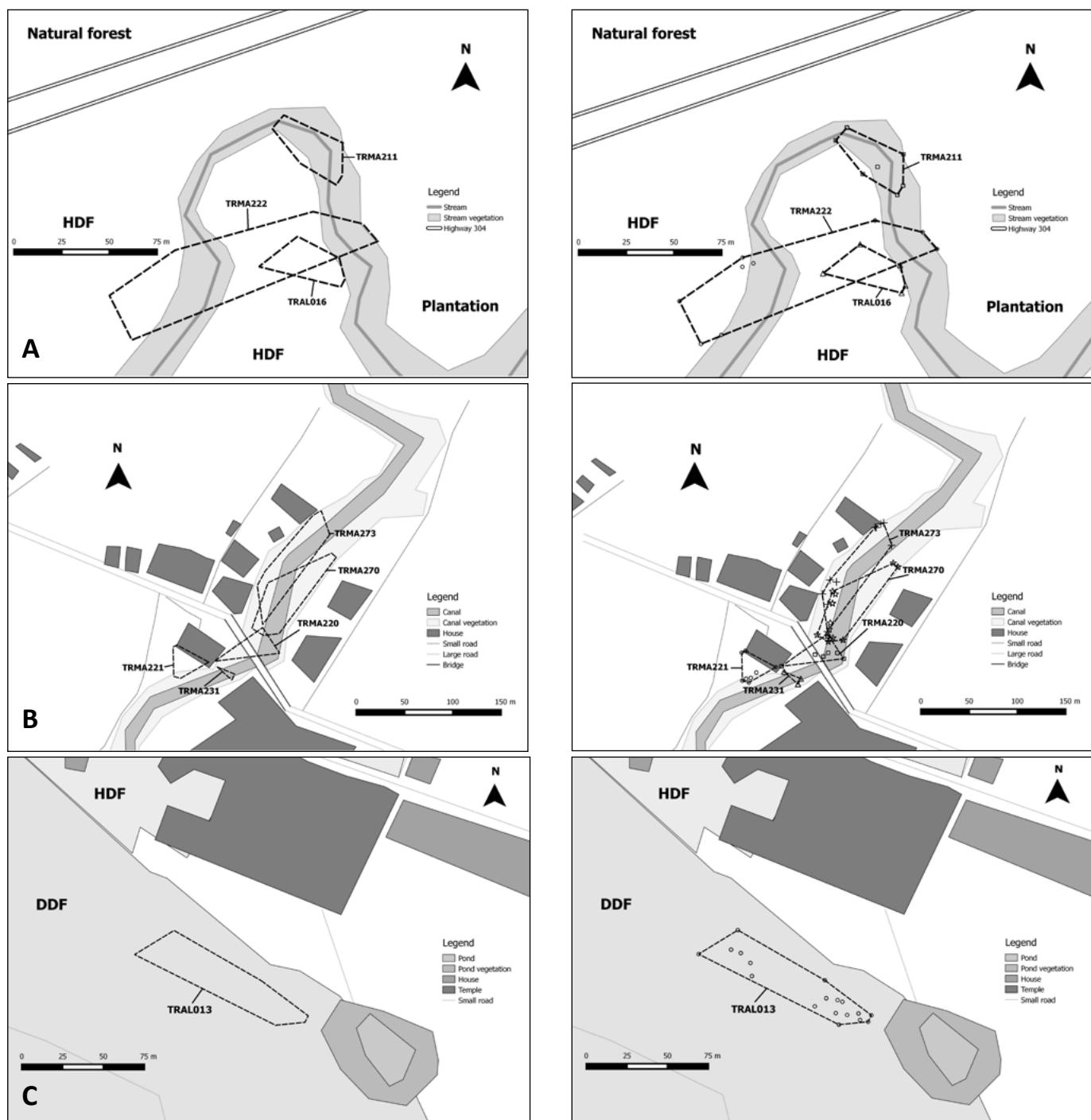
Viper ID	Site	Start	End	Morphometrics			Tracking		Movement			Home range			
				SVL	Mass	SMI	Days	Fixes	Moves	Distance	MDD	MCP	FK50	FK99	
TRMA222	PL	12/10/15	02/28/16	500	33.0	50.59	78	48	11	50.42	0.65	0.423	0.277	2.118	
TRMA229	CA	02/02/16	06/16/16	438	38.6	93.21	134	84	4	17.55	0.13	0.009	0.003	0.024	
				Mean	469	35.8	71.90	106	66	7	34.0	0.38	0.216	0.140	1.071
				SE	31.0	2.8	21.31	28	18	3	16.43	0.26	0.207	0.137	1.047

**Table 3.** Summary of female *T. macrops* (TRMA) radiotracked, tracking site (canal- CA, plantation- PL), tracking start and end date, whether gravid or not, snout-vent length (SVL, mm), mass (g), standardized mass index (SMI, using Strine et al., 2015 as comparison population), number of days tracked (days), number of fixes recorded (fixes), number of moves (moves), mean distance between moves (distance, m), mean daily displacement (MDD, m/day), minimum convex polygon (MCP, 100 %, ha), and 50 and 99 % fixed kernels (50 and 99 FK, ha, respectively) with means and standard error (SE).

Viper ID	Site	Start	End	Gravid	Morphometrics			Tracking		Movement			Home range				
					SVL	Mass	SMI	Days	Fixes	Moves	Distance	MDD	MCP	FK50	FK99		
TRMA211	PL	11/17/15	02/07/16	N	518	42.4	57.97	80	40	10	17.78	0.22	0.075	0.067	0.425		
TRMA220	CA	12/05/15	06/15/16	Y	580	91.3	88.56	190	149	8	39.03	0.21	0.095	0.092	0.570		
TRMA221	CA	12/02/15	01/23/16	Y	612	86.4	70.43	51	34	9	15.60	0.31	0.112	0.065	0.425		
TRMA231	CA	02/23/16	06/16/16	Y	520	47.0	69.84	110	98	4	13.20	0.12	0.006	0.010	0.083		
TRMA232	CA	02/25/16	05/03/16	Y	612	109.0	99.46	88	64	1	30.68	0.35	0.006	0.024	0.165		
TRMA270	CA	10/31/16	01/08/17	N	590	83.5	93.46	90	69	10	28.08	0.31	0.222	0.132	0.884		
TRMA273	CA	11/08/16	01/03/17	Y	593	73.9	82.89	55	67	9	23.62	0.43	0.175	0.163	0.952		
					Gravid	Mean	583.4	81.5	82.23	98	82	6	24.42	0.28	0.079	0.071	0.439
					SE	17	10.3	5.61	25	19	2	4.78	0.05	0.325	0.027	0.156	
					Not Gravid	Mean	554	62.9	75.71	80	40	10	22.9	0.27	0.149	0.100	0.655
					SE	36	20.5	17.74	80	40		5.14	0.04	0.073	0.032	0.230	
					Both	Mean	575	76.2	80.37	94	74	7	23.99	0.28	0.099	0.079	0.500
					SE	15.1	9.1	5.61	18	15	1	3.49	0.04	0.030	0.021	0.125	

**Table 4.** Summary for tests of normality (Shapiro- Wilk) and homogeneity (Levene) of data for *T. macrops*. \* indicates normal, \*\* indicates homogenous

Measure type	Measurement	Normality		Homogeneity		
		W	P	F	P	
Morphometrics	SVL	M/F **, *	0.89	0.18	0.0045	0.95
		Gravid/not **	0.81	0.05	0.281	0.62
	Mass	M/F **, *	0.91	0.32	2.68	0.14
		Gravid/not **, *	0.93	0.54	0.14	0.72
Basic tracking	Fixes	M/F **, *	0.89	0.60	0.30	0.60
		Gravid/not **, *	0.89	0.25	1.101	0.34
	Days	M/F **, *	0.89	0.18	0.022	0.89
		Gravid/not **, *	0.84	0.09	2.1116	0.20
Movement	Moves	M/F **, *	0.86	0.58	0.33	0.58
		Gravid/not **	0.80	0.04	8.95	0.30
	Distance	M/F **, *	0.91	0.29	6.73	0.36
		Gravid/not **, *	0.95	0.76	0.67	0.45
Home range	MDD	M/F *	0.91	0.35	20.24	0.003
		Gravid/not **, *	0.98	0.96	1.35	0.30
	MCP	M/F *	0.85	0.07	17.74	0.004
		Gravid/not *	0.94	0.60	0.39	0.56
	Core area	M/F *	0.90	0.24	18.35	0.004
		Gravid/not **, *	0.96	0.81	0.26	0.63
	Activity area	M/F	0.83	0.05	36.15	0.0005
		Gravid/not **, *	0.93	0.55	0.037	0.86



**Figure 2** Maps of study sites (A. Plantation, B. Canal, C. Pond) with viper locations indicated by unique symbols and outlined by minimum convex polygon (MCP) borders

Similarly, non-gravid female *T. macrops* did not move significantly more frequently than gravid females ( $W = 10$ ,  $P = 0.07$ ). Distance moved by gravid and non-gravid female *T. macrops* was also similar ( $t = -0.18$ ,  $df = 5$ ,  $P = 0.87$ ) as was MDD ( $t = -0.16$ ,  $df = 5$ ,  $P = 0.88$ ).

Mean MCP home range size for all individuals was  $0.14 \pm 0.043$  ha (median = 0.095), and 50 % kernel and 99 % kernel were  $0.10 \pm 0.026$  ha (median = 0.084) and  $0.69 \pm 0.187$  ha (median = 0.554), respectively. Male MCP home ranges were not significantly larger than females ( $t = -0.56$ ,  $df = 1.04$ ,  $P = 0.67$ ). Similarly, core area (50 % kernel) did not differ significantly between males and females ( $t =$

$-0.44$ ,  $df = 1.05$ ,  $P = 0.73$ ), nor did activity area (99 %,  $t = 0.23$ ,  $df = 1.05$ ,  $P = 0.85$ ). Non-gravid female MCP home range size was not significantly larger than gravid female ( $t = 0.87$ ,  $df = 1.42$ ,  $P = 0.51$ ). Core area (50 % kernel) were similar ( $t = 0.59$ ,  $df = 5$ ,  $P = 0.58$ ), as were activity area (99 %,  $t = 0.75$ ,  $df = 5$ ,  $P = 0.49$ ).

Home range overlap between individual vipers was minimal (Table 5). Only 2 sets of MCP home ranges overlapped; minimal overlap (0.041 ha, 14.7 %) was suggested at the plantation site with the male *T. albolabris* (TRAL016) and a male *T. macrops* (TRMA222), but a significant proportion of the two female *T. macrops* at

**Table 5.** Home range overlap between green pit vipers with minimum convex polygon (MCP) analysis of home range overlap (ha and %) and fixed kernel (FK, 99 %) overlap with proportion of home range overlap (HR), probability of a viper being located in another viper's home range (PHR), Bhattacharyya's affinity (BA), volume of intersection index (VI), and utilisation distribution overlap index (UDOI) results presented

Site	Viper ID	Overlap with	MCP		FK99%						
			Overlap (ha)	Overlap (%)	HR 1,2	HR 2,1	PHR 1,2	PHR 2,1	BA	VI	UDOI
Plantation	TRAL016	TRMA211	0	0	0	0	0	0	0	0	0
		TRMA222	0.041	14.7	0.7	0.329	0.196	0.810	0.326	0.170	0.123
		TRMA211	TRMA222	0	0	0.871	0.283	0.183	0.864	0.294	0.152
		TRMA220	TRMA229	-	-	0	0	0	0	0	0
Canal		TRMA229	-	-	0.009	0.041	0.006	0.001	0.002	0.001	1.00E-05
		TRMA231	0	0	0	0	0	0	0	0	0
		TRMA232	0	0	0	0	0	0	0	0	0
		TRMA229	TRMA231	-	-	0	0	0	0	0	0
		TRMA232	0	0	0	0	0	0	0	0	0
		TRMA231	TRMA232	0	0	0	0	0	0	0	0
		TRMA270	TRMA273	0.094	78.9	0.774	0.175	0.217	0.707	0.279	0.152

the canal site (TRMA270 and 273) overlapped (0.094 ha, 78.9 %). Core area (50 %) home ranges did not overlap for any vipers. Activity area (99 %) overlap was minimal for UDOI (range 0-0.124). Interestingly, more sets of vipers (4) overlapped using the activity area method than MCP. Both BA (range 0-0.326) and VI (range 0-0.17) methods similarly suggested minimal activity area home overlap between individuals. All indices (UDOI, BA, and VI) suggested the highest set of home range overlap to be the pair of males at the Plantation site (TRAL016 and TRMA222).

## DISCUSSION

Both species of green pit vipers in our study displayed extremely limited movement and small home ranges (mean MCP size 0.14 ha), which were comparable to the smallest viper in the world (*Bitis schneideri*, mean 0.10 ha for females, Maritz & Alexander, 2012). High levels of activity and movement can increase predation risk of an organism (Gerritsen & Strickler, 1977), which could be a significant factor for vipers in rural communities. Habitat availability could also potentially affect space use, and reduction of natural features such as trees and hedges in non-natural environments may in turn influence arboreal green pit viper home range and movement.

Small home range and limited movement suggest both MCP and kernel methods to be imperfect estimators for green pit viper home range. The MCP method does not take into account movement (or lack thereof) and may include large areas of unused space (Nilsen et al., 2008), while kernels may overestimate overall home range size (Row & Blouin-Demers, 2006). Limited movement also potentially brings issues of autocorrelation between points (Laver & Kelly, 2011); we sought to limit temporal biological autocorrelation by taking data at different activity periods (night and day). Further study is needed to quantify both statistical and biological independence for fixes in snakes exhibiting limited movement and small

home range size. Home range asymptotes were achieved for just over half of the study vipers, an issue traditionally attributed to limited movement combined with low tracking duration (Laver & Kelly, 2011). We were able to track vipers for a mean of 98 days (transmitter life being < 1–4 months), so limited movement (mean 8 moves, 26 m per move) likely influenced home range asymptotes more than tracking duration.

Adult male and female snakes face different challenges which may be reflected in behaviour, movement, and space use (Madsen, 1987; Shine, 2003). Drawing general conclusions between sex and movement and home range size of male and female snakes is difficult, although males are generally more active and exhibit larger home ranges than females (Macartney et al., 1988). Members of the genus *Trimeresurus* are sexually dimorphic (Orlov et al., 2002; Chanhome et al., 2011; Strine et al., 2015; Devan-Song et al., 2017), which likely also influences movement and space use between the sexes. The male *T. albolabris* and male *T. macrops* had higher MDD than females of their respective species (comparison of medians), although not statistically significantly so. Male *T. macrops* also exhibited larger home ranges (medians, not statistically significant) than female *T. macrops*, but interestingly the female *T. albolabris* exhibited larger home range than the male *T. albolabris*. This may be explained at least in part by the longer duration of tracking of the female. Standardising tracking duration for all vipers would be ideal, although would have drastically reduced our already scarce data (number of vipers or days). All of the males were tracked outside of the previously described breeding seasons for their respective species (September to November, Chanhome et al., 2011), which could be one explanation as to why their home ranges were not significantly larger than females. It could also be due to small sample size. However, previous study of Armenian vipers (*Montivipera raddei*) in an agricultural setting also had comparable male and female home range sizes, which were attributed to habitat use in the matrix of fields and natural environments (Ettling et al., 2013).

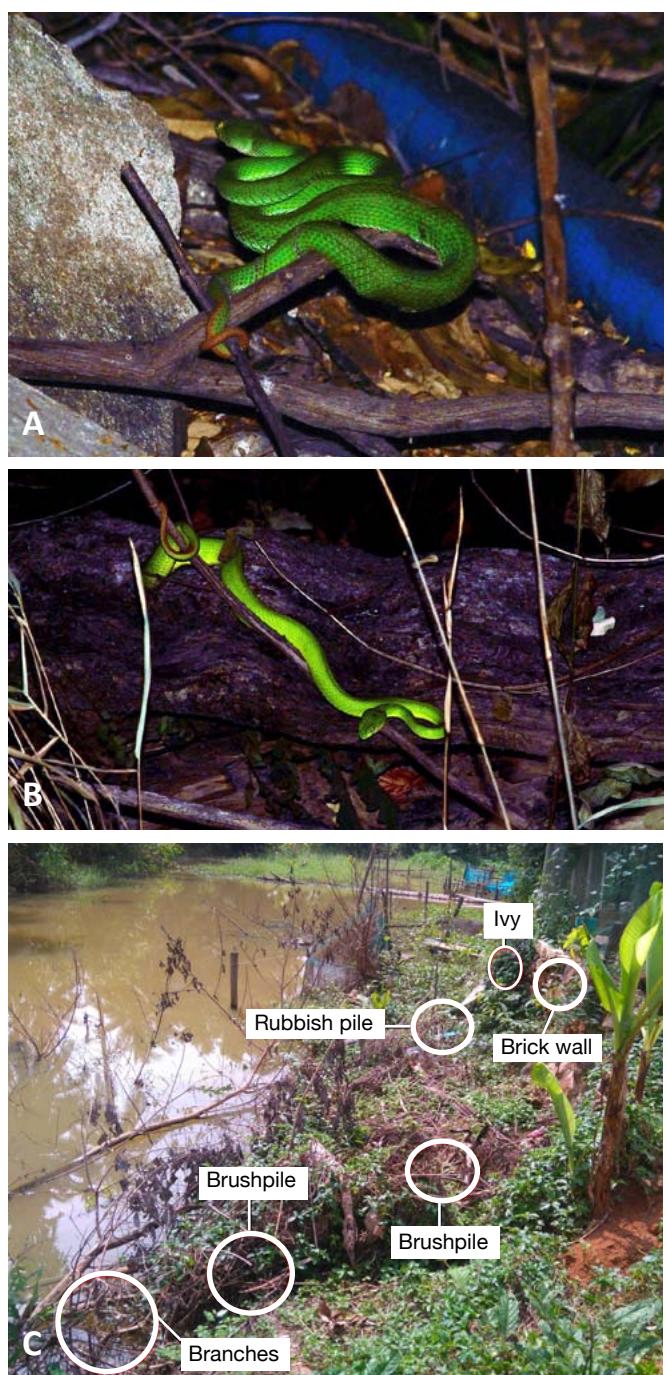
Gravid female big-eye green pit viper movement patterns were observed to be similar statistically to non-gravid females in our study, although median movement and distance was higher for non-gravid females. Most previous studies have found gravid snakes to move less frequently and smaller distances than non-gravid female snakes (Johnson, 2000). Gravidity can pose significant locomotor challenges for snakes, which can be reflected in movement and spatial patterns (Seigel et al., 1987). Further understanding of the benefits and mitigation of costs of similar space use by gravid and non-gravid female *T. macrops* is required.

Snakes are not known to exhibit territoriality and individuals from the same population often display widely overlapping home ranges (see review by Gregory et al., 1987; Weatherhead and Hoysak, 1988; Secor, 1994). Small home range size, and thus limited chance of encounter, may explain the limited overlap we observed between radio tracked snakes. Estimation of density via mark-recapture with concurrent radiotelemetry study could better clarify encounter rate between individual vipers, as our small sample may not be representative.

Tracking males outside of the breeding season may explain limited home range overlap of males with females. Interestingly, the highest UDOI home range overlap observed in our study was with two male green pit vipers of different species. The largest MCP home range overlap, however, was by two female *T. macrops* (TRMA270 and 273). This large overlap could be due to a general lack of intraspecific sexual antagonism observed between female snakes (Shine, 1994) or different use of resources as one of the vipers was gravid and the other was not (Shine, 1979; Macartney et al., 1988).

Caution is expressed regarding extrapolating our preliminary findings to other populations or species, which may exhibit different patterns in rural communities and other non-natural environments. Multiple previous natural history studies of large bodied vipers in rural habitats have observed both indirect and intentional killing of study animals by humans (Bonnet et al., 1993; Durbian, 2006; Wittenberg, 2012). However, we did not observe human caused mortality to the snakes in our study, which we attribute primarily to the cryptic and perceived inoffensive nature of green pit vipers. Two of the *T. macrops* in our study spent extensive time (TRMA221 and 273, >2 weeks) less than 10 m from human habitations. The homeowners were tolerant of the vipers' presence when informed by researchers so long as the study animals did not directly enter living quarters. While study vipers were observed in plantations, they did not fully enter agricultural fields (although they did utilize edges) which likely reduced chance of mortality by certain agricultural practices (tilling, harvesting, etc.) which have been observed for other snakes in the SBR (Kneirem et al., 2017). Limited movement and small home range size may facilitate green pit viper persistence and resilience in our study area, although habitat selection investigation is needed for future conservation measures.

Previous studies have suggested that while well intentioned, translocation can have disastrous results for



**Figure 3.** Tracked female *T. macrops* (TRMA221) ambushing amongst concrete rubbish <10 m from a house (A.), tracked *T. albolabris* (TRAL013) ambushing near log she sheltered in during a fire in dry dipterocarp forest (B.; detailed in Barnes et al., in press), and sites used by a female *T. macrops* (TRMA273) for shelter and foraging near a house (C)

“nuisance” snakes moved outside of their home ranges. Irregular movement patterns, increased home range sizes, and decreased survival rates have been recorded for translocated snakes (Fitch & Hampton, 1971; Nowak et al., 2002; Barve et al., 2013). Translocation is generally perceived by the public to be a humane strategy because it does not result in the immediate death of the individual (Riedl et al., 2008), however, serious consideration is required in particular for relocation of snakes which exhibit small home ranges. Previous study of *T. albolabris*

in Hong Kong by Devan-Song (2014) found a 100 % male and 71 % female mortality rate for individuals translocated outside of their home ranges. Green pit vipers are commonly translocated in rural north-east Thailand when encountered in human habitations, and our findings of very small home ranges of *T. albolabris* and *T. macrops* may present significant management implications. Future studies of the effects of short and long distance (within and outside of home range, respectively) translocation on green pit viper health and survival are needed.

While both study species are listed as Least Concern by the IUCN (IUCN, 2012), *T. macrops* were encountered relatively infrequently (9.1 surveyor hours to find one at all study locations, total 228.7 surveyor hours surveyed during study period) and only the 2 *T. albolabris* were observed at the study sites during the entire study period. Overall, there exists a severe limitation in small bodied snakes as trackable individuals are quite rare given mean body masses often fall well below the minimum size for radio transmitter implantation. Mean body mass for *T. macrops* at the SBR is very close to this accepted minimum, particularly males (Strine et al., 2015). Ethical constraints of number of transmitter implantations due to short battery life must be considered also. Although radiotelemetry can prove challenging for study of small bodied snakes, there are many aspects of green pit viper natural history which still require investigation and can build upon our preliminary work.

Thermoregulation and prey availability have previously been suggested to influence snake space and habitat use in rural environments (Durner & Gates, 1993; Shine & Fitzgerald, 1996; Wisler et al., 2008). Future study of thermoregulation, habitat selection, and prey selection of green pit vipers in rural habitats is required. How vipers utilise non-natural prey and habitat features are subjects which need study. The role of edge habitat and roads are also topics to be explored with regards to green pit viper movement and space use. Increased sample size of males and *T. albolabris* over the course of multiple seasons may serve to better explain potential habitat use differences and niche partitioning within the green pit viper taxon sensu, particularly in non-natural environments.

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# Captive breeding of *Pelophylax* water frogs under controlled conditions indoors

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**ABSTRACT** - Water frogs, genus *Pelophylax*, are culturally and scientifically important taxa and as such are frequently collected and traded in large numbers. This, among other factors, has led to water frog species becoming both threatened in parts of the range of the genus and invasive aliens in others, and they are therefore of conservation interest. Captive breeding has been achieved in outdoor enclosures, but indoor breeding with its advantages of greater control over environment, escapes and pathogen movement, is not frequently achieved and is poorly documented. We present data concerning successful captive husbandry and reproduction of four species of *Pelophylax* (*P. perezi*, *P. shqipericus*, *P. lessonae* and *P. kurtmulleri*) at a private collection in the UK and at Norden's Ark, Sweden. Aquaterraria with flat land areas close to the surface of water were used to house frogs and warm, bright and UVB-radiation rich basking spots were provided, based where possible on field data. Frogs were annually environmentally cycled according to their native climate and reproduced in the spring following an increase in temperature and the end of winter dormancy. The critical temperature for breeding all four species was around 23 °C. Frogs were fed on gut-loaded invertebrates of appropriate size dusted with supplement powders and tadpoles were raised in aquaria and fed on mixtures of algae and animal protein. The similarity of husbandry requirements between species supports the use of closely related species as analogues in determining the husbandry requirements of target taxa. The methods presented here may be used to inform captive breeding programmes.

## INTRODUCTION

Water frogs of the genus *Pelophylax* are distributed across most of Europe, the near and middle East, north Africa and south-east Asia. This speciose genus includes a number of klepton-forming taxa (e.g. Crochet et al., 1995; Dedukh et al., 2015; Sanchez-Montes et al., 2016), which, together with great abundance in the vicinity of human settlements in some species, has made it a frequent model organism for research in fields as disparate as ecotoxicology (e.g. Fasola et al., 2015; Marquez et al., 2011), evolutionary biology (e.g. Canastrelli & Nascetti, 2008; Pruvost et al., 2013; Dedukh et al., 2015) and development (e.g. Marracci et al., 2011). The genus is also culturally important in much of Europe, where it is the native taxon most frequently used in the preparation of frog legs dishes. Although some species are widespread and listed as Least Concern on the IUCN Red List, a number of forms are threatened with extinction and other local populations are also at risk. For example populations in Sweden and the UK represent the edge of the wide European range of *P. lessonae* and are in decline (Sjogren, 1991) or extinct (Sainsbury et al., 2016) and the focus of conservation efforts including captive elements (Buckley & Foster, 2005; Baker & Foster, 2015; Sainsbury et al., 2016). Other forms, are quite the opposite and present an important invasive alien threat in parts of Europe (Holsbeek et al., 2010; Harris et al., 2013); in some cases, threatened *Pelophylax* species may also act as alien invaders outside of their natural range (Domeneghetti et al., 2013).

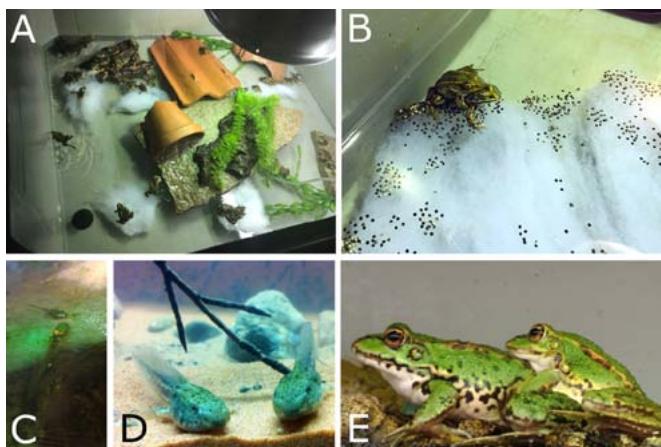
Water frogs are frequently harvested from the wild in large numbers for the laboratory and culinary trades; a search (17/10/17) of the online wholesale trading website alibaba.com identified companies able to export 8 tonnes per month of *Pelophylax* cf. *ridibundus* from Turkey. The reproduction of *Pelophylax* in outdoors vivaria constituting enclosed ponds is well established, although little has been formally published on the subject. Reproduction of these frogs under controlled conditions indoors, however, is not frequently achieved and few if any published data exist. We present some data concerning the captive, indoors husbandry and reproduction of four species of *Pelophylax* in a private collection in the UK and at Norden's Ark, Sweden.

## METHODS

### Species and animal origins

*Pelophylax perezi* and *P. shqipericus* were obtained as eggs from an experimental population at the University of Aveiro, Portugal in 2016, and from a private breeder using outdoors enclosures in 2012, respectively, by CM. Larvae were reared under the conditions outlined below.

*Pelophylax lessonae* and *P. kurtmulleri* were obtained by Norden's Ark, Sweden. *P. lessonae* was obtained under license (dnr 522-1586-08) as wild caught adults (5 pairs) from a wild population in Uppland, Sweden in 2008. *P. kurtmulleri* was collected during a field trip to the island of Milos in 2008. This island population is likely to represent a distinct taxon yet to be named (Vervust et al., 2013). The original group consisted of five adults, five subadults and 100 tadpoles.



**Figure 1.** A. Enclosure used for housing *P. lessonae*. B. Amplexant pair of *P. lessonae* amidst spawn on a mat of filter floss. C. *P. perezi* tadpoles approaching metamorphosis basking under a UVB rich heat spot. D. Advanced tadpoles of *P. lessonae*. E. Amplexant pair of *P. perezi*.

### Environmental measurements

Surface temperatures were measured with non-contact infrared thermometers of various brands and models. Water quality was measured using Salifert re-agent kits. Ultraviolet Index (UVI) was measured using Solarmeter 6.5 handheld units (Solartech, USA). Water temperature was measured using probe thermometers of various brands and models.

### Enclosures

The fundamental characteristics of enclosures used for housing all four species of *Pelophylax* covered here are summarised in Table 1; also see Figure 1A. All enclosures were essentially designed to provide a large amount of open water with low-lying islands allowing frogs to leave the water at both hot and cool ends of the enclosure. These islands were designed to be only just (several mm) above the water line to ensure ease of access. Live and/or plastic plants, as well as filter floss sheeting, provided structures in the water for shelter, calling, oviposition and resting, while plants above the water line provided overhead shelter. Larvae were reared in aquaria of varying size and varying design; water quality and temperature data are presented in Table 1. Water changes were performed as required (as often as daily) to remove waste.

### Heating and Lighting

Frogs were provided with heating and lighting as summarised in Table 2, with overlapping Ultra-violet B radiation (UVB) and thermal gradients. Basking sites were concentrated over land areas and, for *P. shqipericus* and *P. perezi*, also shallow water above mats of algae, aquatic/plastic plants or filter floss sheeting. Ultra-violet index (UVI), collected following the methods used by Michaels and Preziosi (2013), and temperature readings, in June at the University of Aveiro in the ponds where the stock of *P. perezi* originated revealed basking frogs exposed to a maximum UVI of 6 and a basking surface temperature of 30–35 °C and are supported by field measurements of body

temperatures (Meek, 1983). These data were replicated in captivity for *P. perezi*. *Pelophylax shqipericus* occurs at a similar latitude to *P. lessonae* in northern Italy and so the UVI data collected by Michaels and Preziosi (2013) (that is, a UVI up to 3.5) were used to guide maximal UVI exposure; a basking surface temperature of 30–35 °C was used for this species as well.

*Pelophylax lessonae* and *P. kurtmulleri* received similar basking site parameters, which are similar to those recorded for wild *P. lessonae* in France (Meek, 2011) and Italy (Michaels & Preziosi, 2013). Enclosures experienced a drop in both air and water temperature of several degrees, with air temperature falling more; exact changes depended on ambient conditions and time of year.

Tadpole aquaria did not receive direct lighting until animals began to metamorphose and exhibit juvenile, rather than tadpole, skin texture and colouration. Juveniles were maintained in small versions of adult enclosures, with UVB lighting being provided at the same levels and through the same methods as for adults once juvenile colouration was adopted.

### Annual environmental variation and brumation

Annual variation in temperature for *P. perezi* and *P. shqipericus* was achieved through the use of aquarium heaters (various brands and models) connected to external thermostats (Inkbird; various models), allowing manipulation of temperature to 0.1 °C; room temperature was held somewhat lower than aquarium temperature to allow desired values to be achieved without a cooling mechanism. Local maximum/minimum temperature data and photoperiod data from worldweatheronline.com were used to determine the exact environmental values used. Photoperiod and ambient water temperature were adjusted monthly on this basis. Aquaria were also exposed to indirect natural sunlight and associated changing photoperiods. Basking lamps were turned on during the day all year, other than the months of December and January, where no basking sites were provided, to induce dormancy. Frogs were overwintered in the same aquaria in which they were housed the rest of the year.

For *P. lessonae* and *P. kurtmulleri*, from February until end of September the photoperiod was kept at 14 hours. To induce dormancy two months prior to hibernation (October and November) the photoperiod was shortened to 10 hours and basking lamps were turned off. From December until the end of January, *P. lessonae* was moved to hibernation with males and females in separate containers (Samla 78x56x43 cm, IKEA) with ventilation holes drilled in the lid and with a deep layer of moist sphagnum moss as substrate. The temperature was gradually lowered from 15 °C to 5 °C over a five day period and was then kept at this temperature for two months. During the same period the males and the females of *P. kurtmulleri* were maintained in the same boxes as used the rest of the year, but separated by sex. The ambient and water temperature was not reduced for *P. kurtmulleri*, but no basking light was provided, photoperiod was reduced to 10:14 and feeding frequency was reduced.

**Table 1.** Enclosure characteristics for *Pelophylax* species

Species	Adult enclosure type	Adult water depth (cm)	Adult enclosure footprint dimensions (cm)	Adult enclosure furnishings	Filtration	Water chemistry	Maximum adult stocking density (frogs per L)	Maximum larval stocking density (tadpoles per L)
<i>Pelophylax perezi</i>	Glass aqua-terraria (front opening)	15	150x50	Floating cork bark; blocks of filter foam breaking water line; live plants ( <i>Scindapsus</i> , <i>Elodea</i> , <i>Hydrocotyle</i> mats of algae); filter floss	Air-stream sponge filters and internal box filters	Tap water; Alkalinity 180-220mg/L, pH 7.5-8, treated with dechlorinator	0.25	1
<i>P. shqipericus</i>	Glass aquarium (top opening)	15	60x40	Weathered concrete slab resting on bricks to break surface; live plants as above.	None	Tap water; alkalinity <20mg/L; pH 6.5-6.8; treated with dechlorinator	0.3	1
<i>P. lessonae</i>	Plastic box	10-15	100x100	Large flat rocks just above water line; plastic plants; filter floss		Tap water; alkalinity <20mg/L, pH 6.5-6.8, non chlorinated	0.25	1
<i>P. kurtmulleri</i>							0.5	1

**Table 2.** Heating and lighting used for *Pelophylax* species

Species	Lighting array	UVi Gradient (summer maximum)	Basking site temperature (°C)	Age post-metamorphosis at sexual maturity (months)	Winter temperature (°C)	Critical spawning water temperature (°C)	Larval rearing temperature (°C)
<i>P. perezi</i>	T5 fluorescent tubes (D3+ T5, Arcadia; Lightwave T5, Growth Technology) 80W Mercury Vapour lamps (Arcadia).	0-6		8	10		23
<i>P. shqipericus</i>	T8 fluorescent lamp (ZooMed 10% UVB) 80w incandescent lamps, arranged such that great and Uvb gradients were correlated.	2-3	30-35	12	3-5	20-26	23 (but overwintering down to at least 10 possible)
<i>P. lessonae</i>	T5 lamps (Arcadia D3 6%; Arcadia Plant Pro)	3-5		9		20	20-23
<i>P. kurtmulleri</i>	Halogen spots (Osram Halopar Alu 75W)			12	10-15	23-24	

**Diet**

*Pelophylax perezi* and *P. shqipericus* were fed a variety of commercially available insects (mainly *Gryllus* and *Acheta* crickets, *Schistocerca* locusts, *Lumbricus* earthworms), dusted (apart from the earthworms) with calcium and multivitamin/mineral powder (Nutrobal, Vetark), which were placed on the islands for frogs to capture. Food was offered daily during the active months and once a week during cooler periods where frogs were largely dormant. After tadpoles of these species had absorbed yolk sacks, larvae were fed on a mixture of *Spirulina* and *Chlorella* algae, fish flakes (various brands) and spinach until metamorphosis. Initial diets were more plant/algae heavy and slowly the protein content became dominant.

*Pelophylax kurtmulleri* and *P. lessonae* were fed commercially available crickets (*Gryllus bimaculatus*). These were gut-loaded (Superload insect gutload formula, Repashy) 48 hours prior to feeding the frogs. Before

being offered to the frogs they were dusted with calcium (Repti Calcium without D3, Zoomed) and multivitamin/mineral powder (Nutrobal, Vetark). Food was offered three times a week and crickets were placed on the islands for the frogs to catch. After tadpoles of these species had absorbed yolk sacks, larvae were fed on a mixture of turtle food (ReptoMin, Tetra), algae food for fish (Algae wafers, Hikari) and fish fry food (Micron, Sera). Also available until metamorphosis were pieces of cuttlefish bone.

**Collection and treatment of spawn**

Spawn of all species was moved to separate aquaria heated to 20-23 °C, with aquarium heaters if ambient conditions were not warm enough. Spawn was moved without substrate if possible; otherwise small pieces of plant or filter floss sheeting were moved with the spawn and removed, if necessary, once larvae were free swimming. Air-stream sponge filters provided filtration for *P. perezi*

and *P. shqipericus*; the other two species were not kept under filtered conditions.

## RESULTS

### *Pelophylax shqipericus* & *P. perezi*

Calling began in both *P. perezi* and *P. shqipericus* after the end of winter cooling, during which frogs became largely dormant, stopped feeding and rested on the bottom of the tanks underwater. When water temperature began to increase and the basking lamps were turned back on, frogs became active and fed again. Amplexus (Figure 1E) occurred once water temperatures reached 18-20 °C during the day and spawning occurred both during day and night when water temperatures remained between 23-26 °C. Spawning could be readily induced by increasing water temperature to 23-26 °C and heavily feeding animals; dropping temperature again to below 21 °C interrupted breeding behaviour, thereby allowing spawn to be produced on demand throughout the early summer. In *P. perezi*, air temperatures above 28 °C during the late summer caused a reduction in and eventual cessation of calling. Falling temperatures in early September led to a brief resumption of chorusing, but spawn was not produced.

Spawn was deposited in clumps of c. 20-100 eggs on aquatic vegetation and filter floss matting. Spawn hatched within one week and tadpoles began feeding 4-5 days after hatching, having absorbed yolk sacs and become free swimming. Tadpoles were raised successfully in both hard (alkalinity c. 180ppm, pH 7.5-8) and soft (alkalinity <20ppm, pH 6.5) water with comparable success. For *P. perezi*, harder water was used in preference to replicate field data from the source population (a concrete lined pond with alkalinity >200ppm and pH 8). Metamorphosis occurred as quickly as 6-8 weeks in *P. perezi*; tadpoles of *P. shqipericus* sometimes metamorphosed just as quickly; some other individuals overwintered in water as cold as 10 °C before metamorphosing the following spring. Tadpoles, especially those nearing metamorphosis and having begun to adopt adult colouration, frequently basked in shallow water (Figure 1D) with a water temperature of 26-28 °C and a UVi of up to 3. Mortality in tadpoles and juveniles was negligible and largely restricted to animals that were not viable. Animals reached sexual maturity within 6 months, just prior to winter cooling, and reproduced successfully the following breeding season. *P. perezi* juveniles began producing simplified, shortened vocalisations in July at a snout-to-vent length of around 30mm.

### *Pelophylax lessonae* & *P. kurtmulleri*

Calling began in *P. lessonae* shortly after being moved to water with a temperature of 18-19 °C without any extra heating provided. Amplexus occurred once the basking lamps were turned on and the water temperatures reached 21-22 °C. Once filter floss was placed in the water the spawning began. *P. kurtmulleri* needed the basking lamps turned on again and for water temperatures to reach 21 °C and ambient temperature of 22-23 °C to start calling. Amplexus and spawning occurred in *P. kurtmulleri* once water temperatures reached 22-23 °C and with ambient

temperature around 23-25 °C.

*Pelophylax lessonae* spawn was deposited in clumps of c. 20-100 eggs on aquatic vegetation and filter floss matting (Figure 1B), while *P. kurtmulleri* also used stones and roots to deposit their eggs on. Spawn hatched within one week and metamorphosis was achieved within 2 to 3 months. Tadpoles (Figure 1D) were raised without issues.

## DISCUSSION

*Pelophylax* sp. rely on well warmed, sunny areas of relatively still water with rafts of floating vegetation and rarely stray far from water. They are heliophiles and actively bask, exposing themselves to the heat and UVB irradiation of direct sunlight (Michaels & Preziosi, 2013). Historically, indoors enclosures for amphibians were typically lacking in UVB provision and thermal gradients. With increasing understanding of amphibian lighting requirements and the availability of technology to meet them, indoors husbandry for water frogs is now much more easily achievable. Our captive enclosures were designed to recreate the UVB rich, brightly lit and warm environments inhabited by water frogs in nature and these conditions proved successful in maintaining and breeding this genus indoors. Using these methods, we were able to achieve success in maintaining, breeding and rearing *Pelophylax* frogs indoors in captivity. Frogs did not exhibit any noticeable health problems, and did not display any symptoms of metabolic bone disease, a condition which is common in ranid frogs and may result from inadequate calcium or UVB provision (Wright & Whitaker, 2001; Michaels et al., 2015).

Although some field data were available from the microhabitat of *Pelophylax*, which could be used to underpin the husbandry approach taken for housing these taxa, they did not cover the whole year and so some anthropocentric climate data had be integrated into husbandry. Although these sorts of climate data may not even remotely reflect the microclimates used by some amphibians (Michaels et al., 2014), the habitats of *Pelophylax* are typically exposed and so anthropocentric climate databases may broadly reflect the conditions used by frogs. Nevertheless the extremes of anthropocentric climate data were avoided as these may not be the reflected in pool frog microhabitats (Michaels & Preziosi, 2013).

Overwintering temperatures resulting in successful brumation and subsequent reproduction were based on such modified anthropocentric data, and varied according to species reflecting differing geographic origins. However, similar optimal spawning and tadpole rearing of around 23 °C were found in all species, reflecting the similarity in habitat between species. Animals could also be housed under relatively high densities with success, and mortality of tadpoles, juveniles and adults was extremely low. These characteristics are highly favourable to production of large numbers of animals indoors and these methods may prove useful for the generation of captive bred stock for conservation and research. Juveniles did prove to be highly cannibalistic of frogs substantially smaller than themselves, but provided that froglets were size sorted and well fed, cannibalism was avoided.

Indoors reproduction allows animals to be held under controlled environmental conditions, and for more effective barriers to pathogens, predators and escapes to be put in place. Moreover, many laboratory experiments requiring tadpoles produced in captivity resort to artificial crossing requiring the deaths of both parental animals (e.g. Pruvost et al., 2013). By using natural reproduction, parental animals may be kept alive to reduce animal use, to provide the potential for replicate clutches from the same parents, and to improve the quality and viability of progeny, which may be reduced in artificially inseminated spawn (Browne & Zippel, 2007).

The high degree of overlap in the husbandry of these species lends support to the concept of analogue species (Michaels et al., 2014), whereby closely related species may be used to predict the husbandry of closely related taxa. This is likely due to the fact that all European, North African and Near Eastern *Pelophylax* species inhabit similar habitats and speciation is a result of geographic isolation rather than invasion of new niches, which can lead to very different captive requirements (Michaels et al., 2016). Bearing in mind the need to adjust husbandry to reflect subtle differences in local climates, including the overwintering temperature tolerated by different species, relatively common taxa with low extinction risk (here, *P. lessonae*; *P. perezi*; *P. ridibundus*; Bosch et al., (2009); Kuzmin et al. (2009a; b)) may be used as a predictor and training tool to prepare husbandry resources for threatened species (here, *P. shqipericus*; Uzzell & Ivailovic (2009)). It is also important to note that *P. perezi* and *P. lessonae* have very large distributions, incorporating a large range of altitudes and latitudes, and so captive husbandry parameters, especially optimal overwintering temperature, may vary between populations. For example, *P. perezi* in this study originated from coastal Portugal, where winter temperatures rarely fall much below 10 °C and snow or frost are absent; the same species extends into mountainous northern Spain where populations will tolerate snow and ice throughout the winter. The data presented here should therefore be extrapolated with some caution to other populations and species of *Pelophylax*, with field or at least local weather/climatic data, especially surrounding overwintering temperatures, integrated into husbandry strategies.

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# Apparent envenomation of a captive blue dart frog (*Dendrobates tinctorius*) by an eyelash viper (*Bothriechis schlegelii*) without symptoms of toxicity

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Interactions between species are key factors in ecology and evolution (Crawley, 1992; Futuyma & Slatkin, 1983). However, direct observations of interspecific interactions between many reptile and amphibian species are relatively rare in the wild and captive observations can help supplement our knowledge. In the case of venomous predators, toxin resistance in prey has evolved on numerous occasions to facilitate escape (McCabe & Mackessy, 017). Herein we report a case of an eyelash viper (*Bothriechis schlegelii*) biting and seemingly envenomating a blue dart frog (*Dendrobates tinctorius*) in a multispecies enclosure in captivity maintained by one of the authors (MV).

The enclosure measured 80 cm x 40 cm x 50 cm and in addition to the two individuals involved in the interaction housed another two *D. tinctorius*, two *Phyllobates terribilis*, one *D. leucomelas*, and one *Epipedobates tricolor*. Temperature is maintained with a gradient of 25 °C to 32 °C using a spotlamp and relative humidity is kept high (ca. 80-90%) by twice daily spraying of water (including ensuring bromeliads are filled). Water is also provided in a dish which is changed daily. Substrate is ExoTerra Plantation Soil covered by a layer of moss, and drainage is enhanced by means of a layer of clay balls under the soil (separated by an antislip mat). A variety of plants are used to provide shelter (*Anthurium*, *Bromelia*, *Calathea*, *Dieffenbachia*, *Spathiphyllum*, *Codiaeum*, and *Epipremnum*) and dried vines provide opportunities for climbing. The eyelash viper is fed two thawed pinkie mice every two weeks from tweezers. The frogs are collectively fed every other day with fruitflies supplemented with Dendrocare Vitamin and Mineral Powder, and additional supplementation is given by spraying with a soluble vitamin and mineral solution once per week. Occasionally *P. terribilis* are fed additional young dubia cockroaches (*Blaptica dubia*) from tweezers as these frogs compete poorly for food in this enclosure.

On 14 September 2017 at 00:04 GMT+1 during spraying of the enclosure to maintain humidity, the *B. schlegelii* moved away from the spray and fell to the floor of the enclosure near several of the frogs. MV carefully nudged the frogs away from the snake using a snake hook and attempted to move the snake back onto the branches. However, the snake was very active and would not balance on the hook and so remained on the ground. Subsequently, one of the *D. tinctorius* jumped and landed directly on the *B. schlegelii*, which responded immediately by biting

the frog on the proximal part of its rear-right leg (fangs entering on the dorsal surface; Fig. 1). The snake made 'chewing' movements and was attached to the frog for about four or five seconds, during which time the frog did not struggle. Upon release, drops of a straw-coloured fluid were visible on the fangs of the snake and at the bite site on the frog. This seems highly likely to be venom, particularly given the length of time the *B. schlegelii* held on for and the chewing behaviour, although we cannot conclusively discount the possibility of leaking lymph fluid. Nevertheless, we tentatively consider this evidence of envenomation. Approximately five minutes post-bite, the *D. tinctorius* was observed to sit in the water bowl for about 1.5 hours, and at the time of writing (170 hours post-bite) the frog has not shown any adverse reactions or obvious symptoms of toxicity from envenomation.

There are surprisingly few published records of resistance in frogs to snake venoms, making our observations an important addition to the literature. In fact, Minton & Minton (1981) found that frogs were more susceptible to Australian elapid venom than were lizards. Heatwole et al. (1999) reported resistance to *Agkistrodon*



**Figure 1.** Bitten individual *D. tinctorius* approximately 18 hours post-bite. Red circle highlights the location of the bite, though no clearly visible evidence remained at this time.

viper venom in bullfrogs (*Rana catesbeiana*), but although the post-envenomation survival time varied with age, all frogs died from the venom and so meaningful resistance is not clearly demonstrated in this case. Similarly, Gibbs & Mackessy (2009) reported that leopard frogs (*Rana pipiens*) had LD<sub>50</sub>'s for *Sistrurus* rattlesnake venoms an order of magnitude higher than mice or lizards. However, those authors reported an LD<sub>50</sub> of ~90 mg/kg in the frogs, but with the average venom yield they give of 30 mg there seems little chance of a frog surviving a bite, given *R. pipiens* weigh only ~25–45 g (Wright, 2005).

Although *B. schlegelii* has not been previously reported to predate *D. tinctorius* in the wild, we note that few dietary records exist and those that do suggest a broad diet including several frog species (Sorrell, 2009). Furthermore, although *B. schlegelii* is predominantly nocturnal (Sorrell, 2009) and *D. tinctorius* is diurnal (Born et al., 2010), *B. schlegelii* is occasionally active during the day and can catch prey during this time (Sorrell, 2009). Similarly, although *B. schlegelii* is an arboreal pitviper and *D. tinctorius* is predominantly terrestrial, the latter has been found to climb tree trunks to a height of up to 40 m (Born et al., 2010). Hence, these species may interact in the wild, although this is likely to be a relatively uncommon event. Despite the presumed rarity of this interaction occurring in the wild, the observations reported herein suggest that *D. tinctorius* may possess an ecologically-relevant degree of toxin resistance to the venom of *B. schlegelii*, and this is worthy of further investigation.

Finally, despite the incident reported here, the multispecies enclosure used has been maintained for about 5.5 months with no other antagonistic interactions. Hence, we suggest that dendrobatiid frogs and eyelash vipers are generally suitable candidates for multispecies enclosures, particularly when rare antagonistic interactions seem to result in limited clinical effects (as reported here). Even the degree of stress that is likely inherent in any multispecies enclosures may be offset by possible benefits in the form of environmental enrichment (Burghardt, 2013), providing species are compatible.

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## Bird predation by Hispaniolan vinesnakes (Dipsadidae, *Uromacer*)

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Although West Indian dipsadid snakes are known to prey on birds, most occurrences of ornithophagy have been reported in largely ground-dwelling species (Table 1). The snake genus *Uromacer* is endemic to Hispaniola, comprising three species distributed across the main island and several satellite islands. Species of *Uromacer* have green, or brown and green, coloration, are of moderate length (800–1500 mm snout-vent length), and gracile. Among dipsadids, they are the only predominantly arboreal species in the West Indies and are diurnal. They are both active and ambush predators, and their prey is subdued with the aid of venom delivered by fangs situated toward the back of the mouth. The two long-snouted species (*U. frenatus* Günther, 1865 and *U. oxyrhynchus* Duméril, Bibron & Duméril, 1854) are ambush predators known to feed largely on diurnally active lizards (*Anolis*, *Leiocephalus*, *Ameiva*) to the extent of being considered exclusively saurophagous (Henderson et al., 1987). A third species, *U. catesbyi* Schlegel, 1837, is heavier-bodied with a blunt snout and its diet includes a large proportion of frogs (especially the hylid *Osteopilus dominicensis*; Henderson et al., 1987; Landestoy, pers. obs.) that are located by way of active foraging. Recently, however, *U. oxyrhynchus* has also been observed feeding on a frog (*Eleutherodactylus inoptatus*; Landestoy, pers. obs.). To date, only a single incidence of predation on a bird by a species of *Uromacer* has been reported (Henderson & Powell, 2009). Herein we present fortuitous encounters of species of *Uromacer* preying on birds. All observations were made in the Dominican Republic.

*Uromacer* sp.: February 2012: An adult snake was observed with a palm chat (*Dulus dominicus*) in its mouth. The head of the bird was in the snake's jaws and it was vigorously flapping its wings. The bird's struggle diminished over about 15 min. Observations were terminated before swallowing commenced. Birds in proximity to the event appeared agitated (C. Rimmer, pers. comm., 2012).

*Uromacer catesbyi*: October 2002: At 12:00 h, an adult snake (approx. 1.3 m total length) was observed subduing a juvenile bananaquit (*Coereba flaveola*) in the undergrowth (at ~1.5 m) of well-shaded riparian forest. The bird's movements had stopped by 12:05 h (Fig. 1).

30 July 2016: At ~14:05 h, an adult *U. catesbyi* was spotted at the entrance of a Hispaniolan Woodpecker



Figure 1. *U. catesbyi* subduing a young Bananaquit (*C. flaveola*)

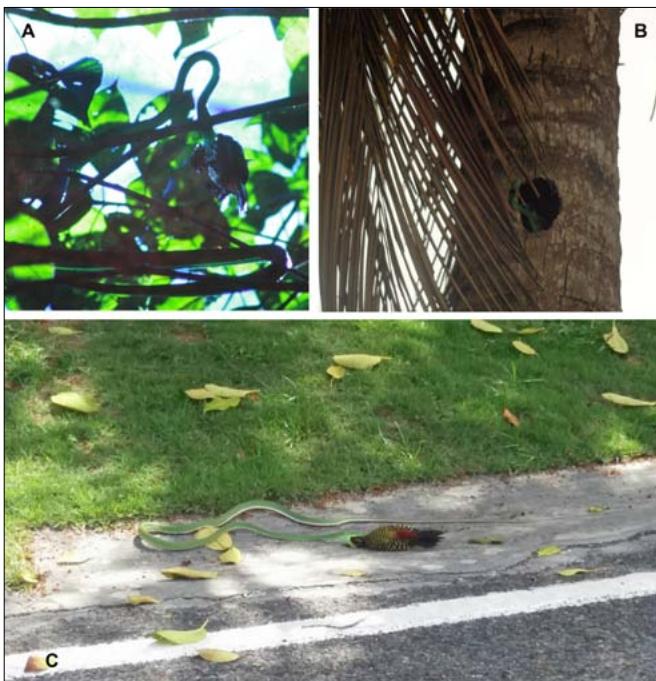
(*Melanerpes striatus*) nest cavity in a coconut palm (*Cocos nucifera*) at the Cap Cana grounds, La Altagracia Province (Fig. 2B). The snake's neck was distended suggesting it had just ingested one of the nest's occupants.

*Uromacer oxyrhynchus*: 2003: At about 12:10 h, and about 6.0 m above ground, an adult snake (approx. 1.6 m total length) was observed holding a Palm Chat (*Dulus dominicus*) by the head (Fig 2A) while other birds (*Mimus polyglottos*, *Phaenicophilus palmarum*, *Quiscalus niger*) appeared agitated and were vocal.

May 2015: At ~11:00 h, a *U. oxyrhynchus* with a total length of 1.69 m was found in a street gutter swallowing an adult Hispaniolan Woodpecker (*M. striatus*) headfirst (Fig. 2C) at the Cap Cana grounds, La Altagracia Province; swallowing was completed within 30 min and the snake then moved into dense vegetation. When first observed, the bird was motionless and the nearest large trees were ~20 m away; consequently, the possibility of scavenging cannot be discounted.

**Table 1.** West Indian dipsadid snakes known to prey on birds. With the exception of species of *Uromacer*, all of the species listed are largely ground-dwelling. Details of *Uromacer* predation appear in the text.

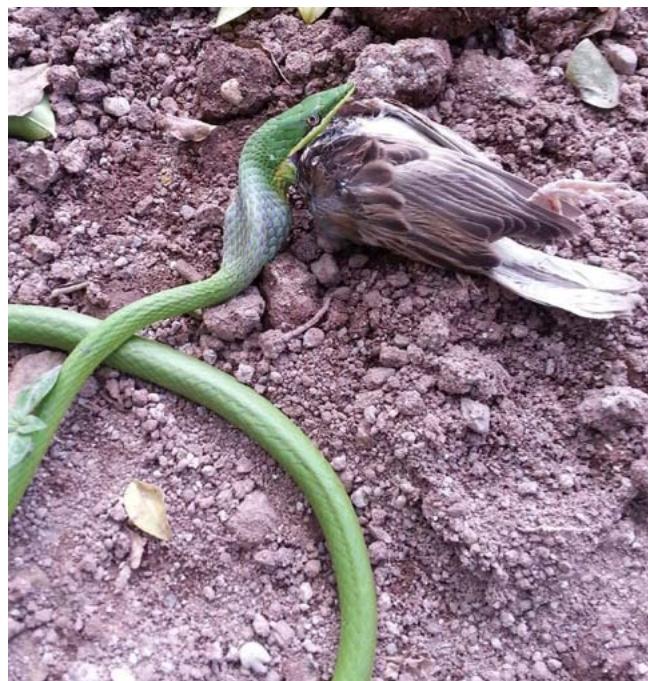
Snake species	Bird species	Island	Reference
<i>Borikenophis portoricensis</i>	<i>Coereba flaveola</i>	Puerto Rico	Perez-Rivera & Laboy-Rivera (1996)
<i>Cubophis cantherigerus</i>	<i>Columbina passerina</i> <i>Turdus plumbeus</i>	Cuba	Reyes-Vazques et al. (2013)
<i>Cubophis fuscicauda</i>	<i>Xiphidiopicus p. percussus</i> “Honey Creeper” (likely <i>Coereba flaveola</i> )	Cuba Cayman Brac (Cayman Islands)	Rodríguez-Cabrera (2017) Grant (1940)
<i>Cubophis vudii</i>	<i>Geothlypis trichas</i> Unidentified	Eleuthera (Bahamas) Cat Island (Bahamas)	Schwartz & Henderson (1991) Henderson & Sajdak (1996)
<i>Haitiophis anomalus</i>	<i>Crotophaga ani</i>	Hispaniola	Landestoy et al. (2013)
<i>Uromacer</i> sp.	<i>Dulus dominicus</i>	Hispaniola	This paper
<i>Uromacer catesbyi</i>	<i>Coereba flaveola</i>	Hispaniola	Henderson & Powell (2009); this paper
<i>Uromacer oxyrhynchus</i>	<i>Dulus dominicus</i> <i>Passer domesticus</i> <i>Melanerpes striatus</i>	Hispaniola	This paper



**Figure 2.** A. *U. oxyrhynchus* holding a palm chat (*D. dominicus*). B. *U. catesbyi* exiting the interior of a nest cavity of a Hispaniolan woodpecker (*M. striatus*). C. *U. oxyrhynchus* with an adult Hispaniolan Woodpecker

3 June 2015: In the yard of a house in the rural countryside (Inoa, San José de las Matas, Santiago Province, elevation 360 m) at 16:15 h, an adult snake (approx. 1.1 m total length) was observed holding a house sparrow (*Passer domesticus*) (Fig. 3). After about 20 min, swallowing stopped and the snake regurgitated the bird.

Although smaller prey (arboreal/scansorial lizards and frogs) make up the vast majority of prey items recorded for species of *Uromacer* (Henderson et al., 1987), it is not surprising that avian prey is occasionally and opportunistically consumed. All of the predated birds were diurnally active, suggesting that captures were made by



**Figure 3.** *U. oxyrhynchus* swallowing a house sparrow (*P. domesticus*). Deglutition stopped after several minutes.

ambush. The lone (possible) exception is of *U. catesbyi* feeding at the nest cavity of a woodpecker. That likely would have required active foraging and, of the three species of *Uromacer*, *U. catesbyi* is the most active forager (Henderson, 1987). Also of note, all of our observations were of adult snakes (juveniles and subadults may lack the size and strength to subdue avian prey), and we lack documentation of avian prey for *U. frenatus*, the member of the genus that feeds most frequently on ground-dwelling prey (Henderson et al., 1987).

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# Absence of chytrid fungus (*Batrachochytrium dendrobatidis*) in an introduced population of the common midwife toad (*Alytes obstetricans*) in Cambridge, UK

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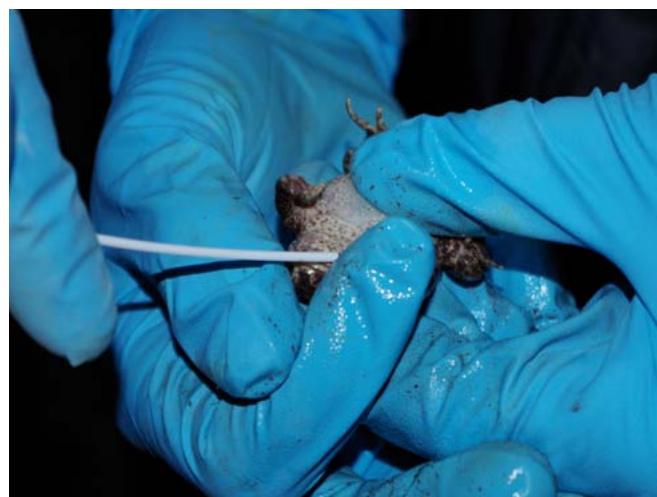
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Amphibian populations globally are currently declining at an alarming rate due to a number of factors, with disease being one of the most troublesome (Stuart et al., 2004). The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) has been implicated in the decline of amphibian species globally and is thought to have been spread through the introduction of non-native species (Fisher & Garner, 2007). Recently a new chytrid fungus (*B. salamandrivorans*) affecting salamanders was discovered (Martel et al., 2014). In recent experiments it was demonstrated that the common midwife toad (*Alytes obstetricans*) was able to act as a reservoir of this new amphibian chytrid fungus without showing any clinical signs (Stegen et al., 2017). In Europe, *B. dendrobatidis* (hereafter *Bd*) has a limited effect on most amphibian species (Duffus & Cunningham, 2010) although *A. obstetricans* is one of the more susceptible species; it is capable of spreading a number of infectious diseases to other amphibians. It is therefore important to monitor and screen any populations which may have an impact on further amphibian species. *Alytes obstetricans* has been established in Cambridge, UK for at least a decade (Baker, 2007) with very little monitoring have taken place before our study.

It is currently unknown where the toads originated from or the detail around their release but it is likely that they originated as pets which escaped into the local environment. The toads are currently restricted to the back gardens of a small block of parallel Victorian houses not far from Cambridge city centre (Baker, 2007). Due to the mosaic of habitats available to the toads, they are able to persist despite being outside of their natural range in northern Europe. The species has been part of the British landscape for over a century (Beebee & Griffiths, 2000) and it is not clear whether or not they pose a threat to our native amphibian species. This study aimed to determine whether or not the toads could be a potential threat as a disease vector in terms of being infected with the amphibian chytrid fungi.

Between June 2016 and May 2017, five gardens were investigated for the presence of midwife toads. This involved the use of call playbacks which were designed to elicit responses from males so that their location could be pinpointed (Allain & Goodman, 2017). Females and juveniles were located by actively searching potential refugia sites within each of the gardens. When midwife

toads were located, they were placed in separate zip-lock bags for a short duration until the search had been completed; this was on average between twenty and thirty minutes. All appropriate biosecurity measures were taken when handling the toads including the use of nitrile gloves (Mendez et al., 2008), the changing of gloves between each site and the use of Virkon S when disinfecting field equipment after use (Young et al., 2007). Before the toads were swabbed, data such as their sex, weight and snout to vent length were taken. A set of digital scales and plastic Vernier callipers were used to collect this data. As part of the swabbing process, each individual was sprayed with a small amount of water in order to free their ventral surface of any detritus which may interfere with the analysis process (Kosch & Summers, 2013). Sterile cotton tipped swabs (Medical Wire & Equipment, MW-100) were used to swab the abdomen, thighs, groin and feet of each individual between 10 and 15 times (Fig. 1). Each of the midwife toads were photographed for future reference before being released at the point of capture.



**Figure 1.** One of the midwife toads is swabbed before being released

Nine individuals were caught and screened; of which 4 were male, 2 female and 3 sub-adults. Midwife toads were found in all of the gardens surveyed and could be heard calling from others in the surrounding area. The snout to vent length of the *A. obstetricans* sampled averaged 3.43 cm (SD = 0.91 cm) and their mean weight was 5.49 g (SD

= 3.05 g). All of the swabs were sent off for analysis using qPCR to test for the presence of both amphibian chytrid fungi. The swabs were tested at the Institute of Zoology at the Zoological Society of London in July 2017. All samples were qPCR negative for both *Bd* and *Bsal*. The samples were refrigerated at 5 °C before they were sent to the lab for analysis. Our initial results are promising but further sampling is required to rule out infection in the rest of the population. The individuals sampled make up approximately 10 to 20% of the suspected post-metamorphic population. We are yet to sample any tadpoles, mainly due to difficulty in identifying the breeding pond(s). While the toads are cryptic in nature (Beebee & Griffiths, 2000), determining their potential location became easier as we developed our search image for the species. It is unlikely that the toads will spread further without anthropogenic intervention due to barriers that have been built in the environment, such as walls and especially roads, which have been shown to limit amphibian dispersal (Carr & Fahrig, 2001).

## ACKNOWLEDGEMENTS

We thank the local residents for allowing us access to their gardens as well as Matthew Perkins of the Institute of Zoology, Zoological Society of London for carrying out the qPCR analysis and Liam Smith for helping us to get the project underway. The work carried out was funded by the Amphibian and Reptile Groups UK through the 100% Fund.

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# An updated distribution of the Arabian cat snake

## *Telescopus dhara* in the United Arab Emirates

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The Arabian cat snake *Telescopus dhara* is a rear-fanged venomous member of the colubridae family. Adult Arabian cat snakes are typically reported to be between 60 and 70 cm long, with the largest individual cited in literature measuring 113.2 cm total length (Egan, 2007). The known distribution of this species includes the mountain ranges of Arabia, from the Gulf of Aqaba in the north down through the western and central mountains of Saudi Arabia; the range continues through Yemen and throughout rocky and mountainous areas of Oman and the UAE (Gasperettii, 1988; Gardner et al., 2009; Soorae et al., 2010). Usual body colour of the Arabian cat snake varies from shades of brown to orange, often with darker transverse markings on the dorsal surface. The ventral surface is cream-coloured and otherwise not pigmented (Gardner, 2013).

The first record of *T. dhara* from the UAE was published by Egan (2007). This record pertains to an orange-coloured specimen caught in Sharjah. Egan (2007) suggested this specimen was probably transported in date palms from northern Oman. In the following 10 year period there appears to be only a handful of subsequently published records (Gardner et al., 2009) and a handful of anecdotal reports from the UAE. Potential habitat of *T. dhara* exists throughout the Hajar mountain range and multiple records from both the more northerly Musandam Peninsula and Oman to the south lend further support to a potentially more continuous area of occupancy within the UAE.

This communication reports on two further records of *T. dhara* from Wadi Helo in south-eastern UAE. The first snake was observed by GT and GB on the night of 21 July 2017 at 22:12 hrs within Wadi Helo ( $25^{\circ} 00'50''$  N,  $56^{\circ} 21'40''$  E). It was found on a tree stump in farmland at 383 masl when discovered and was of the 'orange' colour morph, which has previously been recorded in the UAE. The second individual was observed during a night time search in Wadi Helo on the north-eastern facing slope of the western edge of the valley ( $25^{\circ} 01'10''$  N,  $56^{\circ} 12'10''$  E). The snake was seen at around 01:00 hrs on the morning of 3 October 2017. When discovered it was moving slowly across a loose rocky slope at 555 masl. This specimen of *T. dhara* (Fig. 1) was approximately 50 cm total length and of the known 'orange' colour morph. These findings support the suggestion by Gardner et al., (2009) that the majority of northern Hajar *T. dhara* may be of the orange colour morph. On both occasions, after taking photographs, these snakes were released where they were found.



Figure 1. Arabian cat snake from 3 October 2017, Wadi Helo



Figure 2. Habitat of *T. dhara* in Wadi Helo

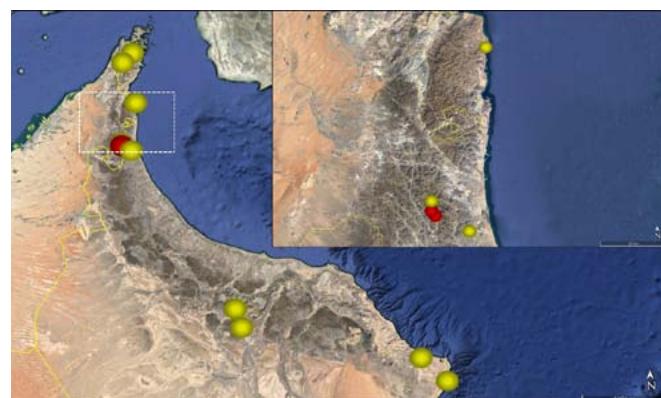


Figure 3. A map of the current published records for the Arabian cat snake in the Hajar Mountains of Oman and the UAE. Published records are indicated by yellow icons. Our new contributions from the UAE are indicated in red.

A specimen of *T. dhara* from Wadi Helo is shown in figure 1 and the typical habitat of this species is shown in figure 2. Figure 3 shows a map of the known records of Arabian cat snake from the Hajar Mountains of Oman and UAE, with an inset showing the known UAE records.

While not representing any significant range extension, the findings reported here do appear to be the only published records of *T. dhara* from the UAE within the last 8 years (Gardner, 2009) and prior to the year 2007 there were no records from the UAE (Egan, 2007). The two new records provided in this note add important data to the current known distribution and status of the Arabian cat snake within the UAE and suggest that there is a stable population in at least the south of the country and that Wadi Helo represents an important area for *T. dhara* in the UAE, with three of five records from the country being from this location. However, it is possible that there is a sampling bias at this site due to its more accessible nature when compared to other parts of the Hajar Mountains. The few records of the Arabian cat snake from the UAE suggest that the species may be uncommon and we agree with the suggestion of Gardner et al. (2009) that this species is deserving of listing on the UAE Red Data List of species of conservation concern.

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# Winter aggregations of adult red salamanders (*Pseudotriton ruber*)

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Information on the winter ecology of amphibians is important for understanding their biology, management and conservation, particularly in temperate climates where amphibians may spend several months overwintering (Irwin, 2005). However, few data are available on winter ecology of many amphibians, especially salamanders (Irwin, 2005; Lannoo, 2005). *Pseudotriton ruber* are medium-sized, semi-aquatic salamanders occurring generally from New York State to Louisiana and westward to Indiana in the eastern United States (Petraska, 1998; Hunsinger, 2005). Typically, adult *P. ruber* move from terrestrial to aquatic habitats (e.g., streams, springs) during late fall, and presumably overwinter at these sites (Bishop, 1941; Bruce, 1978; Petraska, 1998). While abundant in these aquatic habitats during the fall and subsequent spring, *P. ruber* have been noted to be difficult to locate during winter (Bruce, 1978; Pfingston, 1989; Hunsinger, 2005) and typically disappear from the surface cover of streams and springs, leading to the suggestion they may occupy inaccessible, subterranean retreats within streams and springs during the winter months (Pfingston, 1989; Hunsinger, 2005).

During winter 2016-2017, two instances of adult *P. ruber* congregated underneath a single rock within two separate springs were observed in the South Branch Roaring Creek watershed, Northumberland County, Pennsylvania, USA (40.831°N, 76.502°W, WGS 84 grid). On 27 December 2016 at 14:00 h (United States Eastern Standard Time) at an air temperature of 4 °C, eight adult *P. ruber* were congregated underneath a small (~20 x 15 cm diameter) rock within an unnamed spring. On 16 January 2017 at 13:45 h (United States Eastern Standard Time) at an air temperature of 0 °C, 22 adult *P. ruber* were found congregated underneath a medium-sized (~40 x 30 cm in diameter) rock within an unnamed spring (Fig. 1). During each observation, the salamanders were active and began dispersing from their aggregation immediately upon removal of cover (Fig. 1). In each case, the rocks were carefully returned to their original position and the salamanders gently coaxed back underneath the retreats.

Few reports have appeared in the literature regarding aggregations of adult *P. ruber*. Niemiller et al. (2006) found 14 adult *P. ruber* collectively underneath several rocks within a cave stream whilst Walker (1931, in Pfingston, 1989:272) found a concentration of 22 adult *P. ruber* within “a few square meters” in a spring in Ohio during March. During the present field work further rocks and other cover



**Figure 1.** Aggregation of adult *P. ruber* underneath a single, uplifted stone within a spring in eastern Pennsylvania during January 2017

(e.g., woody debris) at each spring were gently lifted but no more adult *P. ruber* were found. However, adult and larval salamanders of *Desmognathus fuscus* and *Eurycea bislineata* were found underneath other cover objects within each spring. Thus, it is unclear why a number of adult *P. ruber* aggregated underneath a single cover rock when other (presumably) suitable cover was present within each spring. Niemiller et al. (2006) found nest attending *P. ruber* in their cave aggregation; suggesting these individuals aggregated for reproduction. However, no nests were associated with the aggregating *P. ruber* in Pennsylvania. Additionally, because breeding in *P. ruber* occurs outside of the coldest winter months (i.e., breeding season of this species varies geographically but is thought to occur outside of the cold winter months, Petraska, 1998), this aggregation appears to be unrelated to mating. Climate records for Northumberland Co., Pennsylvania show that temperatures during winter 2016-2017, including the months of December and January during which the present observations took place, were warmer on average than previous years (<https://www.usclimatedata.com/climate/elysburg/pennsylvania>). Thus, it is possible that above-average temperatures might have stimulated mating later in the season. Walker (1931, in Pfingston, 1989) suggested that the aggregation of *P. ruber* he observed was related to hibernation. Because the observations reported occurred during winter and

presumably outside of the known breeding season for *P. ruber*, it seems more likely that these aggregations were due to overwintering, although mating might also have been involved. Perhaps some unknown physical factor made these particular rocks more suitable for overwintering than other cover available within each spring. These observations of adult *P. ruber* aggregating underneath rocks at the surface of springs, while limited, contribute an important observation regarding the winter ecology of this species.

## ACKNOWLEDGEMENTS

I thank Andrea Swartz for assistance in acquiring literature references and thank Suzanne M Coombes, Roger Meek and an anonymous reviewer for comments on the manuscript. Observations reported herein were conducted incidentally while collecting data as a volunteer for the Pennsylvania Amphibian and Reptile Survey (PARS). The salamanders were released unharmed and the author complied with all Pennsylvania regulations.

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# Observations of yawning behaviour in the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*)

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**Y**awning behaviour has been observed in a variety of vertebrate taxa, including fish, mammals, reptiles, amphibians, and birds (reviewed by Baenninger, 1997). Yawning by salamanders has been documented in the families Ambystomatidae, Plethodontidae, and Salamandridae (reviewed by Bakkegard, 2017). While conducting an ex situ, conservation-driven study regarding behaviour of eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*), large (up to 74 cm total length), fully aquatic salamanders native to cool, highly oxygenated streams and rivers in the eastern United States (Nickerson & Mays, 1973), we incidentally observed instances of behaviour consistent with yawning.

As part of a larger study, we used GoPro Hero 4 camcorders (GoPro Inc., San Mateo, CA) to record video of *C. a. alleganiensis* within raceways set up to simulate the source stream of the hellbenders. Following recording, we reviewed video footage and quantified behaviour of *C. a. alleganiensis*. During the video review, we noted four instances during which a *C. a. alleganiensis* produced a “gaping-like” behaviour lasting for one or more seconds (Bakkegard, 2017) and in which a slow opening of the mouth was followed by a more rapid closing (Baenninger, 1997). All four instances of yawning behaviour were displayed by the same individual, an adult *C. a. alleganiensis* measuring 45.0 cm total length. All observations occurred on the same night (30 July 2016, beginning at 23.36 h) over a period of about 1.5 h when the *C. a. alleganiensis* was positioned with only its head protruding from a ceramic tile used by the animals in our study as a source of cover. The *C. a. alleganiensis* yawned four times at intervals of approximately 26, 37, and 34 minutes between yawns, respectively. Yawn duration ranged between 3 and 8 seconds (mean duration = 5.75 seconds).

To our knowledge, this appears to be the first report in the primary literature of yawning behaviour in *C. alleganiensis* and may be the first report in the primary literature for the Cryptobranchidae, although several online videos exist of Japanese giant salamanders (*Andrias japonicus*) engaging in behaviour consistent with yawning (e.g., <https://youtu.be/u5gdoom6wdI>). The causative mechanisms of yawning in vertebrates is presently unclear, but the stimulus and function of yawning likely varies among taxa (Baenninger, 1997). Bakkegard

(2017) observed instances of yawning behaviour in the salamander *Phaeognathus hubrichti* while the salamanders remained with only their heads and forelimbs protruding from burrows, presumably waiting for passing prey. Thus, yawning behaviour in these salamanders was interpreted to function as a means of heightening arousal (i.e., increasing olfaction and/or prepping jaw musculature) in anticipation of feeding (Bakkegard, 2017). The position (i.e., only the head protruding from cover) of the *C. a. alleganiensis* while yawning occurred is consistent with a sit and wait foraging position, a typical behaviour of this species (Nickerson & Krysko, 2003). Thus, both salamander species might employ yawning behaviour for similar reasons. Alternatively, yawning behaviour in *C. a. alleganiensis* may serve as a means of adjusting ballast. *Cryptobranchus alleganiensis* possess lungs, although respiration in this species is primarily cutaneous (~90%). The lungs are thought to serve primarily a hydrostatic function (Nickerson & Mays, 1973). Yawning could change the water volume in the lungs and so adjust ballast. Although our observations are limited and incidental, further observational data could readily be collected in zoos already maintaining salamanders in captivity to further explore details of yawning behaviour in *C. a. alleganiensis* and other salamanders.

## ACKNOWLEDGMENTS

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## First observations of oophagy in a wild population of the sand boa (*Eryx jaculus*)

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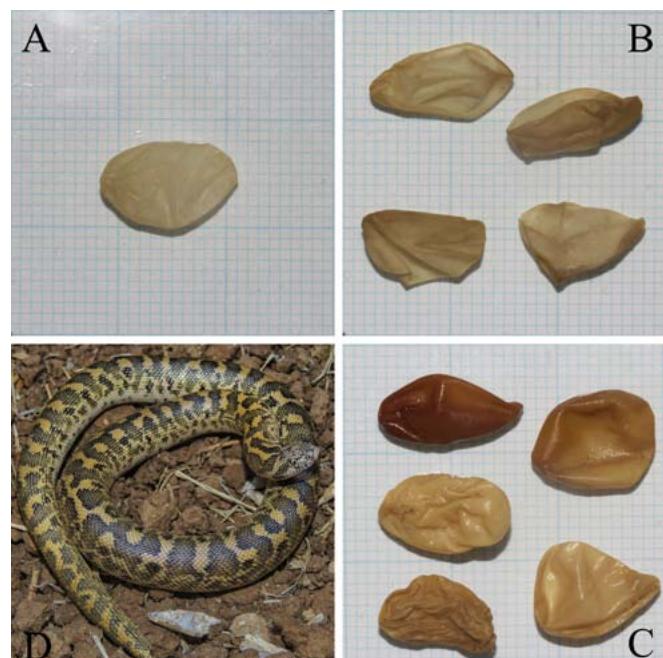
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The sand boa *Eryx jaculus* (Linnaeus, 1758) is found in the southern Balkans, Middle East and North Africa (Sindaco et al., 2013). Only recently the presence of this species has been confirmed in Italy, in a small area of southern Sicily (Insacco et al., 2015). Knowledge of the sand boa in Sicily is limited with few data on geographical distribution, morphology, and habitat (Insacco et al., 2015; Faraone et al., 2017). Even at a global scale the biology of this species is little known due to its secretive habits (Tokar & Obst, 1993).

As predators, sand boas have been reported to adopt both "sit-and-wait" and "active foraging" strategies (Tokar & Obst, 1993) and their prey has been listed as small mammals, lizards and, occasionally, birds, insects, and slugs (Tokar & Obst, 1993; Schleich et al., 1996). Oophagy has only been reported in captivity and only of snake eggs (Schleich et al., 1996). In this paper we report the first field observations of oophagy by the sand boa.

On 7 July 2017, at the locality "Contrada San Francesco di Paola" near Licata (province of Agrigento) (see Insacco et al., 2015), a young female (SVL: 21 cm) that had fallen into an abandoned cistern was rescued. On 13 July 2017, at 9:30 pm, a sub-adult male (SVL: 31 cm) was found lying on the surface of the road SP11 (3 km north-east of Licata) and an adult female (SVL: 41 cm) was found dead close by. All snakes were found in an agricultural area dominated by arable land and olive groves where, if alive, they were quickly released. During a rapid health-check before release, the two live individuals defecated and the faeces were collected and preserved in absolute alcohol. The roadkill female was dissected and the stomach contents were preserved in the same way. Faecal remains and ingesta were analysed using a stereomicroscope. In the faeces of the young female and sub-adult male and in the ingesta of the adult female, one, four, and five saurian eggs were found respectively (Fig. 1). The eggs were rehydrated by the addition of water and were then measured using a digital calliper, with 0.01 mm precision. The average polar diameter was 13.04 mm (SD: 0.77; range: 12.00-14.41). The proportions and the parchment texture of eggs allow us to attribute them to the genus *Podarcis*. Both the Italian wall lizard (*Podarcis siculus*) and the Sicilian wall lizard (*P. waglerianus*) coexist in the area. As a consequence, it is quite difficult to correctly attribute the eggs to specific



**Figure 1.** Lizard eggs in the faeces of the young (A) and sub-adult male (B) sand boas. Eggs ingested by the adult female (C). Sand boa, sub-adult male, July 13 2017 (D).

level. The ingesta of the roadkill female were found in different sections of the gut. One egg seems to have been freshly ingested while the others were close to the cloaca. This arrangement suggests at least two different predation events.

Lizard eggs have been found in the diet of a several species of European snakes. In the smooth snake (*Coronella austriaca*) oophagy of saurian eggs is well known in Spain (Galán, 1988, 1991; Galán & Fernández-Arias, 1993; Amat, 1998; Moreira et al., 2011), and single cases have been observed in the peninsular Italy (Lunghi et al., 2015) and Sicily (F. P. Faraone, unpublished data). This behaviour has also been recorded in the southern smooth snake (*C. girondica*) (Luiselli et al., 2001) and in the ladder snake (*Zamenis scalaris*) (Pleguezuelos et al., 2007).

The cases described in this paper are the first known regarding the sand boa. It is striking that all three specimens had ingested lizard eggs, suggesting that this is a common prey item. *P. siculus* and *P. waglerianus* can lay

eggs throughout much of the spring and summer months (Corti & Lo Cascio, 1999) suggesting that they could be a frequently exploited resource.

## ACKNOWLEDGEMENTS

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# *Hierophis viridiflavus* (Lacépède, 1789)

## feigning death when handled

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Feigning death, often referred to as thanatosis, has been reported for many snake species and is described as a passive defensive behaviour. It involves reduced responsiveness to external stimuli, and is thought to have an anti-predator function, some benefits of which have been proposed by Gregory et al., (2007). With the exception of *Rhinechis scalaris*, thanatosis has been observed in all other colubrid species of metropolitan France e.g. *Natrix natrix* (Gregory et al., 2007); *N. maura* (Fernández-Guiberteau, 2016); *N. tessellata* (Sterijovski et al., 2011); *Coronella austriaca* (Jelić & Vilaj, 2011); *C. girondica* (de Castro-Expósito et al., 2017); *Zamenis longissimus* (Liftme & Liftme, 2014); *Malpolon monspessulanus* (Sannolo et al., 2014); and lastly *Hierophis viridiflavus* (Rugiero, 1999). However, while thanatosis is common among snakes of the genus *Natrix*, it has rarely been observed in the western whip snake (*H. viridiflavus*), which usually flees rapidly from predators and may inflict defensive bites. Rugiero (1999) mentions three cases of tonic immobility in *H. viridiflavus* during handling for morphometric measurements. These three individuals stayed motionless for a few minutes but did not display any other behaviour specific to feigning death. In this note a new observation of thanatosis in *H. viridiflavus*, involving voluntary supination (Gregory & Gregory, 2006), is reported.

The present observation was made close to the village of Chasnais (Vendée, western France, 46°27' N; 1°53' W) on August 5, 2017 at 08.20h (air temperature around 16 °C). An *H. viridiflavus* (see Fig.1) was approached while basking with the body fully exposed. Upon capture, it bit twice on the hands. After two minutes of handling just above the ground, the snake switched from an aggressive behaviour to feigning death. Its muscles relaxed, it became motionless, and then promptly flipped onto its back (Fig. 2) with the exception of its head, which remained prone and partly concealed in grass. Furthermore, its pupils were slightly oriented downward. Other behaviours associated with thanatosis, as described by de Castro-Expósito et al. (2017), were not observed. During thanatosis, the snake did not attempt to bite, even when handled, and kept its mouth closed. In total, the snake feigned death for around five minutes, which included three minutes with physical contact, and two minutes of observation without contact. It then departed suddenly in the direction of the hedgerow where it had been first located.



**Figure 1.** *H. viridiflavus* photographed before thanatosis



**Figure 2.** Same individual feigning death after being handled. Photograph taken four minutes after the beginning of thanatosis. Note that unlike the body the head remains prone but is partly hidden under grass.

It is interesting to note that of more than one hundred *H. viridiflavus* encountered by the author in France (with roughly half of them handled), this is the first time thanatosis has been observed. This is consistent with previous reports (Rugiero, 1999) and indicates that thanatosis is a rare defensive behaviour in *H. viridiflavus*.

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## CONFERENCE CALL

### **Amphibians and Reptiles of Scotland: current research and future challenges**

**University of Glasgow, Saturday 9th June, 2018**

Following the successful publication of Chris McInerny and Pete Minting's *Amphibians and Reptiles of Scotland* (2016), Glasgow Natural History Society is organising a conference aimed at stimulating interest and research. Although the focus of the conference will be on the herpetology of Scotland, we are encouraging talks from farther afield where the subjects impinge on Scotland, such as climate change, disease and road mortalities. The conference will be a contribution to the Glasgow Science Festival 2018. Two keynote speakers have already agreed to participate: Andrew Cunningham (Institute of Zoology) on disease threats; Silviu Petrovan (Cambridge) on road impacts.

This conference call is to alert all those interested of the date/place, and also to invite anyone who wishes to give a presentation to let us know. We are inviting **titles** (and **brief outlines**; no more than 50 words) under three categories:

1. **Standard talks:** 15-20 minutes
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In addition to these three presentation categories, we plan to have at least one **discussion session** that will allow participants to air their views.

**CONTACT:** Anyone interested in giving a presentation should contact Dr. Chris McInerny ([chris.mcinerney@glasgow.ac.uk](mailto:chris.mcinerney@glasgow.ac.uk)). We are keen to get the programme arranged as soon as possible so that we can publicise it fully. So if you are sure you would like to give a presentation, contact Chris as soon as you can. The deadline for presentation proposals is the end of January, 2018.

## Corrigendum

The following shows changes to the original text since publishing:

### **The Herpetological Bulletin 137: 37-38**

**Lunghi E., Deschandol F., Cornago L., Cogoni R. (2016).**

**Dark coloration in Sardinian Grass Snake (*Natrix natrix cetti*)**

In the second paragraph, the text shows:

"The first snake was abudistic and its total length was 109.48 cm (Fig. 1a). The second showed a particular bluish coloration but the darker pigmentation was regular; total length 91.94 cm (Fig. 1b)."

This should read:

"The first snake was abudistic and its total length was **54.74 cm** (Fig. 1a). The second showed a particular bluish coloration but the darker pigmentation was regular; total length **45.97 cm** (Fig. 1b)."

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