The Herpetological Bulletin

THE HERPETOLOGICAL BULLETIN

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Front Cover: Adult *Salamandra algira spelaea*. This salamander is endemic from northeastern Morocco, inhabiting small caves where it is protected from the intense summer drought. Photograph by Daniel Escoriza. See Natural History Note on page 40.

Habitat use of the Aesculapian Snake, *Zamenis longissimus*, at the northern extreme of its range in northwest Bohemia

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ABSTRACT - Habitat use of the Aesculapian snake (*Zamenis longissimus*) at the northern extreme of its range in northwest Bohemia was studied in two areas with different proportions of man-made structures and urban features. Six snakes were equipped with internal transmitters (giving 171 radio locations in total). Compositional analysis at the home-range scale and location-scale revealed that the snakes used habitats and ecotones non-randomly. Man-made structures were preferred significantly over all other habitat types in both study areas with buildings and their surroundings, stone walls and compost heaps preferred microhabitats in both areas. These sites were used mainly as sheltering places or for thermoregulatory activities. In both areas snakes showed a preference for ecotones, transitional areas between biomes. Urban structures were favoured for nesting and overwintering sites. The prevalence of snakes in man-made edge habitats suggests that in climatically challenging conditions, these otherwise heat seeking snakes prefer different habitats than in more southernly areas of this species' range.

INTRODUCTION

Food availability (Madsen & Shine, 1996), availability of suitable overwintering sites (Reinert & Kodrich, 1982; Reinert, 1993), refuges enabling predator avoidance (Webb & Whiting, 2005) and habitat thermal heterogeneity play an important role in habitat selection by temperate snakes. In northern climates selection of suitable habitats and the timing of activities in snakes largely reflects thermoregulatory requirements (Reinert, 1993). An isolated population of the Aesculapian snake (*Zamenis longissimus*) is found in northwest Bohemia (Central Europe), which is at the northern extreme of the species' range (Gomille, 2002). *Z. longissimus* is a diurnal species that has been extensively studied as regards to feeding ecology (Luiselli & Rugiero, 1993; Capizzi & Luiselli, 1996; Capula & Luiselli, 2002), reproduction (Naulleau, 1992; Naulleau & Bonnet, 1995; Capizzi et al., 1996), habitat preferences (Capizzi & Luiselli, 1997; Filippi & Luiselli, 2001) and spatial activity (Naulleau, 1987, 1989, 1993). However, these studies concerned populations in France and Italy (Lelievre et al., 2010), where warmer climatic conditions prevail. No studies have been undertaken in climatically challenging conditions where winters are longer and summers are cooler. In northwest Bohemia part of the *Z. longissimus* population is associated with man-made structures and many individuals permanently inhabit the embankments of a busy road that passes lengthwise through the area (Kovar et al, 2014; Kovar et al., 2016).

In this paper we decribe activity and habitat preferences of *Z. longissimus* living in this northern enclave in two areas with contrasting habitats: an area with a high proportion of man-made structures (occupied house, farm building, stone walls, garden rubbish, compost heaps, apiary, etc.), including a busy road, and an area with a high proportion of semi-natural habitats (grassland, forested areas, full grown shrub, brooks, rocks, etc.). The specific objectives of the study were: (1) to examine habitat use and refuge selection patterns, with emphasis on man-made structures; and (2) to determine if the presence of the road affected the activity of the Z. longissimus.

MATERIALS AND METHODS

Study site and radio-tracking practices
The study area (329-413 m above sea level) is located in northwest Bohemia, near the village of Straz above Ohre. This is an area where the River Ohre (long-term average flow of 29 m3 . s-1, width 35-45 m) creates a deep valley that separates the Krusne Hory mountains in the north and Doupov Range to the south. At the bottom of the valley, in close contact with the River Ohre, runs the E442 (I/13), a busy two-lane road (width of the roadway 8 m; 4,640 cars, 598 light trucks and 1,466 heavy trucks per 24 hours).

The area is sparsely populated with two small villages consisting of both houses and farm buildings and is topographically complex and comprises a varied mosaic of habitats dominated by woodland and extensively grazed and/or hay meadows with numerous trees, gardens around the houses and outbuildings. The area contains numerous stone walls. The snakes we observed had free contact with five macro-habitats in area A, and six macro-habitats in area B (see Table 1). This was reflected in a very wide range of edge habitats (see Table 4).

The study area is located in a warmer river valley surrounded by colder and more humid mountainous areas to the north and south. Climate conditions are characterised by 140 to 160 days per year with an average temperature above 10 ˚C; long-term average temperature in April = 6-7 ˚C, in July = 16-17 ˚C, in October = 7-8 ˚C; long-term average annual precipitation = 712 mm; solar radiation (monthly averages) in September 2007 and June 2008 was 133 W/m2 and 262 W/m2, respectively.

Field work was carried out in two study areas: area A between 15/06/2007 and 09/10/2007 and area B between 08/06/2008 and 28/09/2008. The shortest distance between the sites was 582 m. Snakes tracked were 2 males and 1 female in area A and 3 males in area B. Study area A (GPS centre 50°19′57.168′′N, 13°2′28.835′′E) lies adjacent to the road, and here the snakes being monitored were caught directly in the roadbed to have radio transmitters implanted. On the eastern and western borders are two sets of ten small houses, surrounded by small gardens. Study area B (GPS centre 50°20′35.428′′N, 13°3′12.518′′E) is located out of direct contact with the road (over 250 to 300 m), from which it is separated by a wooded hillside. Besides farm buildings to the west and a small farm to the east of here, no man-made structures are present. For the purpose of determining habitat preferences, study areas A (138,200 m2; the greatest distance inside of area 645 m; total length of edge habitats 8,852 m) and B (425,500 m2; the greatest distance inside of area 927 m; total length of edge habitats 20,822 m) are defined as the areas bounded by the minimum convex polygon of the outermost radio-locations of all snakes. Barriers (e.g., river) were taken into consideration. The snakes were located approximately once/twice a week with a telemetry receiver (Sika, Biotrack) and hand-held Yagi antenna. The locations were found by tracking the signal to their proximity and their position was fixed from multiple directions. We have tried as much as possible to avoid visual contact with the snakes. To avoid data autocorrelation radio-locations were approximately evenly spaced in time. This method allowed accurate location and home range estimates (Börger et al., 2006; Seaman et al., 1999). Snake location findings are shown in (Fig. 1). Snakes were followed when they were active. The locations were photographed and habitat and microhabitat type recorded (see Table 1 and 3) without disturbing the snakes. Details about the snakes captured and monitoring times are given in Table 2. These locations were used for compositional analysis.

Data processing

Compositional analysis of habitat is a suitable method to determine habitat selection when there are several animals and when the resources are defined by several categories (e.g. vegetation types; Aebischer et al., 1993). Because *Z. longissimus* are not territorial and hence there is independence of movement in that one individual will not significantly influence the movement of another and all tagged individuals had equal access to all available resource units, habitat use and habitat available were evaluated using compositional analysis. This was carried out at two levels: selection of the home range within the

Table 1. Characteristics of habitats within study areas and distances (mean + SD, median) of radio-locations from the edges of habitats. Abs indicates radio-locations absent in this habitat.

No.	Habitat type	Description	distance (m)
1	grassland	pasture or meadow without shrubs or trees, well insolated sites without shelters	3.0 ± 1.9, 2.5
2	mixture of shrub and full grown trees	sparce trees with non-continuous canopy with abundant shrubs coverage (Rubus spp.) and no herbaceous layer, shaded sites	abs.
3	full grown trees	forest with closed canopy, small groups of tall trees, solitery tall trees, shaded sites	6.7 ± 3.2, 8.0
4	full grown shrubs	dense tall shrubs with closed canopy (hawthorn, sloe, ...), shaded sites	2.5 ± 1.3, 2.5
5	man-made structures	houses with gardens, farm building, stone walls, fancy mosaic of well insolated and shaded sites, two-line asphalt road (width 8 m) with stony ditches, two culverts in western part of area	3.3 ± 2.9, 3.0
6	water	pond and river banks with grass, shrubs and trees	abs.
7	mosaic of shrubs, open sites and short trees	mixture of short shrubs, open sites and scarce short trees, mosaic of insolated and shaded sites	2 (one location)
8	early successional plants	dense mixture of tall plants, grasses and short shrubs, mosaic of insolated and shaded sites	6.7 ± 1.7, 6.5

study area (second-order habitat selection), and selection of the relocations within the home range (third-order habitat selection). The sampling units for compositional analysis were the 95 % MCP (minimum concave polygons) home ranges. Compositional analysis was performed in R (R Core development Team, Vienna, Austria) using the Adehabitat package (Calenge, 2007). Because the habitats used are linear or spot structures that *Z. longissimus* often exploit in the landscape, we employed minimum concave polygons (MCP) to estimate home range areas. They are better predictors than minimum convex polygons (MCxP) (White & Garrott, 1990) and kernel estimators (KHR) (Seaman et al., 1999; Laver, 2008), for the data used in this study. All but one home range had an elongated (nearly linear) shape.

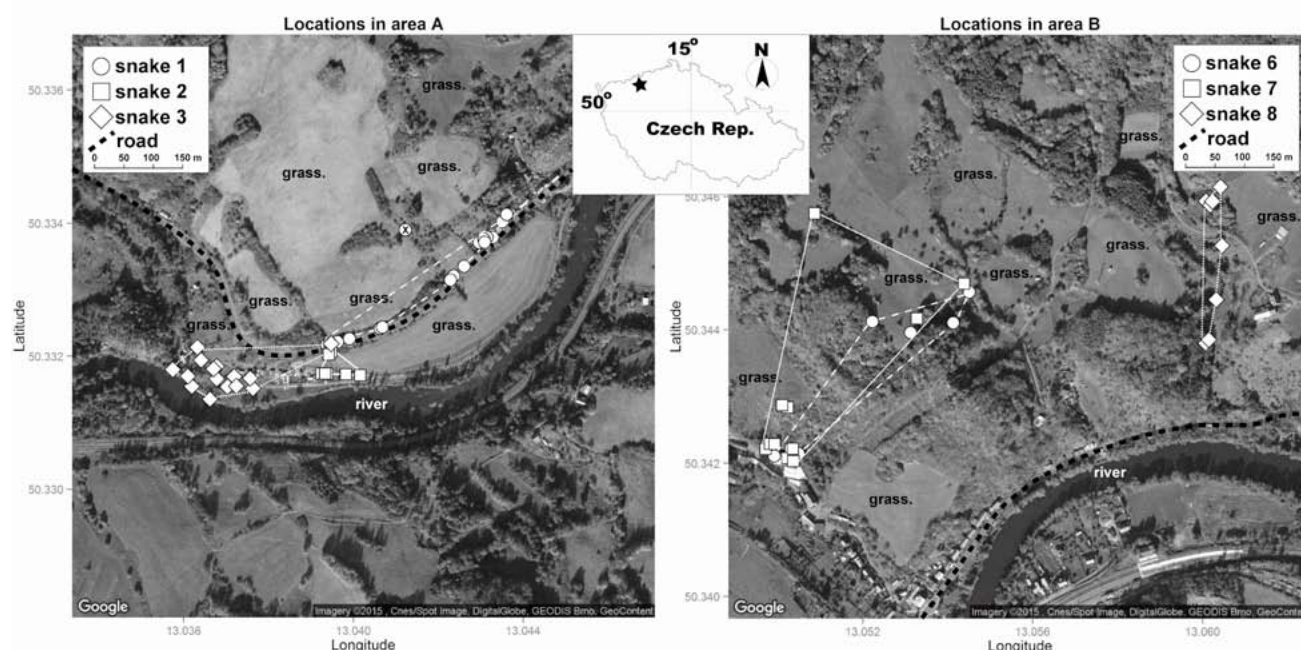Using a line and area measure tools in ArcGIS 10.1 we

Figure 1. Ariel photograph of the study site showing locations of snakes in area A and B. For clarity minimum convex polygons (MCxP) are plotted for each snake and the road is marked by a dashed line. The location of snake 1 marked with "X" in area A was caused by exceptional movement due to disturbance in home range and the snake returned the same day. Grass areas are marked. Insert shows map of Czech Republic with location of the study locality represented by the star symbol.
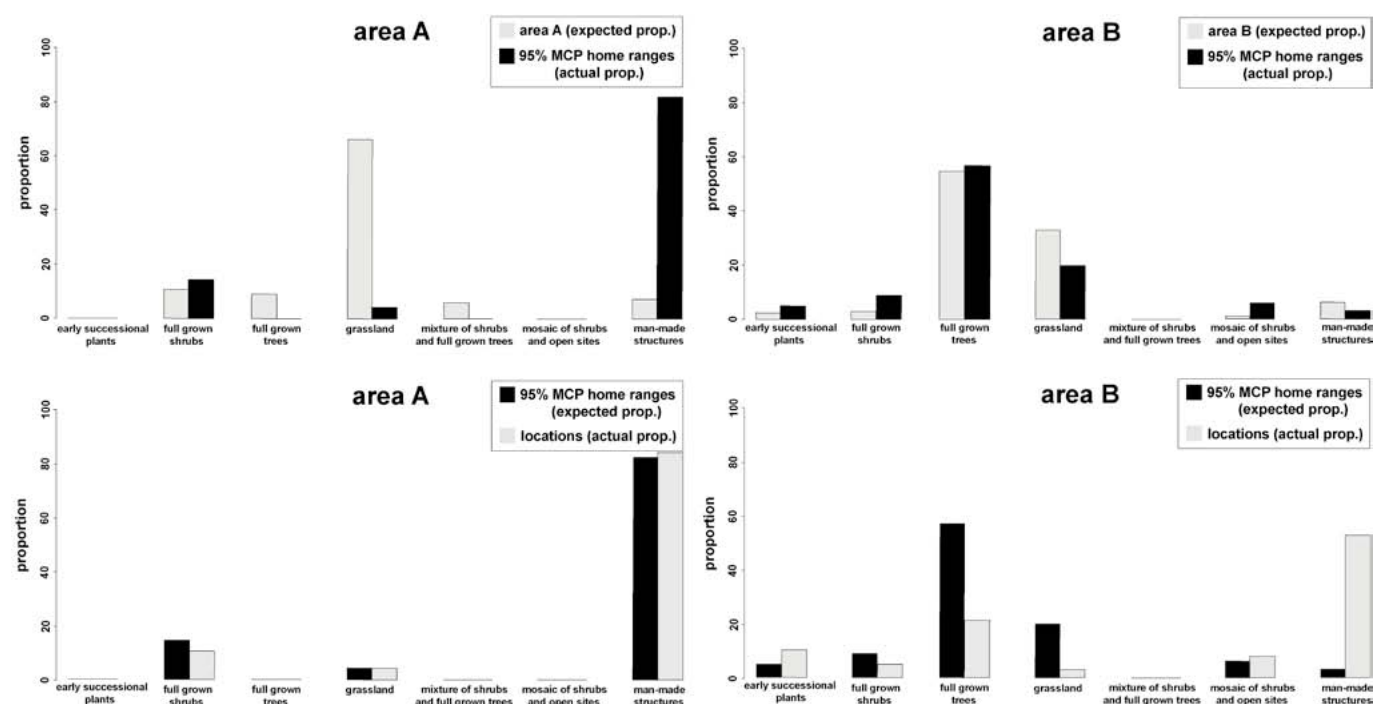

Figure 2. Habitat preferences based on Compositional Analysis (expected vs. actual proportion of habitats). The proportion of habitat types in study area vs. habitat types in 95 % MCP (minimum concave polygon) home ranges (home-range scale) and proportion of habitat types in 95 % MCP vs. habitat types of radio-locations (location scale).

estimated and visualised MCP home ranges and length of all ecotones in our two study areas. These ecotones were classified according to their composition (two types of habitat constituting the ecotone, see Fig. 2). We compared ecotone use to ecotone availablity using compositional analysis (second-order and third-order ecotone selection) (Aebischer, 1993).

Transmitter implantation

The internal transmitters (PIP 2 IMP Biotrack, Biotrack Ltd., United Kingdom, weight 2,1 g) were implanted in the peritoneal cavity under general anesthesia two to three days after capture. The snakes were first weighed and their health status checked. Surgery was performed under sodium pentobarbital anesthesia, and every effort was

Table 2. Data on *Z. longissimus*, times of tracking, distances (m) of radio-locations from the edge of habitat.

Area	A	A	A	B	B	B
Snake No.	1	2	3	6	7	8
Sex	male	male	female	male	male	male
Weight(g)	350	350	400	209	374	150
Tracking period	18/6/07 - 9/10/07	24/6/07 - 25/9/07	15/6/07 - 9/10/07	8/6/08 - 4/9/08	8/6/08 - 31/8/08	8/6/08 - 4/10/08
Number of radio-locations	39	24	27	17	27	37
Distance (m) of radio-locations from edge of habitat (mean; SD; median)	2.5; 2.9; 1.4	1.4; 1.7; 0.7	3.8; 3.5; 4.2	2.0; 0.4; 2.1	4.2; 1.9; 5.1	4.8; 1.9; 5.8

made to minimise suffering. As premedication, we used a combination of 0.25 mg/kg medetomidine (Cepetor inj., 1 mg/ml, CP - Pharma, Germany) + 0.12 mg/kg butorphanol (Butomidor inj., 10 mg/ml, Richter Pharma AG, Austria) + 10 mg/kg ketamine (Narkamon 5 % inj., 50 mg/ml, Spofa, Czech Republic). In 20 minutes, the snakes were sufficiently relaxed, safe to intubate (endotracheal tube, 1.3 x 45 mm, Vasocan Braunüle, B. Braun Meisungen AG, Germany) and subject to inhalation anaesthesia by a mixture of isoflurane (Forane, Isofluranum 100 ml, Abbott Laboratories Ltd., United Kingdom, induction 2 %, maintenance 1 %) and oxygen (0.25 l/min). Following disinfection (Betadine, Iodopovidonum 100 mg/ml, Egis Pharmaceuticals Ltd., Hungary), an incision was made in the transition of dorsal into ventral scales (i.e., laterally at the beginning of the caudal third of the body). A disinfected transmitter was then inserted into the body cavity. The average duration of surgery was 16 minutes. Once the surgical wound was closed, the isoflurane supply was stopped (10 min. after intubation) and the snakes remained on oxygen alone (5 min.). Atipamezole (Revertor inj., 5 mg/ml, CP - Pharma, Germany) was administered intramuscularly as the medetomidine antagonist, followed by extubation, after which the snakes were placed in an incubator (28.5 °C). Recovery from the anesthesia averaged 1 hour and 23 minutes. All snakes were returned to the wild 3-4 days following implantation of transmitters at the same location they were caught.

RESULTS

Habitat preferences
Home-range scale: Compositional analysis demonstrated that 95 % MCP home range selection by snakes was non-random within the study areas ($\lambda = 0.10$, P = 0.018), and indicated urban structures were preferred significantly over all other habitat types in area A; ranks in order of selection: 1) urban structures 2) grassland 3) full grown shrub. The results also indicated that habitats of "mixture of shrub and full grown trees" and "full grown trees" were avoided. In area B "mosaic of shrubs, open sites and short trees" were selected in greater frequency than others. The rankings were in order of selection: 1) mosaic of shrubs, open sites and short trees 2) early successional plants 3) full grown shrubs 4) urban structures 5) grassland 6) full grown trees.

Location-scale: Compositional analysis of selection at the location-scale indicated that the snakes used habitats within their 95 % MCP home range non-randomly ($\lambda = 0.13$, P < 0.01) being located in urban structures more frequently in comparison to other habitat types in area A; rankings were in order of selection 1) urban structures 2) full grown shrub 3) grassland. Habitats with "mixture of shrub and full grown trees" and "full grown trees" were avoided. In area B rankings were in order of selection 1) urban structures 2) grassland 3) full grown trees 4) early successional plants 5) mixture of shrub open stands and short trees 6) full grown shrub (see Fig 1).

Snake behaviour in microhabitats are shown in Table 3. The results were as follows (expressed as a % of the locations), area A: hidden 44 %, basking/thermoregulation 34 %, moving 11 %, unknown 6 %, partly hidden 5 %; area B: hidden 53 %, basking/thermoregulation 47 %.

Linking *Z. longissimus* to edge habitats was essential in both areas. The distance of snake locations from the edges of habitats for individual snakes ranged from 0.0 m to 14.2 m (mean ± SD = 2.6 m + 2.9, median = 1.4 m) in area A and from 0.0 m to 7.4 m (mean ± SD = 4.0 m + 1.9, median = 4.1 m) in area B, respectively (see Table 2). This was significantly different from the average situation in area A (ANOVA, $r^2 < 0.001$, P < 0.001) and B (ANOVA, $r^2 < 0.05$, P < 0.001) respectively - the distances from edges ranged from 0.0 m to 339.4 m (mean ± SD = 56.7 m + 68.4, median = 26.3 m) in area A and from 0.0 m to 725.8 m (mean ± SD = 112. 4 m + 143.5, median = 42.9 m) in area B, respectively. Snakes penetrated deepest into areas with "full grown trees" (mean ± SD = 6.7 m ± 3.2, median = 8.0) and "early successional plants" (mean ± SD = 6.7 m ± 1.7, median = 6.5) (see Table 1). Of the radio-locations in areas A and B, 97 % and 100 %, respectively fell within a 10 m-wide buffer zone along the interfaces. The majority (84 % and 53 %) of the radio-locations in areas A and B respectively fell within a 5 m-wide buffer zone (see Fig. 1). These results indicate that the vast majority of radio-locations in each area were situated in edge habitats (= ecotones).

In areas A and B, 12 and 16 types of ecotone where snakes were found were identified. Some ecotones occurred only in one or the other area, so in total 19 types of ecotone with snakes were identified (see Fig. 3 and Table 4). On a home-range scale Compositional Analysis demonstrated
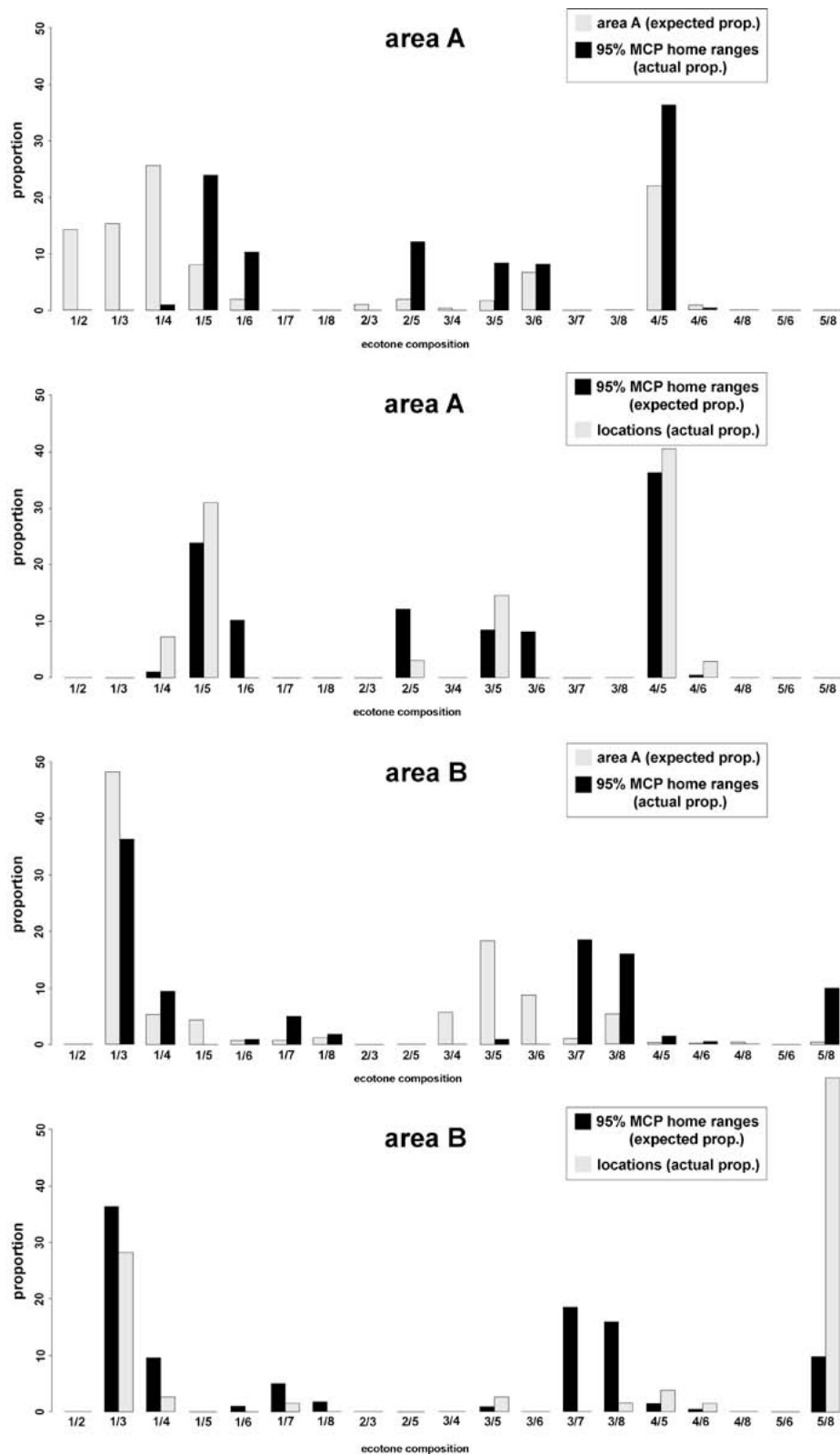
Figure 3. Ecotone preferences based on Compositional analysis (expected vs. actual proportion of ecotones). The proportion of ecotone types length in study area vs. ecotone types length in 95 % MCP (minimum concave polygon) home ranges (home-range scale) and proportion of ecotone types length in 95 % MCP vs. ecotone types of radio-locations (location scale). Figure couples in X axis indicate the two types of habitat constituting the ecotone: 1/2 = grassland / mixture of shrub and full grown trees, 1/3 = grassland / full grown trees, 1/4 = grassland / full grown shrubs, 1/5 = grassland / man-made structures, 1/6 = grassland / water, 1/7 = grassland / mosaic of shrubs, open sites and short trees, 1/8 = grassland / early successional plants, 2/3 = mixture of shrub and full grown trees / full grown trees, 2/5 = mixture of shrub and full grown trees / man-made structures, 3/4 = full grown trees / full grown shrubs, 3/5 = full grown trees / man-made structures, 3/6 = full grown trees / water, 3/7 = full grown trees / mosaic of shrubs, open sites and short trees, 3/8 = full grown trees / early successional plants, 4/5 = full grown shrubs / man-made structures, 4/6 = full grown shrubs / water, 4/8 = full grown shrubs / early successional plants, 5/6 = man-made structures / water, 5/8 = man-made structures / early successional plants.

that ecotone selection by snakes was non-random within the study areas (λ = 0.003, P < 0.001). At location-scale Compositional Analysis at the location-scale revealed that the snakes used ecotones within their 95 % MCP home range non-randomly (λ = 0.016, P < 0.001). Ranks of ecotone preference are shown in Table 4.

DISCUSSION

Habitat preferences (edge habitats)
Both areas in the study localities differed mainly in the proportions of man-made structures (urban features). While they constitute a significant proportion in area A (this area is traversed by the busy road), in area B, they are only minimally represented. Here the major part of the area consists of forest and meadows. Both areas share a high heterogeneity of environments, with a number of transitions between them.

Closed canopy habitat, preferred in the warmer parts of the species range in southern Europe (Lelievre et al., 2011), were avoided in our study localites except when utilising edge habitat. For example, *Z. longissimus* is considered semi-arboreal (Naulleau, 1987, 1989; Luisella & Rugiero, 1993; Schultz, 1996; Lelievre et al., 2010). Pot (1976) found them in trees at a height of between 4 and 7 meters. None of our radio-locations were located on a tree or bush or within any large forested area. Edge habitats exhibit strong edge-oriented thermal gradients, which may allow snakes to adjust body temperature with minimal effort (Wisler et al., 2008). Preference for edge habitats in temperate snakes is a common phenomenon (e.g., Madsen, 1984; Carfagna & Weatherhead, 2006; Row & Blouin-Demers, 2006a; Scali et al., 2008), with high thermal quality of edges, together with prey/shelter abundance, a key factor in selection. This preference is found in both natural and man-made habitats in other species (Blouin-Demers & Weatherhead, 2001). The results also indicate that whilst in optimum climate conditions, *Z. longissimus* operates as a habitat generalist (Lelievre et al., 2011), at the northern limit of the range, probably due to thermal contraints, it selects optimal thermal habitats, such as edges. This is in agreement with data for *Hierophis viridiflavus* (Scali et al., 2008), and *Coluber constrictor* (Plummer & Congdon, 1994). For instance, we considered edge habitat as a 10 meter-wide buffer along habitat interfaces (Row & Blouin-Demers, 2006b; Scali et al., 2008), and 98 % of our radio-locations (irrespective of study A or B) were within this range, with 73 % within a 5 meter-wide buffer. In area A, with a high proportion of urban habitats, this was somewhat higher than in area B. The availability of suitable retreat sites has a major impact on thermal physiology of reptiles (Huey et al., 1989; Huey, 1991) and hence man-made structures may be important in areas with lower temperatures since they offer suitable shelters, basking sites, abundance of prey (e.g., Scali et al., 2008) and potentially low predation pressure by raptors (Meek, 2015).

Our snakes used habitats very unevenly in both study areas with the vast majority of areas inside of their home ranges used only for transits (if at all). Snakes did not move long distances in area A with abundant man-made

structures and did not leave these structures. Snake 1 stayed around a group of houses and inside of stone walls near the road ditch throughtout the study with the exception of movement to the north (outside of the area with man-made structures) probably caused by lawn mowing around the home site. The snake returned the same day. Snake 2 stayed most of the time only around a single house and female 3, except during mating stayed in the vicinity of a single garden manure heap, where she eventually deposited here eggs and probably overwintered. Man-made structures were much less frequented in area B and snakes crossed natural habitats here usually with long, fast and probably well-oriented movements. They stopped subsequently in man-made structures for long periods, or returned after travelling longer distances. The trip of snake 7 to the north is an example, where, inside of semi-natural habitats, was located in a pile of rocks and rubbish but subsequently returned to a barn on the southwest of the home range (see Fig. 1). Snake 6 stayed also at the same site for a long period after travelling from a remote probable over-wintering site (dilapidated wall) in the northeast of the home range and subsequently returned to a nearby wall at the approach of winter. Snake 8, located in an area with a wide range of natural habitats was found exclusively in man-made structures. In general, a sedentary lifestyle was also evident from the rankings of behaviour patterns. Moving snakes were observed only rarely with most in concealed locations or were basking. The presence of the same snakes in the same locations, even after several days were frequent. Compositional analysis at location level,

Table 3. Microhabitats selection and activity pattern in area A and B, respectively (expressed as a % of the locations). Abs indicates microhabitat absent in this area.

Microhabitats type	Area A	Area B
freely at surface	42 %	12 %
inside of building	23 %	12 %
inside of stone wall	12 %	14 %
inside of stone heap	abs.	25 %
grassy vegetation	9 %	19 %
inside of garden compost heap	5 %	abs.
inside of heap of rotten plant material	3 %	0 %
below the surface of roadway	2 %	abs.
garden rubbish	< 1 %	16 %
shrub	< 1 %	2 %
under fallen tree	< 1 %	0 %
Activity pattern		
hidden	44 %	53 %
basking/ thermoregulation	34 %	47 %
moving	11 %	0 %
unknown	6 %	0 %
partly hidden	5 %	0 %

Table 4. The length of ecotones (meters and % of total length of ecotones) in area A and B, respectively and ranks of ecotone preferences based on Compositional analysis at home range scale (HRS) and location scale (LS), respectively.

Ecotone		Area A				Area B			
habitat 1	**habitat 2**	**m**	**%**	**HRS**	**LS**	**m**	**%**	**HRS**	**LS**
grassland	mixture of shrub and full grown trees	1184	13.38			0	0.00		
full grown shrubs	man-made structures	1949	22.02	1.	1.	83	0.40	7.	4.
grassland	man-made structures	720	8.13	2.	3.	906	4.35		
grassland	full grown shrubs	2288	25.85	3.	5.	1031	4.95	10.	6.
full grown trees	man-made structures	158	1.78	4.	2.	3815	18.32	11.	2.
mixture of shrub and full grown trees	man-made structures	177	2.00	5.	4.	0	0.00		
man-made structures	early successional plants	0	0.00			36	0.17	1.	1.
full grown trees	mosaic of shrubs, open sites and short trees	0	0.00			176	0.85	2.	
grassland	mosaic of shrubs, open sites and short trees	0	0.00			96	0.46	3.	7.
grassland	full grown trees	1365	15.42			10068	48.35	4.	3.
full grown shrubs	water	104	1.17			38	0.18	5.	5.
full grown trees	early successional plants	0	0.00			1149	5.52	6.	8.
grassland	water	170	1.92			116	0.56	8.	
grassland	early successional plants	0	0.00			239	1.15	9.	
mixture of shrub and full grown trees	full grown trees	84	0.95			0	0.00		
full grown trees	full grown shrubs	58	0.66			1165	5.60		
full grown trees	water	595	6.72			1784	8.57		
full grown shrubs	early successional plants	0	0.0			97	0.47		
man-made structures	water	0	0.0			23	0.11		

thus probably better reflects habitat preferences of snakes in both areas than the compositional analysis at home-range level.

Road

Different species of snakes (Seigel & Pilgrim, 2002; Andrews & Gibbons, 2005; Jochimsen, 2005; Meek, 2015) and lizards (Meek, 2014) react differently to road traffic, and knowledge of their reaction to the existence of a road can help clarify the significance of the impact of road traffic on the local population (Andrews, 2003; Shine et al., 2004). The busy road passing through the center of our area A was not an impassable barrier for the snakes, but became a part of their home range, and the habitats of many snakes were located very near to it. To cross the roads snakes used the culverts that run under it. While mortality of adult snakes attempting to cross roads is very low, the mortality of newly hatched young snakes is significant (Kovar et al., 2014) suggesting the road could represent an ecological trap. The activity of snake No 1 actually ran parallel with the road. Home ranges along a road is not uncommon in snakes (Fitch, 1999; Sealy, 2002; Shine et al., 2004). The retreats under roads constitute the high-quality refuges and especially at night the Aesculapian snakes sheltering under roads are able to maintain high

and stable body temperature, irrespective of ambient temperatures (Lelievre et al., 2010).

It is recognised that the present results although statistically significant, are derived from limited numbers of monitored snakes (3 + 3 individuals), radio-locations (171) and time (1 + 1 season). However, due to the uniqueness of the local population, it is important that a data base is now available to facilitate additonal research. This could include between-sex differences of habitat preferences, the seasonal changes in preferences and identification of mating, eggs-laying and wintering habitats.

Conclusions for the conservation of Aesculapian snakes

The importance of man-made structures for the survival of the population of *Z. longissimus* in our study area is evident. Since the 1950s there have been significant changes in land use, resulting in the reduction of landscape mosaics, spreading of forest and the gradual disappearance of scattered man-made structures from open, originally agriculture, landscape. Their artifacts (eg. dilapidated stone walls), still remain, but gradually disappear and become overgrown with vegetation. Since we can not expect a return to traditional agricultural land use, conserving the remaining stone walls and other man-made structures is crucial for the conservation of *Z. longissimus*.

Local people do not harm the snakes, however continuing concentration of snakes around human settlements could become a biological trap if this were to change. Enhancing the ecological conditions of the surrounding habitats is one way to reduce this risk.

ACKNOWLEDGEMENTS

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Reproduction in the common house gecko, *Hemidactylus frenatus* (Squamata: Gekkonidae) from Hawaii

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ABSTRACT - A histological examination of gonadal material from *Hemidactylus frenatus* indicated males produced sperm (determined by spermiogenesis) during the ten months samples were available. The smallest mature male (spermiogenesis) measured 34 mm SVL, the largest 59 mm SVL. *H. frenatus* females were reproductively active (determined by yolk deposition, enlarged ovarian follicles > 4 mm or oviductal eggs) in all months except November (N = 1). Mean clutch size (N = 40) was 1.95 ± 0.22 SD, range = 1–2. The smallest mature female measured 38 mm SVL, the largest 53 mm SVL. There was a decrease in female reproductive activity in October, when 92% of 13 females had quiescent (inactive) ovaries. A similar decrease in October reproductive activity was earlier documented in the introduced anole, *Anolis sagrei*, on Oahu, Hawaii, suggesting a shared response to changing environmental conditions.

INTRODUCTION

Hemidactylus frenatus Duméril and Bibron, 1836 is found worldwide in tropical and subtropical regions but has been transported widely by man; it is native to southern and/or southeastern Asia (Bauer, 1994). A global list of introductions for *H. frenatus* and supporting literature is in Kraus (2009). In Hawaii, *H. frenatus* was first observed on Oahu in June, 1951 (Hunsaker & Breese, 1951). There have been numerous reports on its reproduction in different parts of its introduced range: Jalisco, Mexico (Ramírez-Bautista et al. 2006); Java, Indonesia (Church, 1962); Ryukyu Archipelago, Japan (Ota, 1994); Taiwan (Lin & Cheng, 1984, Cheng, 1988); American Samoa (Schwaner, 1980); Fiji (Zug, 1991); and Panay Island, Philippines (Gaulke, 2011). The purpose of this paper is to present data on reproduction from a histological examination of *H. frenatus* gonadal material from Hawaii.

METHODS

We examined a sample of 163 *H. frenatus* consisting of 82 adult males (mean snout-vent length = mean SVL = 49.7 mm ± 5.5 SD, range = 34–59 mm), 77 adult females (mean SVL = 46.2 mm ± 3.4 SD), range = 38–53 mm and four juveniles (mean SVL = 32.2 mm ± 1.5 SD, range = 30–33 mm), from Hawaii, USA and deposited in the herpetology collections of the Bernice P. Bishop Museum (BPBM), Honolulu, Hawaii, USA; Museum of Zoology, University of Michigan (UMMZ), Ann Arbor, Michigan, USA; and Natural History Museum of Los Angeles County (LACM), Los Angeles, California, USA (Appendix). The *H. frenatus* were collected in the period 1963 to 2007 from Hawaii, USA: Hawaii (N = 62), Kahoolawe (N = 14), Kauai (N = 6), Lanai (N = 2), Maui (N = 31), Molokai (N = 24), Oahu (N = 24).

We opened the abdominal cavity and removed the left testis or ovary for histological examination. We cut histological sections at 5 μm and stained them with hematoxylin followed by eosin counterstain (Presnell & Schreibman, 1997). We examined slides of testes to determine the stage of the testicular cycle, and ovaries to detect the presence of yolk deposition. We counted enlarged follicles > 4 mm and oviductal eggs. We deposited histology slides at BPBM, LACM and UMMZ. We measured the snout-vent length (SVL) of each lizard from the tip of the snout to the anterior margin of the vent. We compared *H. frenatus* male and female mean body sizes (SVL) using an unpaired t-test in the program Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

RESULTS

Males of *H. frenatus* averaged larger than females (unpaired t-test = 4.79, df = 157, P < 0.0001). Three stages were noted in the monthly testicular cycle (Table 1): (1) Regression: seminiferous tubules contain spermatogonia and interspersed Sertoli cells; (2) Recrudescence: a renewal of germinal epithelium has commenced for the next period of spermiogenesis, primary spermatocytes predominate; in late recrudescence, secondary spermatocytes and occasional spermatids may be present; (3) Spermiogenesis: the lumina of the seminiferous tubules are lined with sperm or clusters of metamorphosing spermatids. The smallest reproductively active male (showing spermiogenesis) measured 34 mm SVL (BPBM 6573) and was collected during August on Molokai. The seminiferous tubules of one smaller male (SVL = 33 mm) (BPBM 28112) exhibited recrudescence, and it was classified as a subadult. Virtually all males showed spermiogenesis, which occurred in all months for which samples were available (Table 1).

Four stages were noted in the monthly ovarian cycle

(Table 2): (1) Quiescent: no yolk deposition; (2) Early yolk deposition: basophilic yolk granules in ooplasm; (3) Enlarged follicle: follicles > 4 mm (4); Oviductal eggs. Mean clutch size (N = 40) was 1.95 ± 0.22 SD, range = 1–2. (2 clutches of 1 egg each, 38 clutches of 2 eggs each). The smallest reproductively active female (with 2 oviductal eggs) measured 38 mm SVL (BPBM 12706) and was collected on Oahu during July. There was no evidence (oviductal eggs plus concomitant yolk deposition in the same female) to indicate that *H. frenatus* produces more than one clutch in the same year, which may reflect our small sample sizes. However, in view of the extended period of female reproduction (Table 2), it seems possible for a female to have deposited a clutch and subsequently initiated yolk deposition for a second clutch. Three subadult *H. frenatus* (30, 33, 33 mm SVL) were collected--two from July, and one from August.

DISCUSSION

The reproductive cycle of *H. frenatus* is similar to that of other tropical lizards in exhibiting an extended period of sperm formation and egg production (Baker, 1947; Fitch, 1982). This was shown to occur in Bandung (6.9176°S) Java, Indonesia, where *H. frenatus* reproduces throughout the year without undergoing a definite seasonal cycle (Church, 1962). On Panay Island (11.7104°N) Philippines, *H. frenatus* reproduces throughout the year (Gaulke, 2011). However in northern populations, *H. frenatus* exhibits seasonality in the reproductive cycle. For example, on Kita-Daitojima Island (25.9500°N) of the Ryukyu Archipelago, Japan, *H. frenatus* produces eggs from April to September (Ota, 1994). In Taichung (24.1500°N), Taiwan, *H. frenatus* females are reproductively active from late March to September (spring and summer) (Lin & Cheng, 1984). Thus *H. frenatus* may possibly switch from year-round to seasonal reproduction under climates and resultant ecological conditions associated with slightly higher latitudes (24–26°N), than in Hawaii (18.9–22.2°N). It is interesting that 92% (12/13) of *H. frenatus* females from October exhibited quiescent ovaries (Table 2). Only (11%) 2/19 of *H. frenatus* females from October to March contained oviductal eggs, in contrast to (33%) 19/58 from April to September. Similarly, there seems to be a reduction in rate of spermiogenesis in male *H. frenatus* from November to March (Table 1). A similar reduction in female reproductive activity was also observed in *Anolis sagrei* on Oahu, Hawaii, with 78% (7/9) of females from October inactive (Goldberg et al., 2002). October is the start of the cool-wet season in Hawaii, which runs from October to March (U.S. Climate Data 2015). Whether the October decrease in *H. frenatus* and *A. sagrei* reproductive activity is in some way associated with conditions during the start of the cool-wet season in Hawaii merits subsequent investigation. But this skewed reproductive patterns suggests that Hawaii may be a transition point in climate between the tropics, which allow for yearlong reproduction and the more northern latitudes of Taiwan and the Ryukyus, which restrict reproduction to a clearly seasonal pattern.

Table 1. Monthly stages in the testicular cycle of 82 adult male *H. frenatus* from Hawaii.

Month	N	Regression	Recrudescence	Spermiogenesis
Feb	1	0	0	1
April	9	0	0	9
May	10	0	1	9
June	6	0	0	6
July	6	0	0	6
Aug	28	0	0	28
Sept	11	0	0	11
Oct	9	1	0	8
Nov	2	0	0	2

Table 2. Monthly stages in the ovarian cycle of 77 adult female *H. frenatus* from Hawaii.

Month	N	Quiescent	Early yolk deposition	Enlarged follicles > 4 mm	Oviductal eggs
Jan	4	1	1	1	1
Feb	1	0	0	1	0
April	13	4	3	3	3
May	5	3	1	0	1
June	2	0	0	1	1
July	8	1	1	3	3
Aug	21	7	3	1	10
Sept	9	7	0	1	1
Oct	13	12	0	0	1
Nov	1	1	0	0	0

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Appendix: Museum accession numbers for *Hemidactylus frenatus* from Hawaii examined by island from the Bishop Museum (BPBM), Honolulu, Hawaii, USA; University of Michigan, Museum of Zoology (UMMZ), Ann Arbor, Michigan, USA; and the Natural History Museum of Los Angeles County (LACM, Los Angeles, California, USA.
BPBM Hawaii Island: BPBM 6540–6542, 8405, 8585, 11273, 11274, 21714–21717, 21723–21733, 23950, 23956, 23957, 23968–23970, 27132; LACM 145395–145403, 137525–137527, 137529–137538, 138317; UMMZ 225220. 225225, 225244, 225248-225250, 225252, 225253, 227359, 275251; Kahoolawe: BPBM 11577–11579, 11580-11586, 11590, 11592, 11597, 13509; Kauai: BPBM 11249, 11251, 27319; UMMZ 227360, 227361, 227363; Lanai: BPBM 13846, 27320; Maui: BPBM 11074, 13946–13953, 13955–13960, 13974–13976, 13987, 13989, 13990, 13992, 13996; UMMZ 225215, 225219, 225221–225223, 225228–225230; Molokai: BPBM 6573, 6610–6614, 6632–6644, 28108–28112; Oahu: BPBM 2012, 6186, 6550, 6559, 8405, 8557, 10559, 11234, 11235, 11239, 11240, 12680, 12685,12691,12692, 12695, 12706, 12987, 13101, 13102, 24175, 28111, 28112, 28668, 31902: UMMZ 227022.

The first record of *Amolops himalayanus* (Anura: Ranidae) from Bhutan.

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ABSTRACT - During a series of surveys carried out in areas of broadleaf forest in the eastern Himalayas in early spring, *Amolops himalayanus* was identified for the first time in Bhutan. Information is provided on the ecology, habitat, reproduction, and geographical distribution of the species together with notes on the water chemistry of the collection site. A brief synopsis is given of the morphological differences between *A. himalayanus* and *A. formosus,* with which latter species *A. himalayanus* is often confused. *A. himalayanus* has been reported hitherto from seven localities in northern India and Nepal but only the original description of the taxon from Darjeeling is based on incontrovertible data.

INTRODUCTION

The 49 species of Cascade frogs belonging to the genus *Amolops* occur from Nepal and northern India to western and southern China and south to Malaysia (Frost, 2015). In Bhutan, *A. mantzorum* has been recorded at Choetenkora in Trashiyangtse District (Wangyal, 2013), *A. marmoratus* at Sershong in Sarpang District (Das & Palden, 2000), and *A.* cf. *monticola* at Ririchu in Wangdue Phodrang District (Wangyal & Gurung, 2012). Wangyal (2014) predicted the occurrence of *A. himalayanus* (and *A. formosus*) in Bhutan on the basis that the country was suitable for these two species both geographically and climatically.

During a series of amphibian surveys undertaken in Trashigang District in March and April 2015 by a team from the University of Bhutan's Sherubtse College, eight *A. himalayanus* (four males and four females) were identified in a stream near Khaling.

This is the first record of *A. himalayanus* from Bhutan and the only distinct, published collection of the taxon since its original description by Boulenger (1888).

SYSTEMATIC REVIEW

Amolops himalayanus (Boulenger, 1888)
Rana himalayana Boulenger, 1888: 507. "Darjeeling", India. Common names: Himalaya Cascade frog; Himalaya Sucker frog; Himalayan frog.

Variation

A. himalayanus was considered by Dubois (1974) to be a subspecies of *A. formosus* (Günther, 1876) but the taxon *himalayanus* was returned to specific level by Yang (1991: 12) based on diagnostic differences between the two forms (Frost, 2015).
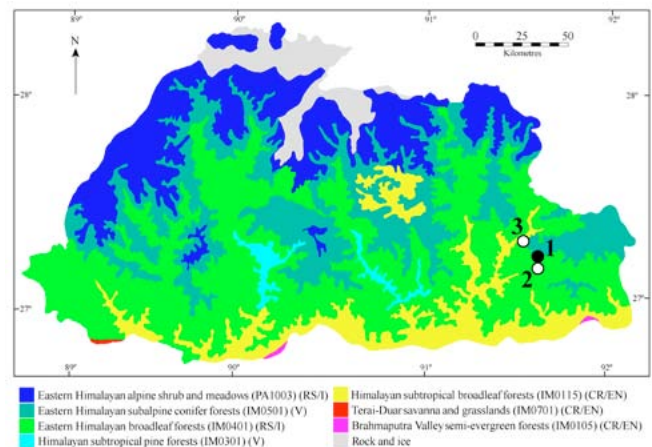
Figure 1. Delineation of the seven terrestrial ecoregions occurring in Bhutan. The conservation status of the ecoregions is represented by the following abbreviations: RS/I – relatively stable/intact; V – vulnerable; CR/EN – critical/endangered (www.worldwildlife.org (2)). Localities: 1 – Jere Chhu; 2 – Khaling stream; 3 – Bodidrang stream.

MATERIALS AND METHODS

Study Area

Bhutan lies within the Himalaya Hotspot as defined by Conservation International (www.cepf.net) and Khaling is located in the Eastern Himalayan Broadleaf and Conifer Forests (Global 200 ecoregion no. 67) and, more precisely, in the Eastern Himalayan broadleaf forests (Fig. 1), which are outstanding globally for both species richness and endemism (www.worldwildlife.org (1)).

As a part of a Rufford Small Grant project, a team comprising five members surveyed Jere Chhu (*Chhu* = river, water), on 28 March, 5 April and 19 April 2015 from 7.00 pm to 10.00 pm. Jere Chhu is located on the outskirts of Khaling town in Trashigang District, eastern Bhutan (Fig. 1).

Further observations of individuals considered to be *A. himalayanus* (Fig. 2E and F) were made in Trashigang District at Bodidrang stream (27° 17′20.33″ N, 91° 30′56.28″E) on 19 July 2014, 28 February 2015 and 22 May 2015 and at Khaling stream (27° 11′26.18″N, 91°36′09.40″E) on 29 June 2015. Bodidrang stream lies 12.1 km north-west of Khaling town while Khaling stream, into which Jere Chhu flows, is located 1.7 km south of the town (Fig. 1).

Specimens and measurements
Eight individual *A. himalayanus* were collected and photographed *in situ* using a Nikon D5100 camera with an AF-S Micro Nikkor 40 mm lens.

External measurements of the eight individuals were taken to the nearest 0.01 mm using Vernier callipers. The measurements, which are listed in Table 1, are defined as follows: SVL – snout to vent length; HL – head length; HW – head width; and TL – tibia length. Sex was determined by size and the nuptial pad on first finger following Boulenger (1920).

Six of the specimens were released and two (one male and one female) were sacrificed humanely in accordance with the U.K. Government's Code of Practice issued under Section 21 of the Animals (Scientific Procedures) Act 1986 (www.gov.uk(1)). The two frogs were placed individually into secure plastic containers containing cotton wool impregnated with chloroform. Upon proof of permanent cessation of circulation, the specimens were preserved in 70% ethanol and deposited in the scientific collection at Sherubtse College Museum, Kanglung, Trashigang District, Bhutan. The two specimens were sacrificed specifically for the following reasons:

1. To permit the taxonomic identity of the species to be determined with certainty under laboratory conditions.

2. To provide material for the scientific reference collection at Sherubtse College, which the lead author is trying to establish as a centre of excellence for the study and conservation of amphibians in Bhutan (there is no such centre in Bhutan at present). This reference material is invaluable in enabling students to identify the species in the field and for comparative purposes during the course of research into the protection of the species, particularly with regard to differentiating *A. himalayanus* from similar taxa (e.g. *A. formosus*).

Six water samples were taken from Jere Chhu from 9 am to 10 am on 29 March at 100 m intervals between the collection site and Jigme Sherubling Higher Secondary School. Results were obtained using a G.R.E.E.N. (Global Rivers Environmental Education Network) water quality monitoring kit. Nitrate and phosphate levels and the pH balance of the water were measured as follows:

Nitrate test – 5 ml of stream water were transferred to a plastic bottle, in which one Nitrate Wide Range CTA TesTab was dissolved. After five minutes, the resulting water colour was compared with the G.R.E.E.N. nitrate colour chart supplied and the corresponding level (in parts per million (ppm)) was recorded.

Phosphate test – 10 ml of stream water were transferred to a plastic bottle, in which one Phosphate TesTab was

Table 1. Measurements (in mm) of *A. himalayanus* from Jere Chhu.

Field no.	Accession no.	SVL	HL	HW	TL	Sex
TND003	–	72.8	24.6	26.0	45.0	♂
TND004	–	74.1	26.8	27.3	48.6	♂
TND005	–	73.0	24.7	25.4	47.1	♂
TND012	SC-AMP-012	76.7	24.0	25.0	46.8	♂
TND001	–	83.4	28.3	28.6	51.0	♀
TND002	–	80.5	26.4	26.8	46.0	♀
TND008	–	89.0	27.0	27.6	52.7	♀
TND011	SC-AMP-011	89.0	26.0	31.0	53.0	♀

dissolved. After five minutes, the resulting water colour was compared with the G.R.E.E.N. phosphate colour chart supplied and the corresponding level (in ppm) was recorded.

pH test – 10 ml of stream water were transferred to a plastic bottle, in which one pH Wide Range TesTab was introduced. When the Testab had dissolved fully, the resulting water colour was compared with the G.R.E.E.N. pH colour chart supplied and the corresponding level recorded.

Air temperature was taken at 7.45 pm on 28 March and water temperature was taken at approximately 9 pm on 28 and 29 March 2015. Water and air temperatures were recorded in degrees Celsius (ºC).

RESULTS

New material
1 ♂ (adult), SC-AMP-012; 1 ♀ (adult), SC-AMP-011; Jere Chhu (27° 12'21.90" N, 91° 36'12.20" E), Khaling, Trashigang District, Bhutan, 2,073 m above mean sea level, 28 March 2015, collected by T. Nidup, N.T. Lepcha, Penjor, D. Gyeltshen, and S. Dorji.

Diagnosis and description
A. himalayanus can be identified by its large size and a combination of characters, the most significant of which are horny spicules on the side of the head and body and in a cluster at the base of the forelimbs; the lack of grooves on the first finger; dark banding on the limbs; and an olive skin colour with consistent brown patches on the dorsum. Measurements of females are greater than males (Table 1). The morphological characters of specimens from Jere Chhu agree in all respects with the description of the taxon given by Boulenger (1888) with the exception of the SVL values of SC-AMP-011 and TND008 (Table 1), which are 89.0 mm. Boulenger (1888) gives a simple SVL value of 80.0 mm for the four specimens he examined, a figure he revised upwards to 83.0 mm subsequently (Boulenger, 1920: 220). The difference of 6.0 mm between Boulenger's maximum SVL value and that of SC-AMP-011 and TND008 could be explained by shrinkage of the Darjeeling specimens during their (presumably lengthy) retention in preserving fluid between collection and examination.

In his study of the phylogenetic systematics of the *Amolops* Group of ranid frogs of south-eastern Asia
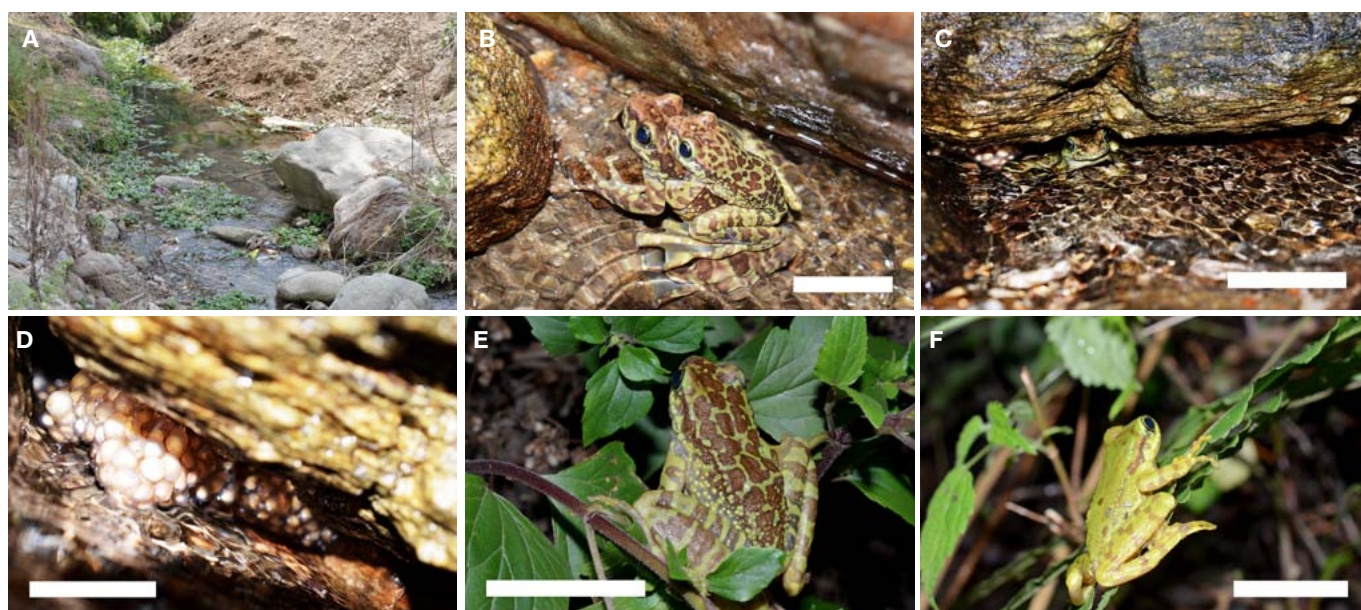
Figure 2. **A** – Jere Chhu showing discarded plastic containers. **B** – Amplectant *A. himalayanus* in Jere Chhu. Scale = 50 mm. **C** – Male *A. himalayanus* next to an egg clutch beneath a rock in Jere Chhu. Scale = 50 mm. **D** – Egg clutch of *A. himalayanus*. Scale = 25 mm. **E** – *A.* cf. *himalayanus* observed at Bodidrang stream. Scale = 50 mm. **F** – Juvenile *A.* cf. *himalayanus* observed at Khaling stream. Scale = 50 mm.

and the Greater Sunda Islands, Yang (1991) gives a maximum length of 88.0 mm. The author does not refer to individual specimens but does list those institutions whose collection of specimens he examined, which includes the British Museum of Natural History (now the Natural History Museum (N.H.M.)), where the type series of *A. himalayanus* is held (Boulenger, 1890). It would not seem possible for Yang to state a measurement of 88.0 mm were that measurement not of Boulenger's holotype or one of the type series. Our own measurement in 2015 of the SVL of the single female *himalayanus* in the collections of the N.H.M. was 78.75 mm, a value that corresponds, conversely, more with the SVL measurements given in Boulenger (1888).

Ecology and habitat
At Jere Chhu, *A. himalayanus* was recorded sitting on rocks, at the water's margin, and in the stream's free-flowing waters. The stream was heavily polluted, the same caused principally by the discharge of effluents and waste water directly into the watercourse from the kitchens of Jigme Sherubling Higher Secondary School. Tangible pollutants comprised plastics, papers, and cloths (Fig 2A). Measurements taken at the stream indicated a mean water temperature of 16 ºC, an air temperature of 18.9 ºC, a nitrate level of 5 ppm, a phosphate level of 4 ppm, and a pH value of 8.

At the collection site, the stream is bordered on either side by cultivated fields supporting maize and potato crops. Other than algae, watercress (*Nasturtium officinale*) was the sole aquatic plant noted in the stream, itself. Floral species in the surrounding area included the Himalayan Coral tree (*Erythrina arborescens*), the Yellow Himalayan raspberry (*Rubus ellipticus*), and the herb, Indian madder (*Rubia manjith*).

Individuals considered to be *A. himalayanus* were found in intact forest at both Bodidrang stream and Khaling stream. At both locations they were observed above ground sitting on the stems and leaves of the invasive plant species, *Eupatorium adenophorum* (Fig. 2 E,F).

Reproduction
Between ten and fifteen *A. himalayanus* were found in Jere Chhu on each of the three study visits. Amplectant individuals were recorded on 5 April (Fig. 2B). A single *A. himalayanus* that was consistent in size with a male was observed beneath a rock in the middle of the stream next to an egg clutch (Fig. 2C), which appeared to be attached to the underside of the rock. The upper section of the clutch was above the water line while the lower section was either resting on the water's surface or was partially submerged (Fig. 2D). The flow of the freshwater stream was constant. The diameter of individual eggs within the clutch was estimated to be 5 mm or less.

Tadpoles and juvenile *A. himalayanus* also were present. At Bodidrang stream, tadpoles were evident on 22 May and a single tadpole was collected by hand and released back into the stream after its dorsolateral and ventral surfaces had been photographed (Fig. 3A,B). Clearly visible in Fig. 3B is the ventral sucker, which is present in the tadpoles of very few amphibian genera but which is a principal character of the genus *Amolops* (see Pham et al. 2015). On 19 April, no mating couples were found and no egg clutches were apparent. Individuals were observed sitting on rocks and at the water's edge.

DISCUSSION

Outside Bhutan, *A. himalayanus* is known currently only from Indian records (Fig. 4), which comprise the type series of four specimens collected between 1872 and 1887 at Darjeeling in West Bengal (Boulenger, 1888), two of

which were referred initially by Boulenger (1882: 70) to *Rana formosa* (Günther, 1876) [= *A. formosus*] (see Boulenger, 1888: 508); unverifiable documentation of the species' presence in four districts of Himachal Pradesh (Mehta, 2005); a field observation in northern Sikkim in 2012 (Barkha, 2015); and an entry on the collections database of the Field Museum of Natural History recording a single preserved specimen, also from Sikkim. A report of the species' occurrence in Nepal (Kripalani, 1961) is likely to be based on a tadpole of another *Amolops* species (see Schleich et al., 2002; Frost, 2015).

Mehta (2005) reports the presence of *A. himalayanus* in the districts of Kangra, Kullu, Shimla, and Sirmour in the Indian state of Himachal Pradesh but does not give verifiable details (either of individual specimens or of localities). The need to substantiate Mehta's locality records of the species was identified by Frost (2015). In addition, the identification key appearing in Mehta (2005: 270-272) would seem to be of questionable accuracy as some of the characters of specimens Mehta regards as *A. himalayanus* disagree with the description of the taxon by Boulenger (1888, 1920), for example the relationship of head width to head length, the degree of prominence of subarticular and inner metatarsal tubercles and of the tympanum, and relative finger length.

A solitary photograph of a ranid frog that, visually, compares favourably with *A. himalayanus* was posted on the India Biodiversity Portal website by Barkha (2015). The image was taken in May 2012 at approximately 27° 49'25"N, 88° 33'23"E, a position north of Chungthang in the Indian state of Sikkim. No measurements, description, or scale accompanied the photograph and the taxonomic identity of the frog cannot be verified.

The Field Museum of Natural History in Chicago records a single specimen of *A. himalayanus* (FMNH 15806) on its collections database (www.fieldmuseum. org). The record of the preserved specimen, which was collected by Herbert Stevens in 1931, is not accompanied by any data other than the collection locality of "Sikkim, India".

Kripalani (1961) reported the occurrence of a tadpole of *A. himalayanus* (as *Staurois himalayana*) from Chagna in Nepal, the same having been collected during the course of the Indian Cho-Oyu Expedition in 1958. Schleich et al. (2002: 80) doubted this record as the tadpole was found in the same habitat as an adult *A. marmoratus* while Frost (2015) states that the tadpole is "likely assignable to *A. formosus*."

Kripalani refers to the Nepalese locality as Chagna and, elsewhere in the paper, as Changa. In a list of the collecting stations visited in Nepal by the 1958 Indian Cho-Oyu Expediton, Datta (1961) refers to Chagma. The true locality is most probably Chyangma, which lies at the approximate co-ordinates of the collecting station (27° 40'N, 86° 18'E) given by Datta (1961).

The collection of *A. himalayanus* in Bhutan is the only published record based on actual material since the original description of the taxon by Boulenger in 1888 although the species may have been collected unwittingly during the period it was deemed synonymous with *A. formosus*. The
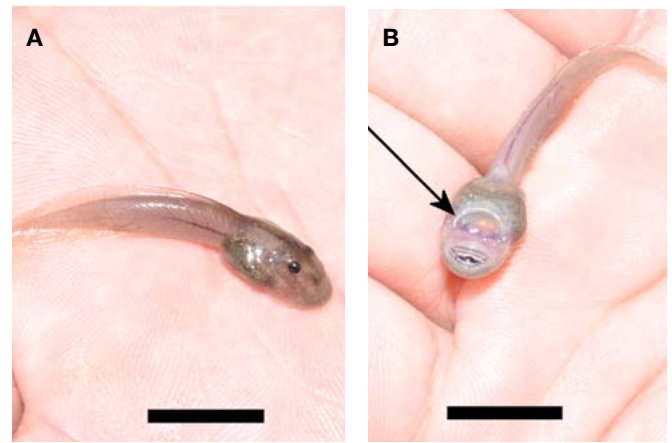
Fig. 3 Dorsolateral (A) and ventral (B) views of a tadpole of *A. himalayanus* collected from Bodidrang stream. The ventral sucker (arrowed), is a principal character of **Amolops** tadpoles. Scale = 10 mm.
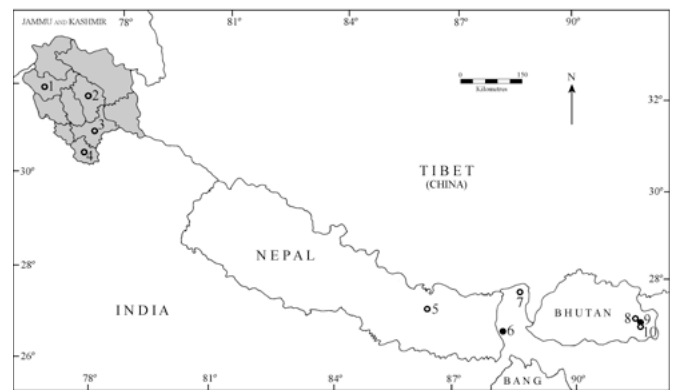
Fig. 4 Map showing the recorded distribution of *A. himalayanus*. The identification of individuals from the localities marked with a black circle is not supported by substantive data. The Indian state of Himachal Pradesh is represented by the shaded area. Localities: 1 – Kangra District; 2 – Kullu District; 3 – Shimla District; 4 – Sirmaur District; 5 – Chyangma; 6 – Darjeeling; 7 – Chungthang; 8 – Bodidrang stream; 9 – Jere Chhu; 10 – Khaling stream. BANG. = Bangladesh.

record of *A. himalayanus* from Khaling extends the taxon's range 330 km to the east. The distance stated, however, warrants a *caveat* insofar as it is not ascertainable whether the original collection locality of "Darjeeling" refers to the town or the district of that name.

The accession of *A. himalayanus* to Bhutan's known fauna increases the number of confirmed amphibian species in the country to 40, a figure that includes one salamander (*Tylototriton verrucosus*) and one caecilian (*Ichthyophis sikkimensis*) (Wangyal, 2014).

Bordoloi et al. (2004), who consider *A. himalayanus* and *A. formosus* to be conspecific (see Appendix I for the principal morphological differences between the two taxa), highlight the lack of information available on egg deposition and larval ecology. Accordingly, it is of note that breeding pairs and an egg clutch together with juveniles and tadpoles were observed in Jere Chhu on 5 April. By 19 April, no mating couples were seen and egg clutches were no longer evident. It would appear likely, therefore, that breeding occurs in late March or early April.

Measured against The World Health Organisation's maximum recommended nitrate level (in drinking water) of 50 ppm (www.who.int (a)), the 5 ppm concentration of nitrate in Jere Chhu is unremarkable as is the pH value of 8, which is within the same Organisation's suggested optimum range of 6.5–9.5 (www.who.int (b)). The phosphate level of 4 ppm in Jere Chhu is, however, of some concern as the reading is 40 times higher than the annual mean phosphate concentration in rivers of 0.1 mg per litre (0.1 ppm) recommended by the U.K. Government (www.gov.uk(2)). Phosphate pollution is caused principally by human activity, often through the treatment of crops with fertiliser (which may run off into adjacent watercourses) or by the discharge of effluent or detergents (White & Hammond, 2006). It is not known whether phosphate-based fertilisers are applied to the agricultural land bordering Jere Chhu but it is unlikely owing to the remoteness of the location, the cost of the chemicals, and the low income of the rural farming communities. A more probable cause of the high phosphate reading is the direct discharge into the stream of effluent, detergents, and other pollutants from Jigme Sherubling Higher Secondary School. The high levels of both tangible and chemical pollution in Jere Chhu seemed not to have a detrimental effect on the frogs' occupation of the locality.

The occurrence of *A. himalayanus* in freshwater habitats at three sites within an area of approximately 6.3 km^2 in Trashigang District points to the species being at least locally common. Extrapolation of these locality data suggests that *A. himalayanus* would be encountered reasonably often in similar habitats at an elevation of approximately 2,000 m (the elevation of Darjeeling is 2,042 m) (www.darjeeling.gov.in) both in Bhutan and in the wider Himalayan region.

It is recommended that further field research be undertaken in Bhutan, particularly in areas of Critical/Endangered Himalayan subtropical broadleaf forest, in order to augment existing knowledge of the composition, distribution, and conservation requirements of the country's amphibian fauna.

ACKNOWLEDGMENTS

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www.gov.uk(1) – see www.gov.uk/government/uploads/system/uploads/attachment_data/file/229022/0193.pdf

www.gov.uk(2) – see: www.gov.uk/government/uploads/system/uploads/attachment_data/file/162164/defra-stats-observatory-indicators-da3-120224.pdf

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Appendix I. The principal morphological differences between *A. himalayanus* (Boulenger, 1888) and *A. formosus* (Günther, 1875).

A. himalayanus	A. formosus	Authority
Tibia as long as the trunk	Tibia considerably shorter than the trunk	Boulenger (1888)
Tibio-tarsal articulation of the hind limb reaches beyond the tip of the snout	Tibio-tarsal articulation of the hind limb reaches the nostril or the tip of the snout	Boulenger (1888, 1920)
Web of toes feebly notched	Web of toes deeply notched	Boulenger (1920)
Nostril nearer to eye than to tip of snout	Nostril midway between eye and tip of snout	Yang (1991)
Tympanum indistinct	Tympanum distinct	Yang (1991)
Small granules on side of body and temples; belly and posterior half of lower surface of thighs granulate	Belly granulate	Boulenger (1920)
	Granules on side of head absent	Yang (1991)
Spinules limited to side of the head and base of the forelimb		Yang (1991)
Olive or greyish above with indistinct darker spots on the body	Bright green above with sharply defined black or blackish spots on the head and body	Boulenger (1920)
Hinder side of thighs purplish brown	Hinder side of thighs marbled with black	Boulenger (1920)
Lower parts brownish or pale olive	Lower parts and web between toes brown or marbled brown and whitish	Boulenger (1920)
Ethmoid rounded in front	Ethmoid obtusely pointed in front	Boulenger (1920)
Snout to vent length (males, n. 2): 74-75 mm	Snout to vent length (males, n. 1): 53 mm	Boulenger (1920), Yang (1991)
Snout to vent length (males): 76 mm maximum		Yang (1991)
Snout to vent length (females, n. 2): 80-83 mm	Snout to vent length (females, n. 6): 53-75 mm	Boulenger (1920), Yang (1991)
Snout to vent length (females): 88 mm maximum		Yang (1991)
Head length (males, n. 2): 24-24mm	Head length (males, n. 1): 18 mm	Boulenger (1920)
Head length (females, n. 2): 27-27 mm	Head length (females, n. 6): 19-23 mm	Boulenger (1920)
Head width (males, n. 2): 25-27 mm	Head width (males, n. 1): 18 mm	Boulenger (1920)
Head width (females, n. 2): 28-29 mm	Head width (females, n. 6): 20-24 mm	Boulenger (1920)
Tibia length (males, n. 2): 47-49 mm	Tibia length (males, n. 1): 32 mm	Boulenger (1920)
Tibia length (females, n. 2): 50-52 mm	Tibia length (females, n. 6): 32-41 mm	Boulenger (1920)

Observations on the distribution of melanistic snakes in Britain

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ABSTRACT - Records of melanistic British snakes were sourced from several databases and individuals. Of the three native snake species there were 217 records of melanistic northern vipers (*Vipera berus*), nine grass snakes (*Natrix n. helvetica*) and four smooth snakes (*Coronella austriaca*). The geographical spread of melanistic individuals appeared to reflect the general countrywide distribution of their cryptically coloured counterparts.

INTRODUCTION

Melanism has been reported from a wide range of taxa and in snakes the phenomenon has a wide geographical distribution (Bittner, 2003). One of the best known examples is that of the northern viper (*Vipera berus*) where melanistic individuals have been found across most of its range and as far north as the Arctic circle in Sweden (Andrén & Nilson, 1981; Luiselli, 1992; Monney et al., 1996).

Melanistic snakes differ from their regularly coloured counterparts by being significantly darker or entirely black. Such colour difference is fairly easy to observe and can be seen in sloughed skins (King, 2003; Strugariu & Zamfirescu, 2009). In melanistic snakes there is greater production of melanin by the melanocytes in the stratum basale layer of the skin (Bechtel, 1978), under the influence of a melanophore stimulating hormone. It is generally considered that a recessive 'loss of function' gene mutation is responsible for melanism in snakes and in the garter snake *Thamnophis sirtalis* inheritance follows a recessive Mendelian pattern (Lawson & King 1996; King 2003).

Melanism is a phenotypic polymorphism and its occurrence would appear to be of adaptive significance (Strugariu & Zamfirescu, 2009). For snakes, three adaptive benefits have been postulated; thermoregulation - where snakes could benefit from faster solar heat absorption (Gibson & Falls, 1979; Lawson & King, 1996; Clusella-Trullas et al., 2007); predator avoidance - where snakes could avoid predation by visual searching predators (Andrén & Nilson, 1981; Luiselli, 1992; Forsman, 1995; Lindell & Forsman, 1996; Bittner, 2003; Wüster et al., 2004; Tanaka, 2005); and reproductive fitness - where melanistic snakes could obtain larger, more sexually competitive body sizes (Forsman & Ås, 1987; Capula & Luiselli, 1994; Bittner et al., 2002). In this study we obtained records of melanistic snakes from the databases of several British organisations and have plotted them to facilitate an analysis of geographical spread.

MATERIALS AND METHODS

Records of melanistic snakes in the British Isles were accessed through consultation with Amphibian and Reptile Conservation Trust (ARC-Trust) which maintains a repository of British herpetological data. The ARC-Trust database was explored using the search term 'melani', which captured records of melanism, melanistic and melanic. The search term 'black' was deliberately excluded due to complications of overlapping phenotypic descriptions (i.e. black pattern coloration of northern vipers).

Additional records were requested from individual Amphibian and Reptile Groups (ARG-UK) and from volunteers of the National Amphibian and Reptile Recording Scheme (NARRS). Where possible, records were verified through accompanying photographic evidence and by comparing the geographical locality against known species ranges. Records have been plotted at low resolutions on map figures to protect sensitive site locations.

RESULTS

Melanism has been recorded in all three native British snakes; northern viper *V. berus* (Fig. 1), grass snake *Natrix n. helvetica* (Fig. 2) and smooth snake *Coronella austriaca* (Fig. 3).

Melanistic northern viper records totalled 217 and these ranged from the south coast of England to the Scottish Highlands (Fig. 4). With the exception of the Isle of Arran, the majority of the records were present in the south of England. Although present throughout Great Britain, melanistic northern vipers appeared to be more prevalent in coastal regions.

There were nine records of melanistic grass snakes (Fig. 4), predominantly in the south of England. A notable exception was a recent record of a sub-adult specimen from Derbyshire. Regretfully, shortly after receiving the record, the animal in question was found dead from an undetermined cause.

Figure 1. Melanistic *V. berus* (photograph John Baker).

Figure 2. Melanistic *N. n. helvetica* (photograph Todd Lewis).

Figure 3. Melanistic *C. austriaca* next to a cryptically coloured specimen (photograph Stuart Woodley).

The earliest record of a melanistic smooth snake in Britain dates from the 19th century and until recently was considered an anomaly (Cambridge, 1894). More recently there have been further records (Pernetta & Reading, 2009; Woodley, 2015) increasing the total to four, all from Dorset (Fig. 4).

DISCUSSION

Some details of melanism in British snakes have been given in the current standard work on the British herpetofauna (Beebee & Griffiths, 2000) but the distribution information within is limited. This is updated and extended by our synthesis of data from three different recording schemes and individual recorders.

The observed occurrence and scarcity of melanistic smooth snakes is consistent with the very restricted range of this species in southern England (Pernetta & Reading, 2009; JNCC, 2010; Woodley, 2015). It is possible that melanistic smooth snakes are under recorded in Britain not least due to their very secretive nature. Other reports of melanistic smooth snakes have come from southern France (Reich, 2012) and a single specimen has been recorded in Russia (Idrisova, 2014).

There were very few melanistic grass snake records, and of these, most were from southern England. Melanistic grass snakes are known from wider Europe with recent reports from Bulgaria (Naumov & Tomovic, 2005; Mollov, 2012), Croatia (Zadravec & Lauš, 2011), Finland (Terhivuo, 1990), Montenegro (Gvozdenovic & Schweiger, 2014), Slovakia (Jandzik, 2004), Sweden (Nilson & Andrén, 1981; Andrén, 2004), Switzerland (Reich, 2012) and Turkey (Habiboglu et al., 2016). On the Baltic island of Gotland, melanistic grass snakes comprise up to 50% of the population (Andrén, 2004). However, despite being a readily encountered and abundant snake in Britain, the numbers of melanistic individuals seemed disproportionately low; the situation on the Swedish mainland appears to be similar (Andrén, 2004). Perhaps, as active foragers, natural selection particularly favours the

normal cryptic colouration of grass snakes.

Unlike the other two species, there were a much greater number of northern viper records, largely from southern England. Greater numbers of populations with melanistic individuals have been recorded on the Isle of Arran and within Exmoor National Park than elsewhere, and the small clusters of melanistic northern vipers in Norfolk, Exmoor, Scotland and East Sussex are interesting. However, all these cases could result from surveyor bias as much as a

Figure 4. Geographical spread of the records of melanistic snakes in Britain. Note that a single locality record may represent more than one individual melanistic snake.

genuine exceptional frequency of melanism.

Despite the few high density clusters of melanistic northern vipers, it appears that the overall geographical pattern of melanism in Britain is correlated with the general abundance and distribution of the three snake species. Although melanism has previously been associated with habitat type (Clusella-Trullas et al., 2007), the metadata with the British records are insufficient for us to draw any conclusions. More detailed mining of the National Amphibian and Reptile Recording Scheme (NAARS) database, to contrast melanistic with cryptic snake populations, may be profitable. Furthermore, in the face of rather scarce data on snakes, the inclusion of melanistic lizards may help to provide the critical mass of data needed to draw further conclusions.

ACKNOWLEDGEMENTS

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Piebaldism in Roatán Spiny-tailed Iguanas, *Ctenosaura oedirhina*

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ABSTRACT - Abnormal colouration has been reported in both wild and captive reptile populations. In wild populations, piebaldism is a type of leucism where individuals have normal-coloured eyes, but exhibit white patches, giving them a blotched or mottled appearance. It is a common form of abnormal colouration. Some species show an ontogenetic shift in this patchy colouration, while others start life with a piebald appearance. We studied colouration across populations of Roatán Spiny-tailed Iguanas (*Ctenosaura oedirhina*), a species endemic to the island of Roatán, Honduras. In general this lizard exhibits an ontogenetic shift in colouration from grey with green mottling in hatchlings to dark grey/black with white or cream patches in adults; however, there is substantial colour variation among both adults and populations. Based on an ordinal regression analysis there is a significant positive trend between size (snout-vent length) and percentage of white colouration, suggesting an ontogenetic shift toward increasing piebaldism. Additional data, specifically recapture and genetic information, are needed to elucidate the mechanisms responsible for these patterns.

INTRODUCTION

Piebaldism is a type of leucism, where an individual has normally coloured eyes but lacks pigment on some parts of the body, giving a spotted or blotched pattern, which often changes over time. Leucism is caused by anomalous pigment cells in the skin that lack colour. In most cases the specific causes of leucism have not been identified, but it may result from dominant Mendelian inheritance, ontogenetic expression (vitiligo), or post-inflammatory leukoderma (Dyrkacz, 1981; Bechtel, 1995).

Albinism is a complete lack of pigment and is known to occur in many species of reptiles (see Bechtel, 1995 for a review), though it is thought to be rare (Krecsák, 2008). Piebaldism, however, appears to be one of the more common forms of abnormal coloration across reptiles (Bechtel, 1995), but generally occurs as an atypical coloration. For example, in *Tarentola boettgeri bischoffi*, a lizard endemic to several islands in the Selvagens archipelago in Portugal, piebaldism has only been documented once (Rocha & Rebelo, 2010). In contrast, in taxa such as *Sauromalus varius*, all adult males show conspicuous reticulated or banded piebald coloration (Hollingsworth, 1998).

Reptiles with albinism or piebaldism are usually rare in nature for many reasons, most notably it is presumed that they are more visible to predators (Krecsák, 2008). They may also have health impacts, directly via associated detrimental genetic mutations, or indirectly via compromised thermoregulation (Bechtel, 1978; Krecsák, 2008). Abnormal coloration can also be caused by nutritional or environmental factors altering pigmentation (Hayley-McCardle, 2012). *Conolophus marthae* has a distinctive pink coloration, which may be indicative of piebaldism. Gentile & Snell (2009) showed that when a pink scale was removed, the resulting blood loss caused it to lose its pink colour, appearing white. This may indicate that the pink coloration is an effect of blood circulating to achromatic scales. They also found that these iguanas have lower levels of vitamin D and seem to avoid the sun more than other similar species (G. Gentile, pers. comm.), which could have negative health consequences.

Ontogeny may also play a role in lizard coloration, reflecting a change in diet, habitat, predation risk, or sexual maturity (Booth, 1990). For example, Piebald Chuckwallas (*S. varius*), found on two islands in the Gulf of California, exhibit an ontogenetic change from brown as hatchlings to yellow/orange with mottled black as they grow (Hollingsworth, 1998). This unusual coloration may have been the result of a bottleneck in the island population (McAliley et al., 2006). The Acklins Rock Iguanas (*Cyclura rileyi nuchalis*) also exhibits a colour shift from
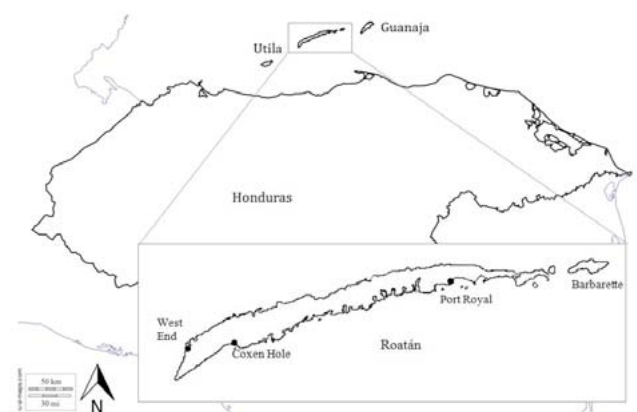

Figure 1 Map of Honduras. Inset shows close-up of the island of Roatán, with some larger towns identified. Adapted from Goode et al., 2016

hatchling to adult, which can vary over time. A colour shift within the adults seems to relate to size, with larger individuals tending to be lighter in colour (Iverson et al., 2016).

Though both ontogenetic colour change and piebaldism have been observed in iguanas, the combination of ontogenetic piebaldism has not yet been described. Delayed onset piebaldism has, however, been seen in wild-type corn snakes (*Pantherophis guttata*) bred in captivity, and in Burmese Pythons (*Python molurus bivittatus*) (Bechtel, 1995).

Roatán Spiny-tailed Iguanas, *Ctenosaura oedirhina*, are endemic to the island of Roatán, off the northern coast of Honduras (Fig. 1). They are listed as Endangered by the IUCN and are on Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (Pasachnik et al., 2010; Pasachnik & Ariano, 2010). In general, hatchlings are grey with green mottling. Most adult individuals are black and grey with "cream to white blotches arranged in bands" (de Queiroz, 1987). There is, however, great variation in the proportion of white observed in adults, ranging from the extremes of almost completely white to completely black (Figs. 2–4).

Differences in coloration among populations of the same species could indicate differences in genetics, environment or a combination of the two. Identifying and understanding these differences would be useful for future conservation efforts involving captive breeding or translocation of individuals. To understand these observations in the context of ontogeny, piebaldism, and sexual dimorphism, we quantified the relationship between colour and size, sex, and location. We also discuss potential mechanisms of colour change realizing that additional data are needed to fully elucidate these mechanisms.

METHODS

We collected colour data on 561 *C. oedirhina* over the course of four years from 2010-2014. Upon capture we measured snout-vent length (SVL) to the nearest millimeter using a tape measure and mass to the nearest gram using Pesola hanging scales. We sexed each individual by cloacal probing and an evaluation of external sex characteristics, including femoral pores, head shape, and dorsal crest spines. We photographed the left side of each individual before releasing it at the point of capture. Total processing took no longer than 20 minutes. Using the photos, one of us (SAP) later ranked each individual for percentage of white on a scale of 1-6 (1 = 0 % white, 2 = 1–25 %, 3 = 26–50 %, 4 = 51–75 %, 5 = 76–99 %, and 6 = 100 %) (see Figs. 2–4 for examples). We considered only the portion of the body from posterior of the front legs to anterior of the back legs on the left side, as this was typically the area with greatest variation across individuals. All photos were scored at the same time and each photo was scored independently at least two times. Once both scores were recorded we selected the photos with a discrepancy between scores and scored them once again, taking the majority rule as the final score. This was the case for 18% of the photos. Six photos were scored an additional, fourth time, due to discrepancy between the

Figure 2. A predominantly dark individual *C. oedirhina* (122 mm SVL, sex unknown as individual is a juvenile) from Roatán, Honduras. Colour ranking 1. Photograph by Stesha A. Pasachnik.

Figure 3. An intermediately coloured individual *C. oedirhina* (222 mm SVL, female) from Roatán, Honduras. Colour ranking 3. Photograph by Stesha A. Pasachnik.

Figure 4. A predominantly white individual *C. oedirhina* (270 mm SVL, male) from Roatán, Honduras. Colour ranking 6. Photograph by Stesha A. Pasachnik.

Figure 5. An individual *C. oedirhina* from Roatán, Honduras, that presumably suffered from an incomplete tail wound. The "second" tail appears to have regenerated with no pigment. Photograph by Stesha A. Pasachnik.

first three scores, and once again majority rule was used for the final score decision. We also noted all instances in which an individual had an injury resulting in a lack of pigmentation in the area of regrowth, such as regenerated tails (Fig. 5), combat wounds, and injured digits.

We used an ANOVA to determine if there was a significant difference in SVL across populations and then the Tukey's HSD post hoc test to determine differences among the populations. We performed an ordinal regression of SVL, sex, and location on colour ranking, using the six locations that had ≥ 10 individuals (n = 546) and six levels of coloration (see above). We also ran the same analysis with four levels of coloration (1 = 0–25 % white, 2 = 26–50 %, 3 = 51–75 %, and 4 = 76–100 %) to compare the effect of equal bin size. In the analysis, we compared five study locations to a sixth, reference population to evaluate the effect of location condition (e.g. disturbance, hunting pressure). The reference population was completely protected from illegal hunting, had little to no habitat alteration, and no supplemental food was available to the iguanas, thus it was likely to be the closest to the natural and historical population structure of this species. Fifty-six individuals were recaptured during the study. Nearly all of the recaptured individuals were larger adults (SVL mean = 223 mm, range = 150–283 mm). Individuals were considered to be mature if they had a SVL greater than 150 mm and to be hatchlings if they had a SVL < 60 mm (Pasachnik, 2013). We used qualitative statistics to determine any difference in growth, recapture intervals, or colour change because of the small sample size of recaptured individuals. All analyses were conducted in R Console and run with packages: foreign, ggplot2, MASS, Hmisc, and reshape (R Development Core Team, 2013).

The locations of our study sites are not identified by name due to the endangered status of the species, but can be obtained from the authors upon legitimate request.

RESULTS

Whether using four or six categories of coloration, the same variables were found to be statistically significant with only slightly different P values and regression coefficients (Table 1). We thus report the results using six bins, as we feel that those more accurately reflect the natural colour categories of the individuals. When compared to the reference population, we observed a significant positive trend in which larger (SVL) individuals had a higher percentage of white coloration (P < 0.001; Table 1, Fig. 6). Colour did not vary significantly by sex (P = 0.06; Table 1). However, colour did vary significantly across Locations 1, 2, and 3 (P < 0.001; Table 1, Fig. 7). In these three locations, regression coefficients were significant. These coefficients indicate the approximate coloration of the population (the more positive the coefficient, the more white the individual). Location 3 had the highest occurrence of white individuals and Location 2 had the highest occurrence of dark individuals (Table 1, Figs. 6 and 7). Size of individuals varied significantly between locations (P = 0.0001, Fig. 8). There was a significant SVL*Location interaction identified for Locations 1, 2, and 5 (P < 0.01 for all three locations; Table 1). This interaction term had a small, negative coefficient for all three locations.

Of the 56 recaptured individuals, four had more white coloration than at initial capture. The average change in SVL between recaptures for these four individuals was 37.7 mm (range = 3–86 mm). The average time between
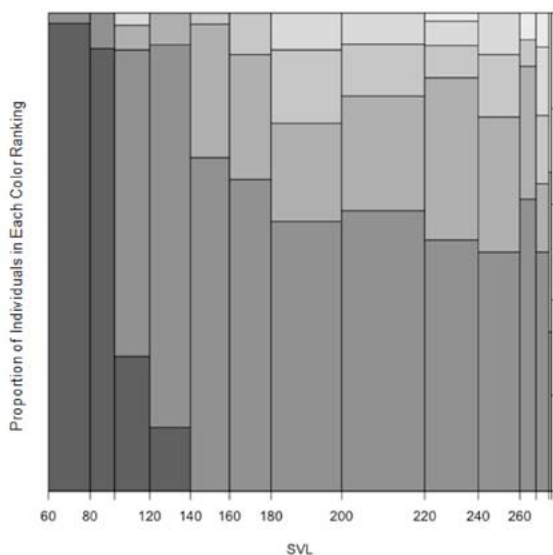
Figure 6. Ordinal regression of snout-vent length (SVL) to colour category in *C. oedirhina* from Roatán, Honduras. SVL sizes were binned by 20 mm increments and the width of bar is relative to number of individuals in that bin. Heights indicate the proportion of individuals in that size class that are in each colour category (percentage of white on a scale of 1-6, see Methods) with the darkest gray indicating individuals ranked 1 (0% white) and the lightest grey indicating individuals ranked 6 (100% white).
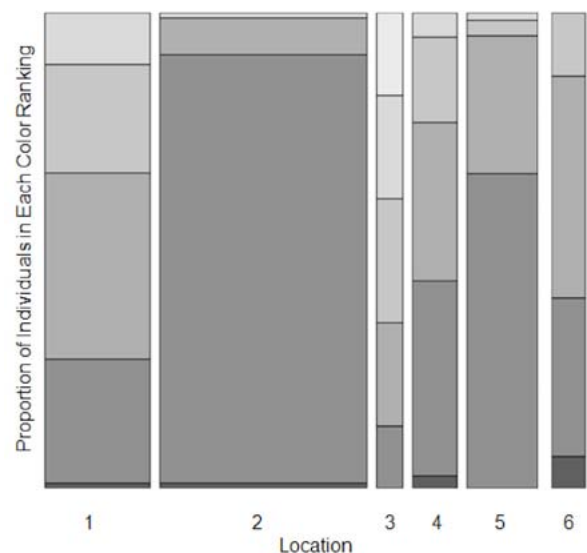
Figure 7. Ordinal regression of colour category by location in *C. oedirhina* from Roatán, Honduras. Height of bar segments indicates proportion of individuals in that size class that are in each colour category (percentage of white on a scale of 1-6, see Methods) with the darkest gray indicating individuals ranked 1 (0% white) and the lightest grey indicating individuals ranked 6 (100% white). Location 6 is an untouched population in pristine habitat, and was used as a reference population for comparison.
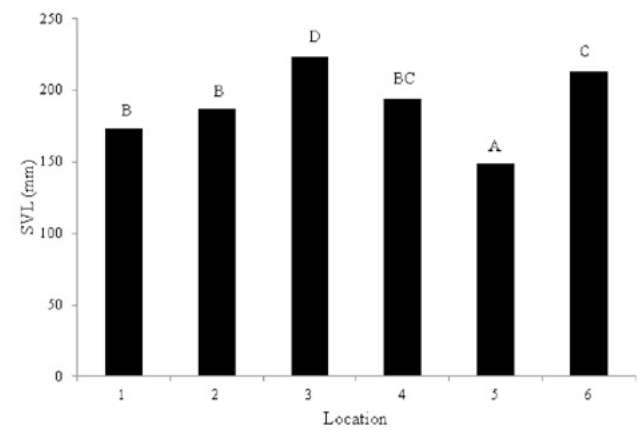
Figure 8. Average SVL of *C. oedirhina* at Study Locations in Roatán, Honduras. Letters indicate Turkey's HSD post hoc analysis results.
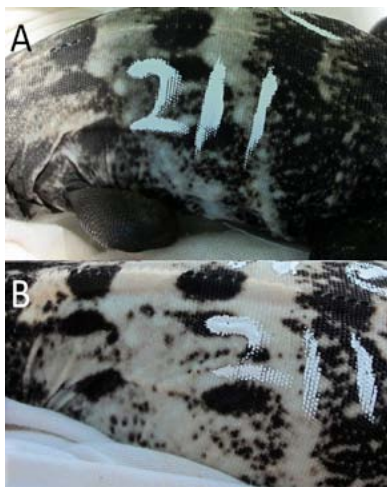

Figure 9. This *C. oedirhina* from Roatán, Honduras grew 31 mm over 160 days, a growth rate of 0.19 mm/day, nearly four times the average. A.) Initial capture on 28 February 2011 at a SVL of 134 mm. B.) Recaptured on 4 August 2011 at a SVL of 164 mm. Photographs by Stesha A. Pasachnik.

Table 1. Significance of Regression Variables in Four Level and Six Level Analysis. (*Indicates significance at α < 0.05)

	Four Level Analysis		Six Level Analysis	
Variable	**Coefficient**	**P value**	**Coefficient**	**P value**
SVL	0.0227	< 0.001 *	0.0248	< 0.001 *
SEX	0.325	0.18	0.439	0.0611
Location - 1	1.648	< 0.001 *	1.923	< 0.001 *
Location - 2	-2.683	< 0.001 *	-2.253	< 0.001 *
Location - 3	2.301	< 0.001 *	2.744	< 0.001 *
Location - 4	0.407	0.384	0.576	0.227
Location - 5	-0.322	0.475	-0.0168	0.971
Location - 6	0.348	0.18	0.462	0.0611
SVL*Loc - 1	-0.0015	0.773	-0.0154	0.0056*
SVL*Loc - 2	-0.0017	0.807	-0.02	< 0.001*
SVL*Loc - 3	0.011	0.17	0.003	0.712
SVL*Loc - 4	-0.006	0.462	-0.013	0.149
SVL*Loc - 5	-0.009	0.173	-0.253	< 0.001*
SVL*Loc - 6	0.008	0.18	0.0189	0.0611

their recaptures was 681 days (range = 160–1043 days). Their average growth rate was 0.0568 mm/day. The other 52 recaptures received the same colour ranking in their first (n = 43), second (n = 8), and third (n = 1) captures. Their average change in SVL was 21.9 mm (range = 1–110 mm), average time between these recaptures was 386.3 days (range = 6–1315 days), and average growth rate was 0.0554 mm/day. The recaptured individuals with the increased white coloration had SVL and time between recaptures within the range of the other group and a very similar growth rate. One individual exhibited rapid colour change over a short period (Fig. 9A & B). We noted observations of injuries or regenerated tails that were abnormally white (n = 3, Fig. 5) in three captured individuals.

DISCUSSION

Though piebaldism has been observed in many reptile taxa, the patterns of occurrence and underlying mechanisms have not been rigorously assessed. Further, delayed onset piebaldism is something rarely observed, even in captive populations (Booth, 1990; Bechtel, 1995). The positive relationship between SVL and colour category found in *C. oedirhina*, elucidates a pattern of larger individuals exhibiting a higher degree of piebaldism. Hatchlings are exclusively dark in colour and usually have some green pigmentation, while individuals observed to be completely white are exclusively large adults. In addition, limited recapture data show a very slow increase in piebaldism over time. Given these patterns, we suggest an ontogenetic increase in the frequency of piebaldism in this species, although some individuals remain dark their entire lives. This pattern seems relatively clear, however understanding the underlying mechanisms is much more complex.

In Piebald Chuckwallas (*Sauromalus varius*) an ontogenetic shift in coloration has been reported to be the result of a genetic bottleneck in their island populations (Hollingsworth, 1998). In the present study, the effects of location, population structure, and genetics may all be influencing the observed colour patterns. Population structure varies by location. This is supported by the significant interaction term associated with three of the locations. Some locations also have human-supplemented food available, which may cause faster growth and in turn may skew the occurrence of piebaldism because of the increased number of larger individuals. While all of the study locations offer protection from hunting, some are better protected, and some have been protected longer and or more consistently. These factors could lead to populations with predominately older or larger individuals. Locations 1–5 have the added factor of hunting pressure outside of the site boundaries, limiting migration and reducing gene flow (Goode et al., 2016 ; Pasachnik & Hudman, 2016).

In order to better understand these various factors we used Location 6 as a reference population as it appears to be large, is not supplemented fed, and is less likely affected by inbreeding than many of the other populations (Pasachnik & Hudman, 2016). The physical isolation of this location and the presence of security eliminate hunting and introduced predators as a threat. Thus, the demographics

of this population are likely more natural than the other locations. As predicted, the degree of piebaldism is relatively evenly distributed within this population (Fig. 7).

This species occurs primarily in isolated populations across the main island of Roatán (Pasachnik, 2013; Goode et al., 2016). There is little migration among populations, resulting in low genetic diversity and inbreeding depression (Pasachnik & Hudman, 2016). This population genetic structure may account for at least some of the differences seen in the occurrence of piebaldism in *C. oedirhina*. In a randomly mating, wide-ranging species, one may expect to find equal variation in traits, such as colour, across all populations. However, this is not what we observed when comparing Locations 1–5. Location 4 had a varied colour distribution but a large percentage of extremely piebald individuals (Fig. 7), and was by far the smallest of our study populations. Location 2 on the other hand, had mostly darker individuals, and is one of the locations most affected by inbreeding. The isolation, size, differing predation histories, and inbreeding in these populations may have resulted in certain colour patterns becoming more prominent in these populations.

When considering protection, Location 4 had the longest and most consistent protection and also provides supplemental food. This is one of the smallest sites, hatchings were rarely seen in this area, and the average SVL is on the larger side (Fig. 8). Thus, this population may exhibit more piebald individuals due to the increased number of larger or older individuals. Location 2 also provided supplemental food and was afforded protection, but to a lesser degree than Location 4. Many hatchlings and juveniles were seen at Location 2, but adult recapture rate was very low (Campbell & Pasachnik, pers. obs.). It is thus possible that this population was younger and fast-growing, which may account for the lack of piebald individuals. There were large adults in this population; however, since we were unable to age the adults in this study it may be that older, and not necessarily just larger individuals, present a higher degree of piebaldism. Similar to Location 2, Location 5 was made up of mostly darker individuals (Fig. 7) and has the smallest average SVL (Fig. 8). This location was afforded some level of protection, and hatchlings and large adults were present, but supplemental food was not provided. Following the previous logic one would then expect this population to have more darker individuals because the older individuals would be smaller. Given that this was not what was found, it might be the case that genetics is playing a larger role in fixing this population for a darker colour morph.

Some individuals exhibited postinflammatory leukoderma, a condition where injured tissue heals without pigment. An example of this can be seen in Figure 5, where an individual that had lost a portion of their tail had healed with completely white epidermis. Only a few of the captured individuals exhibited this characteristic; many were also caught with previous injuries that healed with normal pigmentation. This could be another indication that the increased loss of pigmentation in certain populations is primarily genetically driven, as the individuals with the postinflammatory leukoderma were of varying sizes.

C. oedirhina adults exhibit size-related piebaldism, but is likely that demographic, anthropogenic, and genetic factors play a role in its expression across locations. The evolution of this species on an island with few natural predators could account for the persistence of the piebaldism in the population. Additional studies should focus on understanding the genetic basis for piebaldism in this species, as well as accumulating more recaptures to determine colour change over time.

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Use of an artificial refuge for oviposition by a female ocellated lizard (*Timon lepidus*) in Italy

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The ocellated lizard, *Timon* (formerly *Lacerta*) *lepidus* (Daudin, 1802), is found in the Iberian Peninsula, southern France and Italy (Doré et al., 2015). It is one of the largest lizards in Europe and the only representative of the genus *Timon* in Italy where its distribution is confined to the Ligurian area, in particular from the French border to the Province of Savona (Salvidio et al., 2011). The species lives in typical Mediterranean ecosystems like shrublands, oak forests (especially *Quercus ilex* and *Q. suber*) and pine woodlands. It is also found in olive groves and near human settlements (Salvidio et al., 2004, 2011; Doré et al., 2015). In Italy it is regarded as "extremely endangered" (Capula, 1998; Salvidio et al., 2004) and is listed in Annex III of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) and considered as "Near Threatened" by IUCN (Pleguezuelos et al., 2009).

The observation here reported arose during a study of a population of this lizard located in the Site of Community Importance "Monte Acuto - Poggio Grande - Rio Torsero" (Savona Province, Italy). In April 2013, six artificial shelters were placed in this area in the framework of the master thesis of one of the authors (MS), done under the umbrella of the Montemarcello-Magra Park project dedicated to the conservation of the minor fauna. The project aimed at facilitating the spread of the species in the environment and to improve its observability by the provision of artificial shelters.

The shelters consisted of simple concrete bricks

Figure 1. a) Artificial shelter. b) Specimen observed. c) Specimen during the egg-laying moment; the arrows show the eggs d) Nine eggs observed.

camouflaged by stones and branches collected in situ to make the shelter as natural as possible. Each shelter had an opening of 12x7 cm with a depth of 40 cm (Fig. 1a, 1b). These were located in the area where *T. lepidus* was observed in the past (unpublished data). After the installation of the shelters in 2013, each site was randomly monitored in the most favourable season to observe the species (i.e. between the second part of March until October) seven times during 2013, once in 2014 and twice in 2015.

On June 6, 2015 a female lizard was observed laying nine eggs in one of the refuges (Fig. 1b-1d). This observation documents for the first time the use of this kind of shelter by *T. lepidus* for oviposition. Unfortunately, at the following inspection, on the 29th August 2015, we found that the shelter had been shattered, probably by wild boar present in the area, and could not find any eggs remnant. In France a different kind of shelter, that simulates the burrows of European rabbits (*Oryctolagus cuniculus*), has been successfully used to facilitate the spread and conservation of ocellated lizards (Grillet et al., 2010), highlighting the general importance of artificial shelters to this aim. The potential effectiveness of concrete block shelters, reported here, warrants further investigation both for aiding monitoring programmes, especially during the reproductive season, and as a conservation tool for this species of lizard.

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Death feigning behaviour in *Aplopeltura boa*

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The blunt-headed slug snake, *Aplopeltura boa* is a member of the Pareatidae (Pyron et al., 2011), which is a group of arboreal, nocturnal and mollusc-eating snakes widely distributed in tropical and subtropical regions of Southeastern Asia (e.g. Das, 2012; You et al., 2015). This species inhabits lowland and sub-montane forest up to 1500 m a.s.l. and is often found in understory bushes and undergrowth. Data about the life history of the species are limited: this paper describes death feigning behaviour have not previously been reported.

On 27th February 2009, the second author (DH) found one adult specimen of *A. boa* inside the Taman Rimba Teluk Bahang area of Pulau Pinang Island, west Malaysia. The individual was found in dense tropical forest located close to a small stream (5.435°N, 100.232°E; WGS84, 332 m elev.) just after 23:00h (local time). The snake was observed on a tree approximately 2 m above the ground. After capture, the individual immediately rolled up the front side of its body into a "coil" (Fig. 1A) and when placed on the ground rolled up the whole of its body (Fig. 1B,C). The individual remained in this position for 10 minutes. The mouth was closed throughout. No defensive defecation or smell was emitted.

For snakes, death feigning includes immobility and mouth gapping, often with the tongue hung out, and in more dramatic situations involves voluntary supination and lack of muscle tone (Gregory et al., 2007). Here we recorded immobility and rolled body without mouth gapping. This is similar to observations of immobility in *Antractaspis*

engaddenensis (see Golani & Kochva, 1988). It is possible that as in *Antractaspis*, the death feigning response in *A. boa* does not always occur with the same intensity. This might explain why death feigning has not been reported for this species previously. However, Taylor (1965) described the immobility at *A. boa* after falling to the ground from a tree. Speculatively, this species may use death feigning in addition to cryptic coloration to evade predation. Death feigning has also been recorded for other genera of Indo-Malayan snakes, including *Coelognathus*, *Macrocalamus* and *Xenochrophis* (Vogel & Han-Yuen, 2010).

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Figure 1. Death feigning behaviour at *A. boa*. A – After first contact the snake rolled up its front side of the body. The individual after it had been caught; B – ventral part; C – dorsal part.

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Vipera berus (northern viper):
Morphometrics of feeding on a common field vole

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The diet of *Vipera berus* typically consists of small rodents, lizards and amphibians (Prestt, 1971; Anders, 1991), although birds may also be eaten (Meyer & Monney, 2004; Sweeting, 2011). *V. berus* tackling sizeable prey has been shown in a photo of a male starting to ingest a merlin chick (*Falco columbarius*) (Frazer, 1983); however actual records of the mass of undigested prey relative to snake mass in free ranging *V. berus* have not been commonly reported. The discovery during regular field work of a dead, recently fed, sub-adult female *V. berus* provided an opportunity to record details of prey type and size relative to the predator. The snake was found in a bracken (*Pteridium aquilinum*) and gorse (*Ulex europeas*) riverine woodland ecotone on August 30th 2015 at Black Knowl in the New Forest, southern England. Length (±1mm) was measured with a tape measure, head length from rostral to the posterior edge of the rear-most supralabial scale with a micrometer (±0.1mm) and weight with a Pesola spring balance (±2g). Total body length was 402mm and snout-vent length (SVL) was 355mm. Head length was 17.4mm. A sizeable elongate bulge was visible mid-way between the head and vent indicating that it had recently fed (Fig. 1). Examination of the snake revealed head trauma, a mid-body puncture close to the spine and tissue damage to the upper left and right flanks of the body. The body was in good condition and exhibited no signs of decomposition or maggots. Opening of the stomach revealed an adult male bank vole (*Microtus agrestis*) (Fig. 2). The prey had been swallowed head first. The head of the vole exhibited minor digestion of soft tissue causing the posterior part of the zygomatic arch of the skull to protrude through the fur. Fur was also detached from the mid body section of the right flank. The prey was otherwise intact. The total mass of adder and prey was 54g. The prey mass was 26g and the snake mass with prey removed was 28g. The maximum girth of the prey was 70mm around the shoulders. Girth around the hips was 65mm and the snout to vent length was 105mm. The adder had therefore consumed a meal equivalent to 92.8% of its body weight and 29.6% of it's snout-vent length.

The relationship between maximum mass of ingested prey items and snake SVL for *V. berus* was investigated experimentally by Forsman & Lindell (1993) using adders with SVLs of 400 to 650mm; rather larger than the sub-adult reported here. They determined the relationship

Figure 1. Body of a sub-adult female *V. berus* showing elongate food bulge and tissue damage at five localities on the upper flanks.
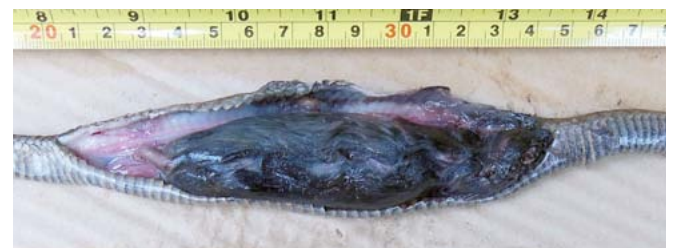
Figure 2. Stomach contents of a sub-adult female *V. berus* showing an almost undigested field vole (*M. agrestis*).

$y=0.15x-35$, where y is prey mass and x is snake SVL. This implies a maximum prey mass of 18.25g for the adder in this study which is 29.8% less than observed here. This would be consistent with an ontogentic shift in the maximum size of prey taken by *V. berus*. however more data would be required to test this hypothesis.

The cause of death is unclear. Carcasses of grass snakes (*Natrix natrix*) have been found at this site with most of the flesh and organs removed and the ribs severed close to the spinal column, but the head and tail are left untouched. These remains are characteristic of buzzard (*Buteo buteo*) kills (Dave Bird, pers. Comm.). Buzzards are common at this site, however no predation of adders by any bird has been witnessed in the many years of recording at this site and no adder carcasses exhibiting characteristics of predation by buzzards have been found. The two other significant avian predators of British snakes, pheasant (*Phasianus colchicus*) and crows (*Corvus corvus*) are rare at this site.

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Nesting and over-wintering sites of Aesculapian snake, *Zamenis longissimus*, in an anthropogenic landscape in the northern extreme of its range

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A relict population of the Aesculapian Snake (*Zamenis longissimus*) survives almost 300 km north of a contiguous distribution area in the northwest of the Czech Republic (Edgar and Bird, 2006) (Map reference: 50°19´57.168´´N, 13°2´28.835´´E; 350 - 450 m.a.s.l.). It has been long hemmed in by the presence of humans but has adapted to anthropogenic structures, in particular compost heaps, low stone walls, agricultural buildings, road culverts and the embankments of roads and railways. In the area of study, small villages and farm buildings are situated along a busy asphalt road that is lined by low stone terraces and undercut by culverts. During June 2007 we radio-tracked two adult females and four males *Z. longissimus* to investigate their selection of nesting and over-wintering sites.

It is probable that the primary substrate selected by *Z. longissimus* for oviposition is rotting wood and stumps of old trees, with some sites subject to mass egg-laying and containing up to 130 eggs, but in human altered landscapes alternative ovipostion sites may be selected (Gomille, 2002). The first female we tracked laid her eggs under the middle of the roadway near the culvert passes under it. A second female laid her eggs in a garden compost heap, where several other clutches and old shed skins were also observed. Egg-laying by *Z. longissimus* in garden compost heaps has been recorded previously (Schultz, 1996; Rugiero et al., 2002). Similarly in the case of *Natrix natrix*, in climatically less favourable areas, heat-generating compost or manure heaps may be used as oviposition sites (Madsen, 1984; Zuiderwijk et al., 1993). This confers advantages of greater hatch rates, earlier hatching, and larger hatchlings with enhanced locomotory performance (Löwenborg et al., 2010). The use of road structures for egg laying by snakes is not exceptional (Schlaepfer et al., 2002; Guiller, 2009; Lelievre et al., 2010) and in our study area a communal egg-laying site was observed on the abutment wall of the road (Figs 1 & 2).

Despite the great range of natural and semi-natural habitats in their home ranges all tagged snakes except one over-wintered in man-made structures. One inside a stonewall near a house, a second actually inside a house,

Figure 1. Photograph showing the abutment wall of the road, which is a frequently used egg-laying site. (Photograph, Radovan Vita)

Figure 2. Photograph showing three *Z. longissimus* entering and exiting the egg deposition site shown in Fig 1. A female was observed basking daily between 6 and 30 June at the entrance with egg-laying occurring on 28 June in the same cavity. Basking was occasionally observed alongside two other gravid female *Z. longissimus* and also on several occasions with a female grass snake, *N. natrix*. (Photograph, Karel Janousek)

and three others inside dilapidated stone walls situated in the middle of semi-natural habitats. The female that laid eggs in the compost heap was killed in the autumn by a bird of prey while at the compost heap, consequently her over wintering site was not determined. This note suggests that *Z. longissimus* is able to benefit from human presence, and even from such potentially hostile man-made structures as culverts and abutment walls of roads.

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Amphiesma stolatum (striped keelback):
Habitat and reproduction

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Amphiesma stolatum (Natricinae) is a non-venomous Asiatic colubrid distributed widely from Pakistan, eastward into Southern Asia, northward into south China and on various mainlands and islands across Southeast Asia. In India, the snake is found up to 2000 feet (610 m). *A. stolatum* is a small species (to 90 cm total length), terrestrial, diurnal, and inhabits well-watered lowland plains, forests, hills, rice paddy, agricultural and rural areas. It has a typical natricine diet and hunts amphibians, fish, invertebrates, small lizards and rodents. Females produce 5-15 eggs per clutch throughout active parts of the year and may also attend clutches. The snake aestivates during hot weather and is more frequently observed during rainy seasons (Whitaker & Captain, 2004).

During monsoon season on 18/07/2014, at midday, we discovered two clutches of snake eggs whilst conducting amphibian refugia surveys along line transects (see Heyer et al., 1994) on an open rocky plateau near Dhamapur, Kolhapur District, Maharastra, India (N 16 1 53.4, E 73 35 2.4; 85 m) (Fig. 1). The plateau is a ferricrete of laterite with multiple patches of small loose rocks (Fig. 2). The clutches of eggs were found under loose rocks situated next to shallow temporary rain pools in the middle of the plateau habitat. One clutch had two neonate *A. stolatum* under the rock, complete with empty egg shells. These empty egg shells were also attached to the rest of the clutch. We therefore surmised that the egg clutches were most likely to be *A. stolatum*. A nearby rock also unveiled an adult female *A. stolatum* underneath, adding to the known presence of the species in the area. We recorded morphometrics of the specimens and eggs (Tables 1, 2 and 3). In the interests of conservation we left the eggs in-situ to hatch naturally, replacing the covering rock. We also released the snakes at the site of capture, under their refugia.

What is interesting to note about this record of egg-laying was the deposition site. Both clutches and snakes were discovered in the middle of an open rocky plateau habitat with nothing but occasional sparse rocks for cover. The rocks were directly adjacent to the edge of shallow temporary pools that were formed by recent rains. These pools were the subject of our initial study for amphibians on the rocky plateaus and contained ample tadpoles and small frogs belonging to *Fejervarya* spp. genera. It is typical for many oviparous snakes to select a location to lay eggs which has an appropriate temperature and humidity range,
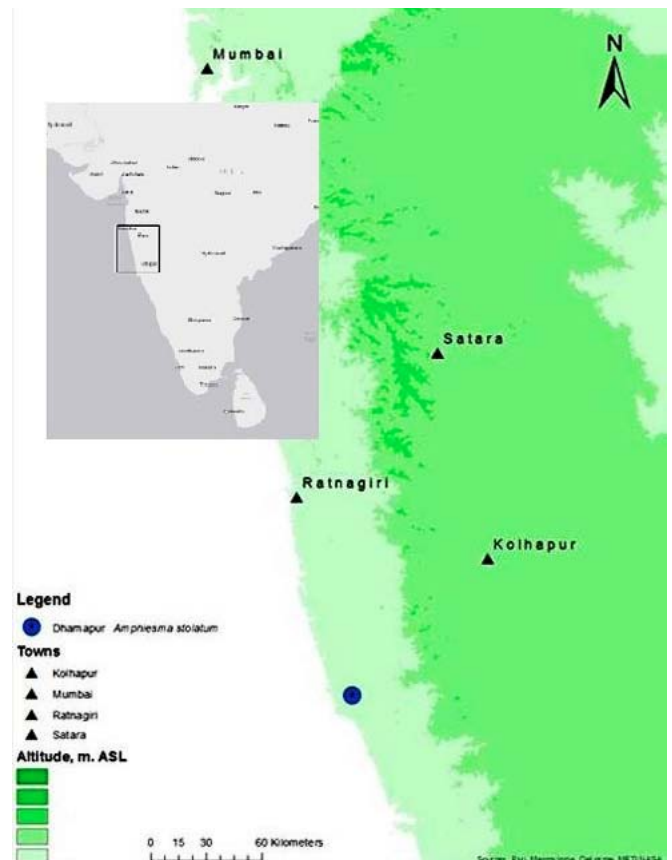
Figure 1. Map showing location of Dhamapur, India.

as well as a substrate capable of maintaining it (Lillywhite, 2014). Such sites are usually warm, moist and free from initial predation so that they may assist in thermal and water regulation of the eggs (Lillywhite, 2014). In this instance, the rock habitat, being out in the open and exposed to full sun, may only have provided temporary resistance to desiccation, and a suitable environment for a limited time. Alternative habitat could have easily been selected nearby in the form of established islands of vegetation (within 300 m of our transect) or the forest edges that surrounded the rocky plateau (within 800 m of our transect). Such alternative locations, close to our transects, contained leaf-litter and shade that arguably might have made for a less precarious egg deposition location. The open plateau rocks had minimal soil and debris under them and although were

Table 1. Egg clutch no. 1 morphometrics and habitat detail.

Clutch 1	Rock: 35x20cm	Temp: 27.1˚C
Egg No.	Length (mm)	Width (mm)
1	37.80	17.65
2	21.10	17.00
3	24.10	16.70
4	23.00	16.60
5	23.00	16.20
6	20.10	16.60
7	25.60	14.60

Table 2. Egg clutch no. 2 morphometrics and habitat detail.

Clutch 2	Rock: 55x40cm	Temp: 25.8˚C
Egg No.	Length (mm)	Width (mm)
1	23.2	12.7
2	20.7	14.5
3	20.1	12.9
4	20.6	14.2
5	19.6	13.9
6	20.5	14.3
7	20.5	15.6
8	22.3	12.9

Table 3. *Amphiesma stolatum* morphometrics.

Snakes	SVL (mm)	Tail (mm)	Weight (grams)
Neonate	11.6	4.8	1.1
Adult F	29.6	11.2	15.05

Figure 2. Rocky plateau habitat near Dhamapur comprising lateritic rock ground with sparse woody vegetation, scattered loose lateritic rocks, shallow soil depressions, temporary streams and pools. Insert: rock partially lifted to reveal *A. stolatum* eggs.

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wet from recent rains, could easily dry out on sunnier days and thus place developing eggs at risk.

Previous observations by Wall (1925) recorded eggs of the species successfully hatching in a garden in Rangoon confirming that this snake will use semi-natural nesting sites. Our observation suggests that this unusual choice of deposition among lateritic rock by *A. stolatum* may be influenced by seasonality (wet season rains), prey availability (amphibians/tadpole in temporary pools), and/or suitable foraging habitat. We also speculate that in this instance the snakes concerned may have chosen to lay eggs in an area with immediate prey availability in preference over traditional substrate as a way of potentially improving offspring survival.

Defensive behaviour exhibited by the green and black poison frog (*Dendrobates auratus*) in response to simulated predation

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Anurans exhibit a broad range of defensive behaviours that have evolved to cope with the risk of predation. Deimatic behaviours are characterised as postures or displays exhibited by prey that are intended to startle or warn predators and ultimately discourage predation (Edmunds, 1974). Body raising is one type of deimatic behaviour in which anurans outstretch their legs vertically or laterally and the body is raised off the ground. In certain aposematic species, this behaviour is thought to enhance the colour signal as a warning to predators. This defensive behaviour has been described in certain members of Hylidae, Leptodactylidae, Leiuperidae, and Bufonidae (Toledo et al., 2011) and one member of Dendrobatidae, *Ameerega flavopicta* (formerly *Epipedobates flavopictus*) (Toledo et al., 2004).

Dendrobates auratus is an aposematically coloured, chemically defended member of Dendrobatidae that is naturally distributed throughout Central America (Savage, 2002). Herein, we report deimatic body raising behaviour by way of vertical stretching of the legs by captive-raised *D. auratus*. Although the frogs were captive-born and raised, they are derived from a Costa Rican lineage.

Between 30 September 2015 and 13 November 2015, and as part of a larger lab-based study on the effects of predation pressure on behaviour, one mature *D. auratus* individual (SVL = 28.3 mm) began exhibiting body raising in response to simulated predation attempts that were conducted daily. No frogs were harmed during the experiment. Simulated predation included gently picking up and releasing individual frogs with a pair of 3" pressure sensitive forceps (Williams et al., 2000). Body raising consisted of the individual *D. auratus* extending its front and rear legs and pointing its snout towards the ground resulting in a back arching stance (Fig. 1). Within one week of the adult's initial behaviour, three juveniles in the same terrarium, exposed to the same predation treatment, began displaying a similar behaviour. Initially, the juveniles only exhibited the behaviour occasionally, but by the end of the 1.5 months of predation treatment all four frogs exhibited the behaviour daily. Furthermore, the behaviour was originally observed only following simulated predation attempts, but by the end of the experiment it was observed prior to the predation treatment, where all individuals immediately body raised when they were exposed from under cover objects in their terrarium. On the basis of our observations,

Figure 1. Adult *Dendrobates auratus* exhibiting body raising behaviour by vertical stretching of the legs.

it appears that body raising in *D. auratus* is in response to stress from predation attempts. Given that the dorsal surface of *D. auratus* contains the highest concentration of alkaloid defences (Saporito et al., 2010), body raising may provide increased exposure of this chemically defended region to a potential predator while increasing the conspicuousness of their aposematic colouration.

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Salamandra algira spelaea (Beni Snassen fire salamander); new distributional records

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Salamandra algira Bedriaga, 1883 is the only species of the genus *Salamandra* present in North Africa (Schleich et al., 1996). Its distribution extends from northern Morocco to northeastern Algeria (Escoriza & Ben Hassine, 2015). In this broad area of distribution there are several subspecies; *S. algira tingitana* in the Tingitana Peninsula (northern Morocco), *S. algira splendens* in the Middle Atlas and Rif mountains (northern Morocco), and *S. algira spelaea* in the Beni Snassen mountains (northeastern Morocco), being restricted the nominotypical form to northern Algeria (Ben Hassine et al., 2016).

The recently described *S. algira spelaea* (Escoriza & Comas, 2007) (Fig. 1), is phylogenetically closer to Algerian *S. algira algira* than to other subspecies in Morocco (Escoriza et al., 2006). It is likely to be scarce in the Beni Snassen massif due to arid conditions (Escoriza & Comas, 2007; Faouzi et al., 2015). During the winter-spring season, over a 5 year period (2009–2014), we performed several surveys in the mountains of Beni Snassen, to improve knowledge of the distribution and ecology of this rare salamander (Escoriza & Ben Hassine, 2014). The surveys were performed following brooks and also lifting stones and logs, with an effort of 1h/Km (Teixera et al., 2001).

In this short communication the first precise distributional records for *S. algira spelaea* in the Beni Snassen massif are presented. The results indicated a limited distribution, mostly located in the valley of Oued Beni Waklane (Fig. 2) where it occurs between 600–1300 m above sea level at coordinates 34.84ºN, 2.26ºW, 34.85ºN, 2.23ºW and 34.84ºN, 2.21ºW. Both adults and larvae were found, suggesting the presence of stable populations. The vegetation included forests mainly of evergreen oaks (*Quercus coccifera* and *Quercus ilex*; Fig. 3) and araars (*Tetraclinis articulata*), with a dense understory formed by *Arbutus unedo*, *Erica arborea*, *Pistacia lentiscus* and *Viburnum timus*. In these habitats *S. algira* breeds in small streams and fountains (Fig. 3) along with *Discoglossus pictus* and *Pelophylax saharicus*. The species was not observed in other areas of the massif, such as eastern and western parts. These areas are transitional towards the semi-arid conditions, dominated by xero-thermal shrub formations (*Ceratonia siliqua*, *Chamareops humilis*, *Olea europaea*) and perennial grasses (halfah grass).

Figure 1. Juvenile of *S. algira spelaea* (Ouartass, Morocco).
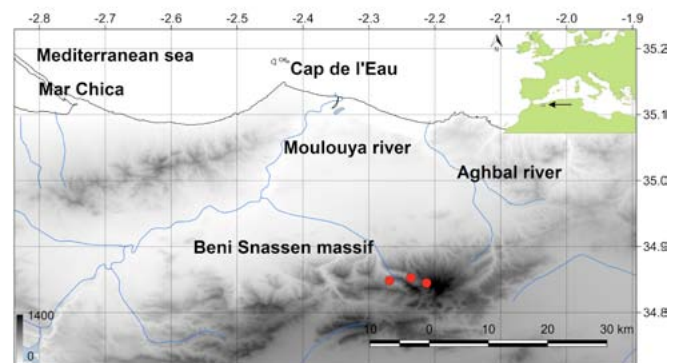
Figure 2. Map (A) of the region showing the distribution (red dots) of *S. algira spelaea* in the Beni Snassen Massif (northeastern Morocco).

Figure 3. Breeding habitat of *S. algira spelaea* in the Beni Snassen massif (northeastern Morocco).

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New records of Gaboon viper (*Bitis gabonica*) in Angola

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The Gaboon viper (*Bitis gabonica*) is a sedentary snake distributed across the tropical and subtropical forests, woodlands and savannas of sub-Saharan Africa (e.g. Angelici et al., 2000; Warner, 2009). The geographical distribution ranges from Nigeria to southern Sudan, through Uganda, western Kenya, southeastern Tanzania, Zambia, Democratic Republic of Congo, the extreme north of Angola (Zaire and Cabinda provinces), northern Zululand, and South Africa (Spawls et al, 1995). Between April 2015 and May 2015 a search was made for *B. gabonica* through four provinces of Angola (South Kwanza, Benguela, Huila and Malange). This included requesting local people to deliver newly-captured individuals (dead or alive). Subsequently eight specimens of *B. gabonica* were found and added to the CIMETOX collection (Table 1, Fig. 1 & 2). All snakes encountered were sent to the Medical Faculty of Malange, Lueji N'konde University. Live snakes were housed in a serpentarium; dead animals were fixed in formalin and placed in the Snake Collection of the Centre for Research and Information on Drug and Toxicology (CIMETOX) in the same department.

These collections expanded our knowledge of the distribution of this species in Angola, to include south into Benguela Province and Malange (Fig.1). The *B. gabonica* from Benguela was collected in a woodland thicket environment, while those in Malange were in a forest of tall bushes and savanna mosaic. According to Marsh and Whaler (1984) *B. gabonica* occupies mostly woodland thicket habitats and this type of vegetation can be found between Malange and Benguela (República de Angola, 2006). More inventories in Angola are needed to confirm these assumptions.
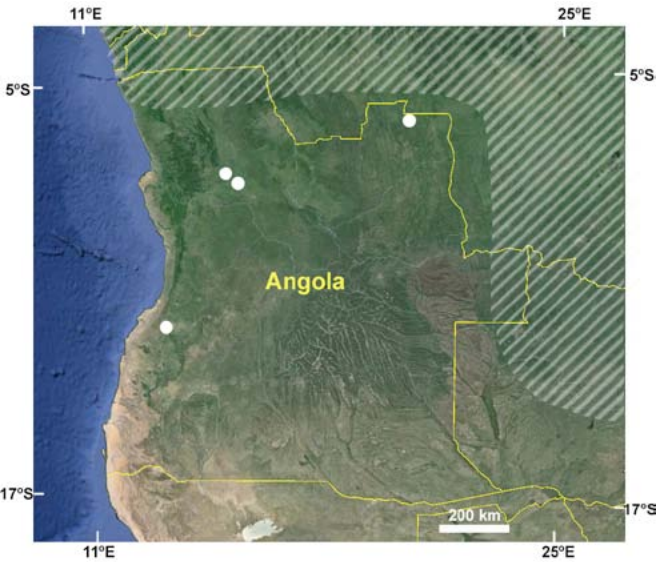
Figure 1. Geographical distribution of *B. gabonica*. The cross hatched area corresponds to the previously known distribution. The white spots indicate locations of new specimens.

Table 1. Dates of collection, location, geographical coordinates, size and sex of *B. gabonica* collected in Angola. F = Female; M = Male; SVL = Snout-vent length; nd = not deposited

Date of collection	Municipality (Province)	Sex	SVL (cm)	Geographic coordinates		Catalogue number
				Latitude	Longitude	CMTOX
April 2014	Cacuso (Malange)	F	115	-9.4994°S	16.1613 °E	0010
25/3/15	Caimbambo (Benguela)	F	125	-13.1677 °S	13.7663 °E	0013
29/3/15	Kalandula (Malange)	F	118	-9.1956 °S	15.9026 °E	0015
30/3/2015	Kalandula (Malange)	M	108	-9.1956 °S	15.9027 °E	0016
01/04/2015	Kalandula (Malange)	M	109	-9.1956 °S	15.9027 °E	0018
02/04/2015	Kalandula (Malange)	F	150	-9.2097 °S	15.8906 °E	0019
02/04/2015	Kalandula (Malange)	F	121	-9.1907 °S	15.9159 °E	0020
04/05/2015	Kalandula (Malange)	M	119	-9.3872 °S	15.9441 °E	0021
30/05/2015	Dundo (Luanda Norte)	M	195	-7.3667 °S	20.8167 °E	nd

Figure 2. Sample of *B. gabonica* collected in Kalandula, Angola (29/03/2015)

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Venomous Reptiles & Their Toxins:
Evolution, Pathophysiology & Biodiscovery
Edited by Bryan G. Fry

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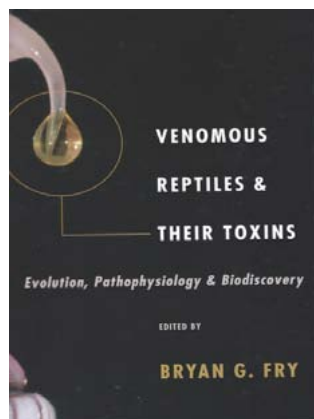
One of the first things that struck me about this book is the number of contributors, 110 to be precise, that were involved on this project. That the editor has brought together such a thorough and international cast shows you immediately that this is a volume of some serious consideration. The second thing that struck me was the style in which the book is written. Unlike other recent books on reptile venoms and toxins (Mackessy 2010; Weinstein et al 2011) that, whilst being in my opinion superb works on the subject, are on the impenetrable side without prior knowledge of the toxicological vocabulary, Fry's book is slightly more manageable for someone starting out with the subject. That is not to say that the book is not technical and precise. It very clearly is.

The first chapter is a detailed account of the controversial reptiles group the Toxicofera, as proposed by the editor. Regardless of which side of the "origins of reptile toxins" fence you sit this chapter gives a thorough overview and synthesis of the single origin argument. It is a very convincing account, as you would expect from one of the main proposers of the Toxiceferan clade at the current time. Although understandably so, given the editors stake in the outcome of that argument, it is shame that the argument for multiple origins is not covered to give a full account of current ideas regarding the subject.

The next three chapters cover the symptoms and treatment of snakebite, antivenom research and ineffective treatments. All are thoroughly researched and presented in the same easy to read style. Chapters five through seven are detailed accounts of maintaining venomous reptile collections, veterinary care of venomous reptiles and research methods, respectively. Again all are well researched and thoroughly referenced. Each contain example protocols where appropriate that serve as excellent reference material and starting points for those creating their own protocols, whether in field or captive settings.

The next section of 17 chapters, the bulk of the book, deals with the different toxin families found in reptile oral secretions. Everything from 3 finger toxins, metalloproteases to phospholipase and CRSPs are covered in the same meticulous detail. Each chapter is dedicated to a different family of toxins and tends to follow the same format, information and current knowledge permitting. Sections in these chapters cover the following subjects: evolutionary origins; structural and functional forms, both ancestral and derived including their physiological affects; current and potential therapeutic uses; and convergence with other toxins.

The final chapter deals with two subjects that are often misunderstood and ill-defined. The first is the subject of poisonous snakes of the genus *Rhabdophis*. These natricine snakes have nuchal glands that sequester the toxins of toads they eat and use them as a defensive mechanism. The genus also happens to include species that have caused human fatalities. The subject of the chapter deals with whether Komodo dragons kill their prey using oral bacteria as a weapon. I won't dwell on the details here as the information is far more interesting read first hand in the book itself.

The book is rounded off with a glossary and a huge 112 page bibliography. The glossary is very thorough, and while it does miss some technical terms included in the text, it is successful in enabling the reader to understand the technical terms in the text. I turned to it on several occasions while reading the book for the review.

Overall, this is a very thorough, well researched and referenced book that is easy to read and relatively easy to follow and understand its understandably technical aspect. A book that is well worth investing in for anyone with an interest in venomous reptiles.

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