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Front Cover: An adult female Chinese cobra (*Naja atra*), the subject of a study of venom spitting presented on page 22. Photograph taken by Alessandro Paterna.

Emigration and seasonal migration of the northern viper (*Vipera berus*) in a chalk grassland reserve

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ABSTRACT - Long-term monitoring of individually recognisable northern vipers (*Vipera berus*) has provided new evidence concerning emigration and seasonal migration. Northern vipers are well known to migrate from their wintering areas in springtime to forage and/or reproduce and then eventually return to the same wintering areas to hibernate. Less well known is their capacity to emigrate and in this study we suggest that this can be recognised when a viper selects a new wintering area at least 150 m from that used previously. Evidence is provided showing that there are two significant opportunities for emigration. First, neonates may wander considerable distances from their place of birth before selecting their hibernacula. Second, in the following spring some of the emergent juveniles disperse from the neonate-selected wintering areas but lack the homing ability to return to them and are consequently obliged to select new hibernacula. The sub-adults and adults developing from them have sufficient homing abilities to return to the wintering areas they occupied as juveniles. Only 45 % of males and 23 % of females showed signs of emigration and, interestingly, males emigrated over significantly greater distances than females. The direction of viper displacements in springtime varied considerably being influenced by the position of the sun, topography, weather, barriers to movement, and corridors of cover. Irrespective of migration direction, the sub-adults and adults appeared to make accurate returns to their wintering areas. The return of many adult males to these areas was commonly prior to their second moult and close to mid-summer; much earlier than reported previously. This is interpreted as an opportunity to occupy a warmer area to facilitate repopulation of the testes with spermatocytes prior to hibernation and is equivalent to the behaviour of gravid females that remain in the wintering area to promote the development of their embryos. But unlike females the males remain hidden from view until the autumn. The significance of neonates and juveniles as the life stages that emigrate is discussed in relation to viper reintroduction programmes.

INTRODUCTION

In springtime, northern vipers (*Viper berus*) may make seasonal migrations away from their established wintering areas for foraging and/or reproduction and eventually return to the same wintering area to select a hibernaculum. Wintering areas are typically warmer, drier habitats receiving extended periods of sunlight (Viitanen, 1967; Prestt, 1971). The seasonal migrations are distinct from emigration that can be recognised by the selection of new, distant, wintering areas. Emigration is an important feature of viper behaviour enabling the formation of new sub-populations and the maintenance of healthy metapopulations (Madsen et al., 1999). Unlike migration, to date emigration has been poorly documented.

The seasonal movements of *V. berus* across the landscape have been reported in several studies (Saint Girons, 1952; Viitanen, 1967; Prestt, 1971; Neumeyer, 1987) which are in general agreement with each other but differ in certain specifics. Northern vipers emerge from hibernation in springtime (usually March and April). Adult males are more tolerant of cold conditions and break hibernation a week or two before the adult females and immatures. Their early emergence allows them to bask in the spring sunshine consequently accelerating spermiogenesis (Viitanen, 1967; Herczeg et al., 2007). They are ready to mate following their first moult, after which they seek out reproductively active females, often over considerable distances from the wintering area (Viitanen, 1967; Hand, 2018). After a period of searching, the males may be guided to their mates if they encounter the scent trails left by the reproductively active females. Following the period of sexual activity the males are reported to seek out places to forage, referred to as the 'summer areas'. Likewise non-reproductive adult females and the immatures of both genders also seek out the summer areas to forage. The foraging vipers generally disappear from view so that they are difficult for humans to detect without aids such as telemetry or artificial refuges. It has been demonstrated recently that they tend to take up positions in deep cover, effectively below ground (Hand, 2018). Following mating, reproductive females seek out good basking locations in the wintering areas to advance the development of the embryos they carry. After giving birth in late summer (August or early September) these females begin to forage, although if food is available then some females may feed whilst they are still gravid (Bauwens

& Claus, 2019). In the autumn (September and October) the vipers move close to their wintering areas and subsequently select a hibernaculum. The vipers may hibernate singly or in groups but Viitanen (1967) noted that some 25 % of the snakes winter separately, many of these being neonates.

How the vipers manage to navigate the landscape remains poorly known but there are some clues. In southern Finland, Viitanen (1967) observed that on trend the vipers travelled south-west in the spring and in the opposite direction, north-east, in the autumn so that they return to the proximity of their previous wintering area. He speculated that they do this by following the sun from mid-day onwards in the spring (photo-positive) and the opposite in the autumn (photo-negative).

In the current study, *V. berus* populations were monitored on a chalk grassland reserve for twelve-years. Data on viper movements were collected by observing them along survey paths and at artificial refuges. The results are presented first as a series of four illustrative case studies of landscape movements by individual vipers, and then analysed to provide summaries of emigration by a larger number of males and females.

MATERIALS AND METHODS

Survey site
The study site was a chalk grassland nature reserve at about 51˚N, 0˚E. The reserve is managed by Kent Wildlife Trust and comprises three distinct areas of grassland totalling 11.1 ha separated from each other by dense woodland (Fig. 1). These three areas have margins and islands of low scrub and generally slope at 10˚ to 15˚ and each area has a different aspect: Area 1 slopes west into a relatively narrow valley, Area 2 slopes predominantly to the south-west, and Area 3 slopes predominantly southwards; the last two areas face into a wide open vale. The grassland is managed by extensive cattle grazing, usually from August to November, and scrub clearance by hand.

In addition to the grassland, there is a further 17 ha of sheep-grazed pasture to the west with hedgerows (Fig. 1)
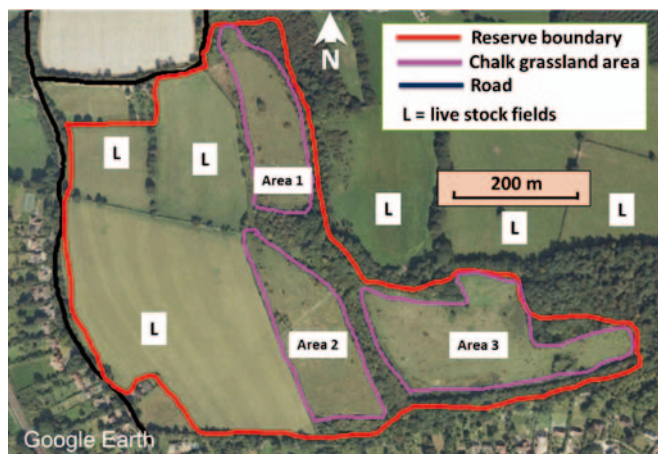

Figure 1. Map of the reserve showing three areas of chalk grassland, livestock fields, housing to the south and west, road to the west and north

along which viper observations are made. Otherwise, the reserve is bordered by open farmland, housing, woodland, and roads. It seems to offer few prospects of connection with other viper populations.

Monitoring methodology
A detailed account of the monitoring methodology is presented by Hodges & Seabrook (2018). The data presented here were collected from 2008 to 2018 during visits to the whole site in the reptile active period from February to October. The number of annual visits varied from 59 to 77. For each visit, the survey followed standard survey paths (total 6 km) and at intervals along the paths there are refuge positions where paired artificial refuges of corrugated iron and roofing felt (each 50 cm x 60 cm) are placed. At the start there were 31 refuge positions, increasing to 50 positions from 2016 onwards; vipers were recorded at refuge locations and along the survey paths. In this study, the advantage of using refuges is that they increase the numbers of observations on immature stages (Hodges & Seabrook, 2016), particularly in the vicinity of the wintering areas selected by neonates and juveniles, as well as on adults that are moulting or digesting food. To facilitate individual recognition, close-up photographs were taken of head-scale patterns (Benson, 1999) when the vipers were located under refuges or, by using a long-focus lens, when they were basking in the open; the patterns were coded and then entered into a database. As of 2018 the database held records of 505 individual vipers but only in the case of 52 individuals (10.3 %) was there sufficient data to contribute to this study. The study involved no animal handling in order to minimise disturbance and stress.

Different life stages were defined as follows: neonates had not yet hibernated; juveniles had hibernated only once; and, the sub-adult stages in the case of males were completed in two years and in females three years. Adults were recognised by having completed a total at least 4 hibernations in the case of males and, normally, a minimum of five in the case of females. In this study, assigning age class to individuals was relatively easy as nearly all vipers had been observed initially as neonates or juveniles, and in a few cases only as fully mature adults. The gender of adults and sub-adults was determined by colouration and body proportions (Smith, 1951; Beebee & Griffiths, 2000) while that of juveniles was assigned by colour and confirmed when they had developed to later stages.

Recording viper positions
The positions of vipers were recorded either at refuges or if along the survey paths then as the distance and direction from the nearest refuge. Observations of vipers emerging in early spring (February to early April) and/or those about to enter hibernation in autumn (September to early October) indicate the wintering areas chosen and the approximate location of their hibernacula (Viitanen, 1967; Prestt, 1971; Neumeyer, 1987); in this study the wintering areas typically had diameters of 20 - 30 m. To observe approximate distances between wintering areas, their locations were plotted on Google Earth and the straight line distance

between them in subsequent years was measured by creating a path on Google Earth (Path Tool). The lengths of paths were estimated using 'KML Path Measurer' (available for free download). If a viper was within 10 m either way of the previous year's position then its movement was treated as the selection of a basking site and not emigration or migration, i.e. recorded as zero. If the location of successive wintering areas was more than 150 m apart this was treated as evidence of emigration.

Statistical analysis

Data on the distances between the wintering areas selected by neonates and those selected by the same individuals in the last sub-adult stage, and the distances between these areas selected by the same individuals as adults in their first or second years, had high values of kurtosis and skewness (>0.8). This indicated that that they did not conform to a normal distribution. Consequently, paired comparisons were made using the Mann Whitney 'U' test (Siegel, 1956), applied as the more conservative two-tailed test. Differences were treated as statistically significant when the probability of them occurring by chance was 5 % or less (p≤0.05). With large sample sizes the data were approximated to a normal distribution (z), while smaller samples were assessed against the Mann Whitney 'U' distribution.

RESULTS

Case studies

The case studies reported below have been chosen as good examples of vipers making long displacements across the landscape of the reserve. They are intended to indicate the potential for emigration and/or seasonal migration, giving details of net directions and annual timings. Such lengthy displacements were observed in few of 505 vipers known to the project but there may perhaps have been many more cases of this if circumstances had permitted greater frequency of observation on these vipers.

Case Study 1 - Emigration and seasonal migration of a male viper (M100) in immature and adult stages

In the four years before M100 became sexually mature, all net displacements across the reserve were to the west of its site of birth (1, Fig. 2). M100 was first observed as a neonate under a refuge tin on 12th September 2012 (1, in Fig. 2) but, in a warm autumn when the snakes were still active even in October, it subsequently selected a hibernaculum about 200 m to the north-west (2, in Fig. 2) where it emerged in early April 2013. The juvenile viper was seen again three months later (July 2013) when it had moved beneath a refuge tin 275 m to the south-west (3, in Fig. 2). M100 was not seen during the two sub-adult stages (2014 & 2015) but the wintering area selected by the second stage sub-adult was evident when in April 2016 the newly emerged adult male was detected 200 m further west (4, in Fig. 2). There is no indication of the path travelled by this snake as a sub-adult but the net displacement between wintering area of the neonate and that of the last sub-adult was 713 m. In 2016 and 2017 the mature M100 dispersed in search of females resulting in net displacements to the north of the wintering area (5 & 7, in Fig. 2) where on both occasions M100 was found with a reproductive female. In both years M100 eventually returned south to the same wintering area (♣ in Fig. 2).

The pattern of emigration shown by M100 was a net westward movement starting with the neonate and continued by the juvenile. The last observation of the
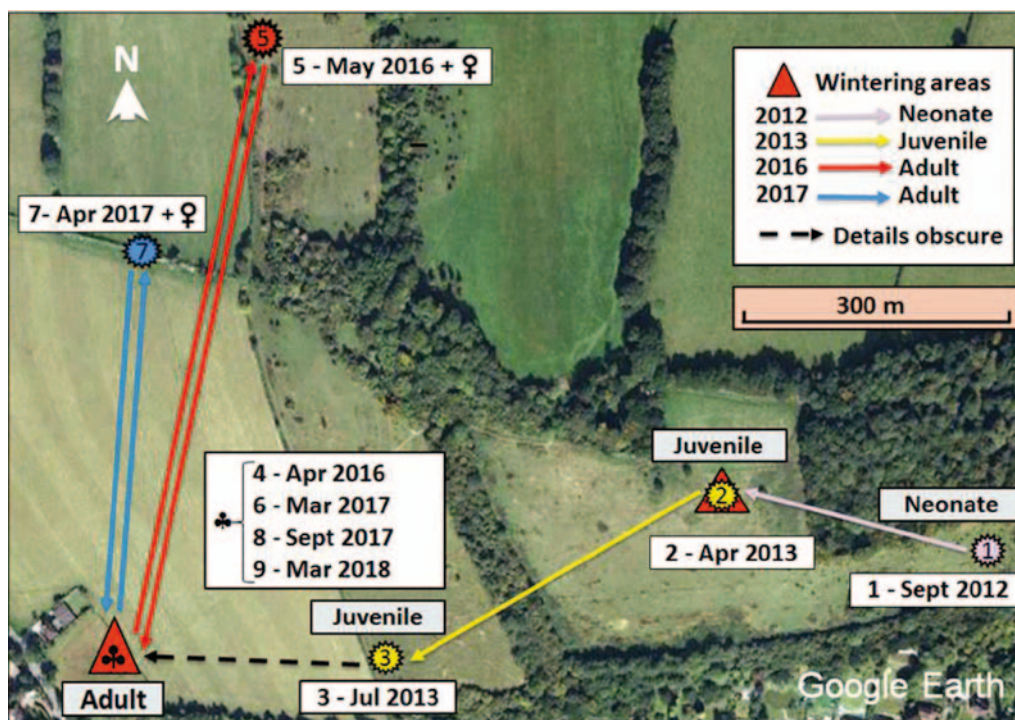

Figure 2. Net displacements across the landscape and the wintering area used by a male *V. berus* (M100) observed as a neonate in 2012, juvenile in 2013, and then as an adult (2016 – 2018). No information is available on the sub-adult stages.

juvenile was in July so that further displacement was still likely that year and indeed may have been to the location that was later observed to be the adult wintering area (♣, in Fig. 2). Further westward movement would not have been possible as this was blocked by a wide, busy road. It is not clear why as an adult M100 moved northwards although the same direction of seasonal migration has been recorded for other males in this locality. The very busy road to the west and housing and open gardens to the south would present significant barriers. The main linear features on the reserve are hedgerows and those that M100 would contact first run north-south (Fig. 2). Open pasture in the area would place reliance on the use of these hedgerows for cover. As movement south along the hedgerow was faced with the barrier of shaded woodland and housing the only option may have been to head north.

Case Study 2 - Emigration and seasonal migration of the immature stages of a male viper (M40)

M40 was observed as a juvenile and in its two sub-adult stages. In this time it demonstrated both emigration and seasonal migrations (Fig. 3). The adult M40 was not observed.

It is not known where M40 was born but as a juvenile it was seen in March 2007 in the wintering area that would have been selected by the neonate (1, in Fig. 3). The juvenile moved 180 m south-west where it was observed beneath a refuge tin (2, in Fig. 3). It seems likely that M40 then moved from here to select a wintering area 215 m to the north-east (3, in Fig. 3). This was not observed directly but has been assumed since M40 in both the first and second sub-adult stages also used this as its wintering area (♣, in Fig. 3) and undertook routine foraging migrations back to the same feeding area used by the juvenile in the south-west

(♠, in Fig. 3). In this example, it appears that the juvenile was responsible for the selection of the wintering area and hence for emigration.

Case study 3 - Seasonal migration of an adult male viper (M39)

The wintering area of adult M39 was at the top of the slope in Area 3 in a very warm sunny but otherwise exposed location. From 2012 to 2014, M39 made springtime migrations and on each occasion located at least one reproductive female. These migrations were made despite there being at least one reproductive female in the vicinity of his own wintering area (2 in both 2012 and 2014, 1 in 2013). These females were eventually paired with males that had hibernated further down the slope and had moved in a north-westerly direction to find them.

The directions of M39's springtime migrations in 2012 and 2014 were similar (Fig. 4 & Table 1). Following the first moult of the year, M39 moved in a westerly direction to the same location in both years (♥, in Fig. 4). In 2012, he was found competing with another male for access to a female while in 2014 he was observed mating with and, the following day, mate-guarding the same female. Subsequently, in both years M39 headed north where in 2012 he was found under a refuge tin (3, in Fig. 4), while in 2014 he was detected somewhat further north in the open (10, in Fig. 4). In both years, this male then returned to the wintering area (♣, in Fig. 4) to be found under a refuge tin undergoing a second moult. In both these years there was a third moult in August.

In the spring of 2013, M39 behaved differently. This viper was first detected in the wintering area on 8th April, somewhat later than usual due to the cool damp weather
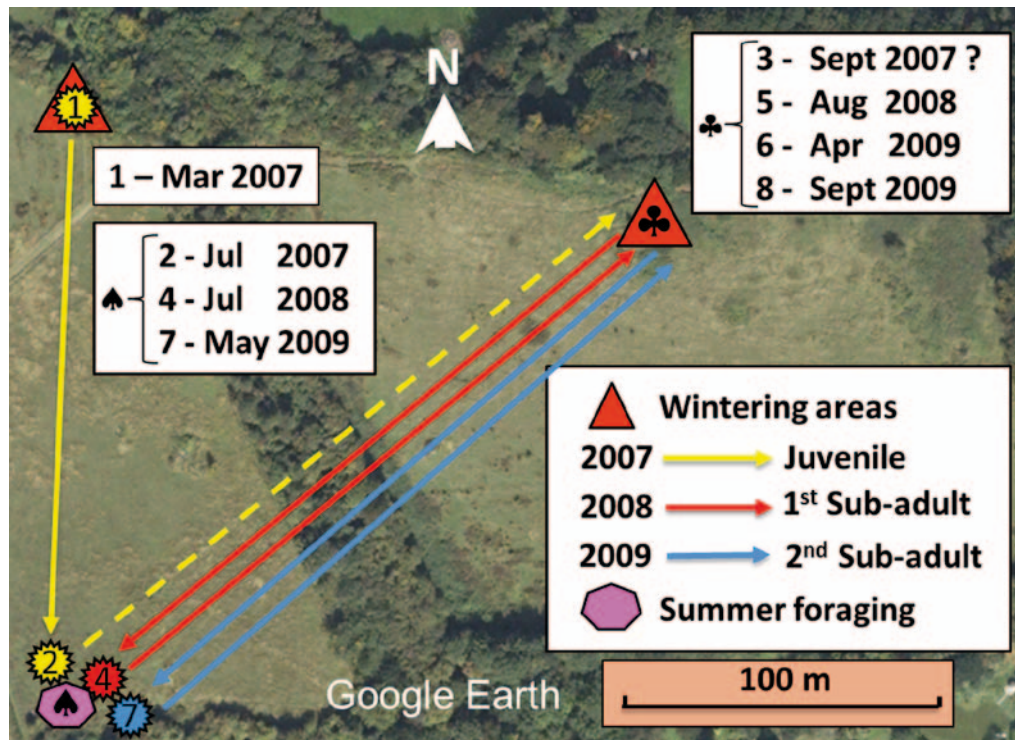

Figure 3. Net displacements of a male *V. berus* (M40) as a juvenile and first and second stage sub-adult
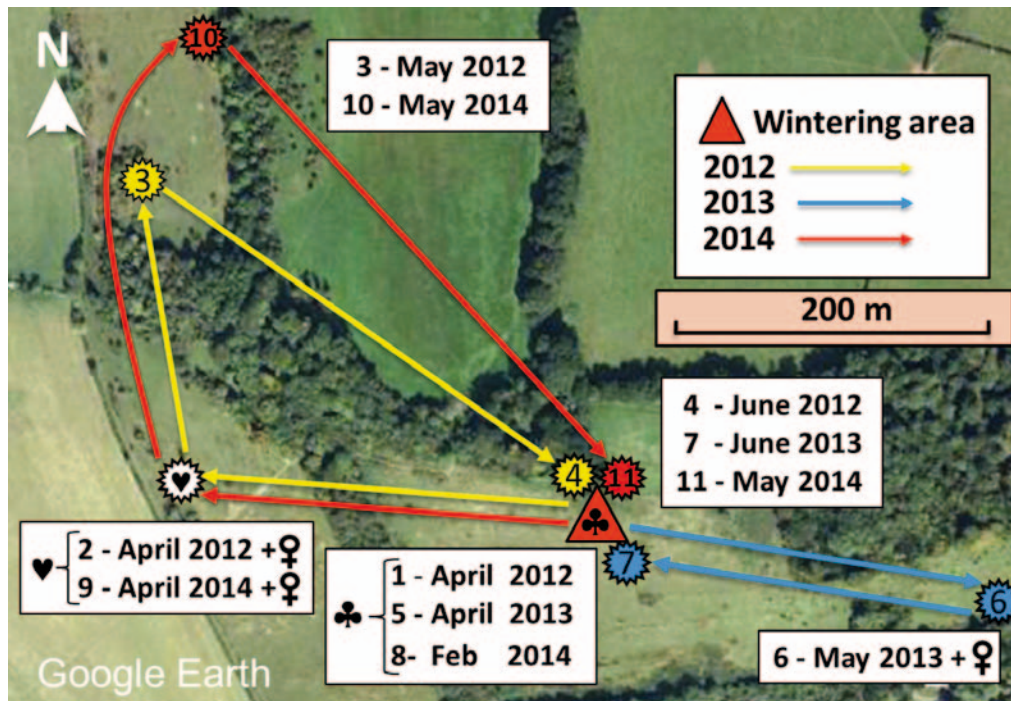
Figure 4. Net displacements of an adult male *V. berus* (M39) in spring and early summer in each of three consecutive years

Table 1. Springtime migrations following 1st moult of adult male Vipera berus (M39) searching for mates and then returning to the wintering area in time for the 2nd moult. For comparison, data from a telemetry study of an adult male (M235) in England (Hand, 2018; Hand, pers. comm., 2019) is included.

Male	Year	Date of 1st moult	Net displacements (m) from, and back to, wintering area with net directions			Date return recorded	Date of 2nd moult
			Outward	Middle	Return		
M39	2012	<16th April	400 m W	270 m N	367 m SE	20th June	25th June
M39	2013	<30th April	330 m SE	-	330 m NW	29th June	>4th July
M39	2014	30th March	400 m W	329 m N	403 m SE	9th May	13th June
M235	2017	10th April	352 m NW	98 m NE	380 m SE	23rd May	31st May

of that year. By 30th April, M39 had moved 30 m south-east and was recently moulted. Ten days later he was seen 330 m south-east of the wintering area (6, in Fig. 4) and was in the presence of a female and other males. By June, M39 had returned to the wintering area and was found under a refuge tin. His second moult was eventually completed soon after 4th July (Table 1) and there was no evidence of a third moult that year. This unusual easterly displacement in 2013 was also observed in a second adult (M75) which emerged from hibernation within 50 m of M39 and was later detected south-east in the same location (5, in Fig 4), in competition with M39 for a female.

A return to the wintering area in advance of the second moult has not been reported previously but the same phenomenon has been detected in a telemetry study of an adult male (Hand, 2018; Hand, pers. comm.) shown in Table 1. This prompted a wider investigation of the data set of adult male vipers to determine whether this might be a more common behaviour. In the case of 14 individuals there were data on the locations of the first and second moult (9 individuals contributed data for more than one year). The first moult was within the wintering area and for most of these adult males the second moult was in the
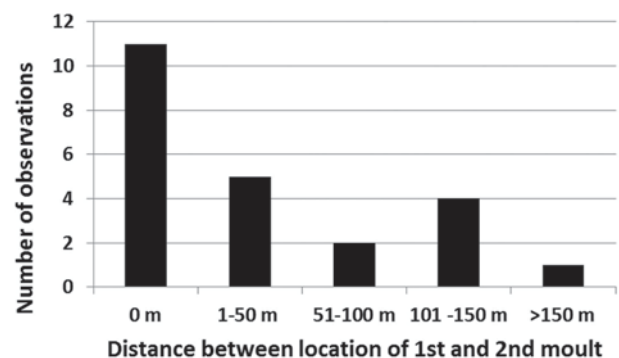

Figure 5. Distance between locations of the 1st moult and 2nd moult of fourteen adult male *V. berus*, some were observed in more than one year (n=23)

same place (Fig. 5). If a distance of less than 51 m between the locations of the two moults is taken as a criterion for remaining within the wintering area then 70 % had either simply remained within this area for both moults or had returned to it (Fig. 5). There was no evidence that these males left the wintering area after the second moult or before hibernation.
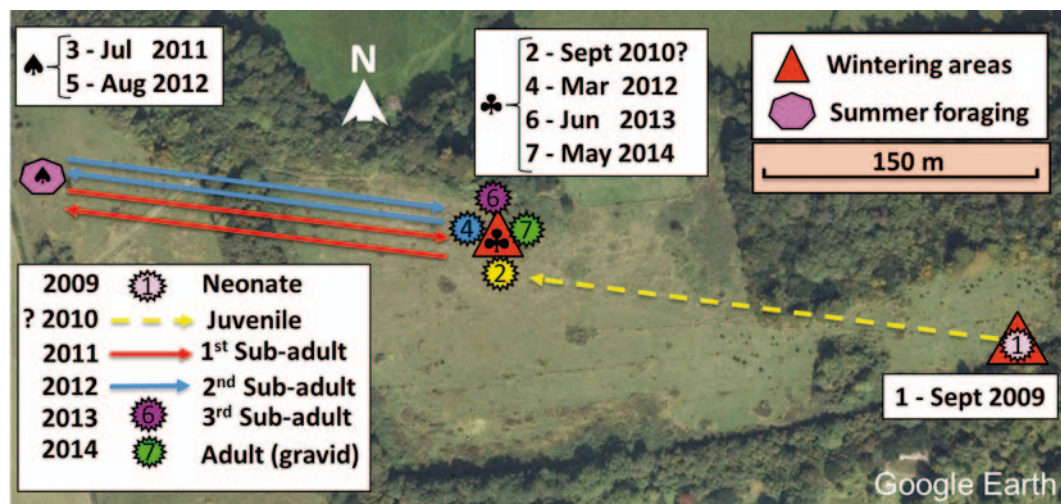
Figure 6. Net displacements of a female *V. berus* (F81) as a neonate, juvenile, in three sub-adult stages, and as an adult

Case study 4 - Emigration and seasonal migration of a female viper (F81) when immature and when adult

Female F81 was oberved for five years, from new born to adulthood but with direct observations on the juvenile stage lacking. From the observed positions of the neonate and first sub-adult stage, it may be inferred that after juvenile emergence from hibernation (1, in Fig. 6) F81 achieved a displacement of 280 m north-west where she probably selected her wintering area in September 2010 (2, in Fig. 6). The first sub-adult stage was detected a further 243 m north-west in a foraging area in July 2011 (3, in Fig. 6) but then returned south-east to the same wintering proposed for the juvenile, where in March 2012 F81 emerged as a second stage sub-adult (4, Fig. 6). This viper then dispersed again to the north-west and was detected in precisely the same foraging area (5, in Fig. 6). Thereafter, the third stage sub-adult (seen x3 in 2013) and the adult itself (seen x16 in 2014) showed no evidence of any movement away from the wintering area (6 & 7, in Fig. 6). F81 bred in 2014 and was observed basking in the open or present under either the felt or tin refuge.

It seems likely that juvenile emigration established the wintering area (♣, in Fig. 6) and then the first and second stage sub-adults established a routine foraging migration to a specific feeding area to the north-east (♠, in Fig. 6) with accurate return to the wintering area. Subsequently, there was a switch in behaviour with both the last sub-adult and adult viper apparently remaining in the wintering area throughout the year.

Emigration revealed by analysis of the distances between wintering areas

To give an indication of the extent of emigration by the immature stages, observations were made of the wintering areas selected by 20 male and 22 female vipers as neonates and then by the same individuals when they had reached the last sub-adult stage. Detection was in autumn, just prior to hibernation or in early spring at the time of emergence. Further observations were made on the wintering area selected by the same, albeit somewhat fewer, vipers as first year and second year adults to give an indication of adult emigration.

Emigration by immature vipers

The distances between the neonate wintering areas and those chosen by their corresponding last sub-adult stages (immature emigration) were highly variable (Table 2, Fig. 7 a & b). Given the criterion that emigration is recognised when the wintering area of last stage sub-adult is more than 150 m from that of their neonate, it would appear that 45 % of males have emigrated compared with only 23 % of females. When the distances between the wintering areas of neonate and last sub-adult stage males and females were compared, the male distances were significantly greater (Mann Whitney 2-tailed test, z = -2.2281, p = 0.025).

Emigration by adults

The distances between the wintering areas of the last sub-adult stage and those selected by the same vipers as first or second year adults (adult emigration) were relatively short (Table 2, Fig. 7 a & b,). When compared, there was no statistically significant difference between the first and second year adults for males (U = 53.5, p >0.05) or for females (U = 25, p >0.05). Consequently, for further testing the data for year one and two adults were combined. When these combined emigration data for adult males were compared with that for adult females, there was no significant difference between them (Mann Whitney 2-tailed test, z= 1.395, p> 0.164), indicating that there was no difference between adult males and adult females in the probability that they would return to the wintering area of the last sub-adult stage.

Comparison between immature and adult emigration

The distances recorded between the wintering areas of the neonates and those of the last sub-adult stages were longer than those between last sub-adult and adults (Table 2) and this difference was statistically significant for both males (Mann Whitney 2-tailed test, z = -3.1168, p = 0.0018) and females (Mann Whitney 2-tailed test, z = -2.587, p = 0.0096). This confirms the immature stages as the most active source of emigration.
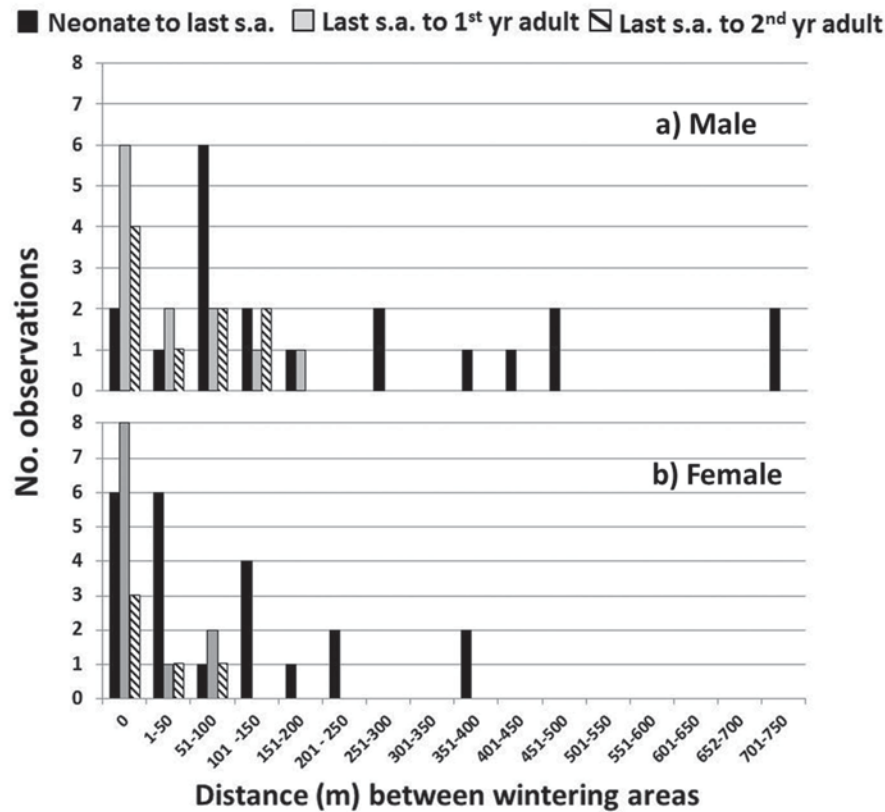
Figure 7. Distances (m) between the wintering areas of neonates and their corresponding last sub-adult stage (s.a.), and between those wintering areas selected by last sub-adult and the corresponding first and second year adults for a) Males and b) Females

Table 2. Summary of distances between the wintering areas of male and female *V. berus* as neonates and in the last sub-adult stage, and distances between the areas selected by same individuals as last sub-adult stage and as first and second year adults

Gender	Stages compared	n	Distance between wintering areas		
			Median (m)	Range (m)	Cases > 150 m
Male	Neonate to last sub-adult	20	131	0 - 738	45 %
	Last sub-adult to adult	21	20	0 - 200	5 %
Female	Neonate to last sub-adult	22	43.5	0 - 434	23 %
	Last sub-adult to adult	16	0	0 - 84	0 %

DISCUSSION

Emigration

For this study, emigration has been defined as the selection of a new wintering area at least 150 m from that used previously. Assessment of the movements of 20 male and 22 female vipers according to this criterion, combined with detailed observation on individuals, reveals that emigration is undertaken mostly by immature stages, in particular neonates and juveniles. There were no observations of juveniles leaving wintering areas and then returning to them; those that had clearly departed selected new wintering areas. By the time vipers had developed to the sub-adult stages, wintering areas appeared to have become more or less fixed. Almost no adult males or adult females adopted wintering areas further than 150 m away from that used by their last sub-adult stage.

Previous accounts of landscape movements by neonates and juveniles are sparse. Emigration by neonates was investigated in an experimental study by Saint Girons (1981) who found that those released early (August) would cover considerable distances (up to 90 m in little over one day) before hibernation but those released later (September) would instead hibernate close to the release site. Similarly, neonate M100 (Case study 1) moved to a wintering area 200 m north-west of where it was first observed in a warm autumn when the vipers were still active even in October. It seems probable that warm autumns and/or favourable summers that result in earlier births will increase the likelihood of neonate emigration. In the case of juveniles, Viitanen (1967) mentions that they "… spend their first summer in the immediate vicinity of the wintering area. In spite of this, only a small number of them find their way back to the common wintering dens", and concludes that "…this age group is especially apt to winter outside the wintering area proper. As they grow older the tenacity to the wintering

area grows stronger." These observations are confirmed by the present study. It would appear that neonates and juveniles have little or no homing instinct (Cases Studies 1, 2 and 4). This may be why many (or most) neonates and juveniles do not venture far from their natal wintering areas. Nevertheless, some juveniles make a springtime dispersal that results in emigration since lacking the homing ability to return to their previous wintering area they are obliged to select a new one. The sub-adults developing from them have sufficient homing abilities to return to the juvenile-selected wintering areas (Cases Studies 1, 2 and 4). Whether or not each year a sub-adult occupies exactly the same hibernaculum in the wintering area was beyond the scope of this study but Viitanen (1967) suggests they do not. There is little evidence that adult vipers contribute to emigration although perhaps occasionally there are adults with poor homing abilities, due perhaps to disease or injury, or as Viitanen (1967) suggests the rapid and early onset of cold weather may occasionally compel individuals to seek a hibernaculum before they have had an opportunity to return to their wintering area.

It is not known why some juveniles disperse over relatively long distances in springtime while most others appear restricted to small areas. An understanding of this difference in behaviour is the key to understanding what drives emigration in *V. berus*. However, it would seem that not all emigrating vipers behave in the same way. A greater number of immature males emigrated than immature females (45 % v. 23 %) and the displacements of males were significantly longer than those of females with the longest for females being about 400 m and for males about 700 m. In the case of the males this may be an underestimate of their emigration potential since the dimensions of the study site preclude recording much greater displacements; if there were greater displacements then the vipers would have moved off site into unsuitable habitat; presumably vipers turn away from this or perish. Such differences are not well known in other snakes but in the case of the slatey-grey snakes (*Stegonotus cucullatus*) hatchling males dispersed away from their release points whereas hatchling females did not, and adult males moved further than adult females (Dubey et al., 2008). It would seem that adult male *V. berus* are naturally more active than females as shown by two recent telemetry studies (Hand 2018; Nash & Griffiths, 2018) where adult males were recorded moving considerably greater distances. Male investment in more active and exploratory behaviour would have selective benefits as there is strong sexual competition between males and Madsen et al. (1993) have noted that the number of matings obtained by male northern vipers is enhanced by the distance travelled during the breeding season. However, this does not explain why pre-reproductive males should also be more active than females. It could perhaps indicate gender differences in foraging strategy which is the case in certain rattlesnakes (Duvall et al., 1990). Further research into this may give deeper insights into the life history strategy of *V. berus*. Viewed from the perspective of the female, there may be strong selective advantages in being more sedentary, perhaps avoiding predation and helping to accumulate the body weight required for successful reproduction.

The observation that emigration is a behaviour of the neonates and juveniles may have some bearing on approaches to conservation. Since these life stages establish the behaviours that become routines in the sub-adults and adults, it would be expected that they are probably more robust colonisers. In this case reintroduction programmes based on young immatures may be particularly successful as they may be more tolerant of being displaced than older individuals. It might be thought that reliance on young immature vipers in a reintroduction programme would risk higher mortality than the use of more mature specimens but recent long-term studies on the mortality rates of *V. berus* on heathland in Belgium suggests that mortality among this group is not especially high (Bauwens & Claus, 2018).

Seasonal migration

Previous accounts of northern viper behaviour suggest that after the adult males have completed their search for mates they move to cooler, moister 'summer areas' to forage and do not return to the wintering area until autumn (Viitanen, 1967; Prestt, 1971; Neumeyer, 1987). An interesting behaviour shown by M39 as an adult was always to return to the wintering area prior to the second moult of the year (34 days earlier in 2014) which usually happens about mid-June. This return happened in three consecutive years suggesting an entrenched pattern of behaviour. Such behaviour has not been reported previously, but in a telemetry study of *V. berus* (Hand, 2018; Hand N., pers. comm.) an adult male made a typical seasonal migration and was back in its wintering area for the second moult (Table 1). Failure to document this behaviour in earlier studies may be due to the fact that moulting snakes are secretive and therefore often remain unseen. In the case of M39 there was a refuge positioned close to the hibernaculum, under which it remained when moulting, so recording this behaviour was easy and in the study by Hand (2018) telemetry facilitated the observation. It seems possible that a return to the wintering area for the second moult is not uncommon; indeed in our study there was evidence that this may be the case in at least 70 % of adult males. Such males may remain unnoticed because they are hidden in deep cover at this time of year (Hand, 2018) and perhaps remain so until there is a need to bask when cooler weather arrives in September. This would give the impression that males have only returned to their wintering areas at this time of year when in fact they were already close by. The early return to the wintering area, typically a warm sunny location, may be connected with reproductive performance. The second moult is believed to coincide with a critical stage in germ cell development; the formation of primary spermatocytes (Volsøe, 1944). The successful proliferation of these cells is essential for the repopulation of the testes prior to hibernation. This will enable spermiogenesis to proceed without delay next spring. However, in some individuals, spermatids may already be present by October (Marshall & Woolf, 1957) and it is tempting to suggest that such males may be those that undergo a third moult, which was the case with M39 in this study. Early advancement in germ

cell production would contribute to male competitiveness per se. It would also have the advantage of apportioning some of the energy costs of sperm production, which are a significant drain on resources in springtime (Olsson et al., 1997), to the prior year at a time when the males are still feeding and now in a warmer zone where physiological process such as digestion and shedding are more rapid. If this is the case then such confinement of adult males to the wintering areas appears equivalent to the confinement of gravid females to the same areas until after parturition. In contrast, it would seem that sub-adult vipers make a slower return to the wintering areas (e.g. Case study 2) and those that have dispersed widely may not return until the autumn.

Direction of movement and homing

The case studies have allowed an assessment of the extent to which the directions taken by emigrating or migrating vipers follow those predicted by the position of the sun as explained by Viitanen (1967), south-west in spring and the north-east in autumn. In the springtime, vipers on the south facing slope (Area 3 in Fig. 1) showed strong westerly (NW, W or SW) displacements as immatures and adults. The behaviour of M39 in 2013 was the 'exception that proves the rule'. In that year the spring weather was cold and damp and the first moult delayed until around 30th April so that migration was late. By this time temperatures had risen steeply so that snake activity could probably be initiated earlier in the day. It would appear that adult males instead of being most active in the afternoon would instead become active earlier in the morning. At this time of day the sun would be in the east and south-east and this would explain the unusual direction of travel. Thus it would appear that the timing of warm weather in springtime can have a distinct effect on the direction of travel of adult male vipers. There were however exceptions to the westward movement. The wintering area of the adult M100 was established on level ground below a south-west facing slope and in receipt of somewhat later sunshine than on the south facing slope. In this situation it dispersed northwards up the valley two years in a row (5 & 7, Fig. 2), presumably following the sun but constrained by the need to keep in the cover of the hedgerows which happen to run in a north-south direction; the importance of such linear features for viper migration has been emphasised by Prestt (1971). Similarly, the migration of adult M39 involved first a typical westward displacement but once this male had moved across the south facing slope to the south-west facing slope the direction of travel was changed and this male also headed northwards up the valley (3 & 10, Fig. 4). Other observations (not detailed here) on males and females at the northern end of valley on a west facing slope were different. The area is backed by shaded woodland and the vipers receive their first direct sunshine from the south, the net directions of their movements were south-west or west. It would seem that at least in springtime, the position of the sun combined with weather conditions, topography, barriers to movement, and corridors of cover have profound influences on the direction of viper movement.

In the autumn (or before in the case of many adult males) migrating vipers return to their wintering areas. The expectation is that their homing behaviour is again mediate by the sun which at this time elicits the opposite reaction to that in the springtime and so results in a reversal of movement back to the wintering areas (Viitanen, 1967). In the case of M100, the return to the adult wintering area involved movement south (Fig. 2), M40 moved north-east (Fig. 3), F81 moved back east (Fig. 6) and in the case M39 there were two returns to the east and one return to the north-west (Fig. 4). It seems that from whatever compass direction vipers left their wintering areas they could make an accurate return journey. This suggests that the simple negative photo-response suggested by Viitanen (1967) is unlikely. For those making the return journey, the actual route used was not clear from the current study. It would have been of interest to know if the vipers had retraced their tracks or used a completely new route. Telemetry studies on at least one case in *V. berus* (Hand N., pers comm.) and in *Natrix natrix* (Madsen, 1984) suggest that return movements do not involve backtracking and investigation of the seasonal migration of rattlesnakes to and from their hibernacula has shown that the movements are directed by orientation to the sun (Landreth, 1973). The ability of sub-adult and adult *V. berus* to return to their wintering areas is impressive and navigation by this species is worthy of closer study especially now that path integration has successfully explained considerable feats of navigation in species of both invertebrates and vertebrates (Biegler, 2000).

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Mining Flickr: a method for expanding the known distribution of invasive species

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ABSTRACT - It is important to map invasive species in order to demonstrate their rate of spread and current distribution. Most recording schemes rely on opportunistic sightings and awareness to collect and gather data. Mining data from online social media and other data sharing platforms has become more prevalent in recent years as increasing numbers of users share more information. In this study, sightings from the image sharing platform Flickr were compared with the records submitted to the national recording scheme Record Pool. This study was completed to determine whether or not there was a significant difference between these two as sources for sightings of fresh water turtles across the UK.

INTRODUCTION

Fresh water turtles as a group are not native to the UK, however they were once part of the British herpetofauna assemblage during the Holocene period (Sommer et al., 2007). In past decades, several turtle species have been introduced into ponds and waterways throughout the country following the Teenage Mutant Hero Turtle craze of the late 1980s and early 1990s (Beebee & Griffiths, 2000). Several species were imported, mainly from North America, where they were bred in vast quantities specifically for the pet trade (Warwick, 1986). By far the most common species was the red-eared slider (*Trachemys scripta elegans*), which under the right conditions can live for 40 years and reach a mature size of 280 mm in just a few years (Wareham, 2008). Due to their voracious appetite and rapid growth spurts, red-eared sliders quickly outgrow the care of their owners and unfortunately many were then released into the local environment. Fresh water turtles are now widespread across the UK (Beebee & Griffiths, 2000), with the red-eared slider being the most frequently recorded species, perhaps due to its popularity as a pet (Langton et al., 2011). Other species such as the yellow-bellied slider (*T. scripta scripta*) and map turtle (*Graptemys geographica*) were also common pets and can be still be encountered in the wild.

Thankfully due to our much colder climate, these introduced turtles are unable to breed (Beebee & Griffiths, 2000). This situation could change as our climate warms and certainly elsewhere in Europe red-eared sliders do breed and have started to compete with native turtle species (Perez-Santigosa et al., 2008; Polo-Cavia et al., 2011). In the UK, it is illegal to release turtles into the wild under the regulations of the 1981 Wildlife and Countryside Act. Since 1997, the trade in Europe of fresh water turtles imported from the USA, such as the red-eared slider and the painted turtle (*Chrysemys picta*), has been restricted following the introduction of Council Regulation 338/97/EC, resulting in a reduction of American species for sale in pet shops. The introduction of non-native species to an ecosystem usually causes some level of disruption, however the effects of turtle introductions into the UK remain largely unknown.

Fresh water turtles are often quite conspicuous in the areas where they have been introduced, basking on logs or banks along canals and lakes etc. (Beebee & Griffiths, 2000). For this reason, they are often photographed by members of the public as they may either be concerned or excited about their sightings. Sometimes these images are uploaded to online file-sharing platforms that can then be mined, and later analysed (Daume, 2016). The records of different animals/plants submitted to various recording schemes are opportunistic in nature and only offer a snapshot at a single moment in time, much like a photograph. This being the case, the potential for the photography sharing platform Flickr (https://www.flickr.com/) was explored as a novel source of verifiable records of turtle sightings across the UK. These records were then compared with the data submitted to Record Pool to determine whether or not there was a significantly greater number of records held on Flickr and whether they differed much in their locations.

METHODS

In order to retrieve suitable images, Flickr was searched using a number of different search terms (see Table 1 for a small example of these) for relevant photos of fresh water turtles. When an appropriate photo was found during a search, the species was identified and recorded along with the number of specimens and their location (including both the county and nearest town/city). If the photos were geo-tagged then this information was used to geo-reference the water body where the turtles were photographed; other photo metadata such as the date the photo was taken were also recorded. For photos that were not geo-tagged, the location information within the description of the photo was relied upon. With this information, the centre of the water body within the given area was taken as an approximate location. For locations with more than one water body, the midpoint between them was treated as the location. This was completed using Grid

Table 1. Some of the search terms used during the study showing the total number of results from the whole Flickr database and the total number from within the search period. Note the diminishing number of results as search terms become more specific, the example used was typical of searching for red-eared slider photos.

Search term	Total results	Results within 2008-18
Terrapin UK	948	870
Terrapin Britain	700	636
Pond terrapin UK	130	120
Pond terrapin Britain	93	84
Red-eared slider UK	66	61
Red-eared slider Britain	**49**	17

Reference Finder (https://gridreferencefinder.com/) was used to provide both the coordinates needed to define the locality as well as its grid reference (to six figures) which gives an accuracy within 100 metres. In the event that a defined place name was not available within the metadata or the description of the photo, the photographer was contacted to help provide further information. Care was taken to ensure that only feral turtles were included in the analysis; those photographed in zoos or wildlife parks were excluded.

RESULTS

In the period 2008 and 2018, a total of 258 fresh water turtle sightings (of 5 species, Table 2) were detected through Flickr compared with only 86 submitted to Record Pool in the same period. The mean annual number of sightings from Flickr was 23.45 and 7.8 from Record Pool. When comparing the data between years (Fig. 1), there were significantly more data gathered from Flickr than Record Pool (paired t-test, t = 5.2002, df = 10, p = 0.0004). There was also a significant difference in the number of records collected from both sources between the different months of the year (paired t-test, t = 3.6193, df = 11, p = 0.004) and the peak number of counts from both systems was in May (Fig. 2).

The data submitted to Record Pool has been slowly increasing through time (Fig. 1) whereas the number of sightings available through Flickr has been more consistent over time (except for 2014). No records were submitted to Record Pool in 2015 whereas there were 27 sightings detected through Flickr. In 2017 and 2018 there was a distinct increase in the number of photos of turtles submitted to Flickr. Despite these differences between the two systems, they show broadly similar geographical coverage (Fig. 3).

DISCUSSION

The total number of fresh water turtle sightings within the UK recorded on Flickr from 2008 to 2018 was significantly greater than those submitted to Record Pool in the same period. This clearly demonstrates the potential of photo sharing platforms such as Flickr as a source of faunal records. Researchers have already successfully used social media to investigate the trade in turtles in Vietnam (Van et al., 2019) and to even evaluate which species of amphibians are most often kept as pets (Measey et al., 2019). Despite the fact
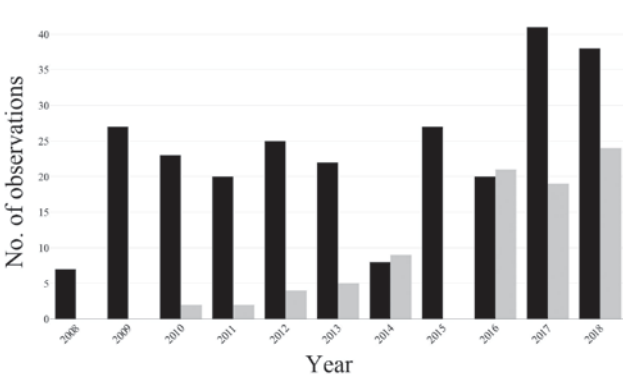

Figure 1. A graph comparing the frequency of turtle sightings from Flickr (in black) with Record Pool (in grey) from within the search period, 2008 – 2018
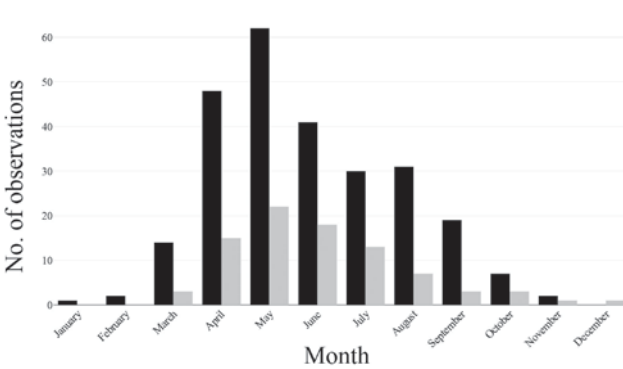

Figure 2. A graph comparing the frequency of monthly turtle sightings from Flickr (in black) with Record Pool (in grey) for the period 2008 – 2018. Both systems show a peak of observations in May.

Figure 3. A map comparing the distribution of sightings across all species from Flickr (black) and Record Pool (white)

Table 2. The species identified during the intensive Flickr search and the number of sightings associated with each species.

Common name	Scientific name	Number of sightings
Red-eared slider	*Trachemys scripta elegans*	163
Yellow-bellied slider	*Trachemys scripta scripta*	89
Map turtle	*Graptemys geographica*	4
European pond turtle	*Emys orbicularis*	1
Common snapping turtle	*Chelydra serpentina*	1

that the searches were completed manually, the data was collected in a relatively short time. The advantage of Flickr over Record Pool is that as every record has a photo, you can identify the species and verify it. Flickr also preserves the photo metadata which can be used to determine when a photo was taken, and in some cases even where. For this project, all of the records submitted to Record Pool were assumed to be correct although there wasn't always the same species-level detail available as there was on Flickr.

Fresh water turtles are most active between April and October (Inns, 2009) although they may be seen throughout the year (Fig. 1). The month with the most sightings from both datasets was May, indicating that this may be an ideal time to implement turtle surveys across the country. The effects of these turtles on British ecosystems are not currently understood but may be significant as they are known to be voracious predators potentially consuming ducklings, fish, amphibians and other wildlife (Beebee & Griffiths, 2000). Further research is needed to confirm the diet of feral turtles as well as their interactions with other species. Fresh water turtles have been introduced into a number of other countries around the world where they have impacted native fresh water turtle species (Ramsey et al., 2007). Here in the UK though, the exact distribution of the turtles is not known and consequently a current citizen science project, the 'Turtle Tally' initiated by Hadlow College and the British Herpetological Society in early 2019, is seeking to fill this gap. The information gathered may be used to help manage feral turtles if later research confirms that they have a negative impact on local ecosystems.

It is important to consider a number of different search terms when searching Flickr as not every photo will have the correct title or the appropriate tags. The careful choice of terms is also important and in the current study the use of 'turtle UK' rather than just 'terrapin' yielded over 5,600 results which within this project would have created an excessive workload. For the future this could potentially be tackled by replacing manual searches with machine learning to automate the process. When using search terms, broad terms as well as specific ones should be used as there is a trend towards fewer results when using more specific search terms (Table 1). The number of records submitted to Record Pool has been slowly increasing since its launch (Fig. 2) whereas Flickr has been more consistent over time although there has been an substantial increase in sightings submitted to Flickr in the past two years (2017-2018) but this may simply be linked to an increase in the number of users rather than an increase in turtle numbers.

There is a large degree of overlap in the wider geographical distribution of sightings between Flickr and Record Pool (Fig. 3) which is quite striking given the large difference between the size of the two data sets. Further analysis is required to determine how closely or by how much the two implied turtle distributions differ but at least in the metropolitan areas such as London and Manchester, where there are both more people and likely more turtles as a consequence, there is relatively little overlap between the records. Most sightings are from the southern half of England with sightings becoming rarer as you get closer to the Scottish border, although there are a small number of sightings from both sources within Scotland. It has previously been stated that within Scotland fresh water turtles are only rarely encountered (McInery et al., 2016), this is confirmed by the current data and probably relates to a poor climate for turtles. There are no sightings of them in the Scottish Highlands but this is likely linked to human distribution with fewer people owning freshwater turtles and fewer people releasing them. There are no reported turtle sightings for Northern Ireland which indicates that further surveys are needed to discover whether or not this reflects reality.

Flickr has helped to provide a vast number of additional records that have contributed to a much more detailed distribution mapping for turtles within the UK when combined with Record Pool data. In order to gain more consistency in the future, outreach should target the sighting of turtles and how to record them properly.

By using data acquired by mining social media it may be possible to make distribution maps of any species more comprehensive (not just fresh water turtles). There is also the need to integrate data uploaded to social media sites with conventional faunal recording systems, such as Record Pool, so that the information obtained can be stored in a common repository. Organisations such as the Amphibian and Reptile Groups UK (ARG UK) and the Non Native Species Secretariat (NNSS) could then use this data for follow-up surveys if they see fit to confirm the presence of alien species. The current social media platforms available for the kind of mining described above include Flickr, Twitter and Facebook.

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On the verge of extinction in Mexico today: Field observations of *Ambystoma ordinarium* and *Ambystoma flavipiperatum* with remarks on their habitat and conservation

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ABSTRACT - Mexico is a hotspot of salamander diversity and harbours 18 species of the genus *Ambystoma* (Amphibia: Urodela) widely distributed from the Sierra Madre Occidental to the Trans-Mexican Volcanic Belt. These species are highly threatened by various factors and some of them are considered nearly extinct in the wild. The Michoacán stream salamander, *Ambystoma ordinarium* and the yellow-peppered salamander, *A. flavipiperatum* are two endemic and scarce species from Mexico living in isolated and declining populations. Few observations have been made on them in their natural habitat since their original description. Here we report new data regarding the biology and habitats of *A. ordinarium* observed at Rio Bello, 2,120 m a.s.l., Morelia city, Michoacán state, and for *A. flavipiperatum* at Sierra de Quila, 2,165 m a.s.l., 100 km south from Guadalajara city, Jalisco state. For *A. ordinarium* we found one neotenic adult male and two dead adult females in a small shallow stream located within a fragmented fir, oak and pine forest. For *A. flavipiperatum* we recorded an adult male and a female both neotenic, one clutch of eggs, and eleven larvae inhabiting a slow-moving stream located through a riparian habitat surrounded by large pine-oak forest. Our observations confirm that both species are highly endangered, capable of facultative neoteny and occur in small slow-moving streams surrounded by coniferous forests. We discuss their morphology, geographical distribution and conservation status.

INTRODUCTION

Mexico is a hotspot of salamander diversity (Petranka, 1998; AmphibiaWeb, 2019) and harbours 18 species of the genus *Ambystoma* widely distributed from the Sierra Madre Occidental to the Trans-Mexican Volcanic Belt (Raffaëlli, 2013; Percino-Daniel et al., 2016; AmphibiaWeb, 2019). These species generally breed in deep volcanic lakes, shallow vernal pools, artificial cattle ponds, and intermittent, fish-free stream pools. Their habitat is mainly associated with temperate climates at high altitudes while *Ambystoma velasci* is the only species that may sometimes be found in arid and semi-arid habitats (Raffaëlli, 2013). Here we report on field observations of two species the Michoacán stream salamander, *A. ordinarium* Taylor, 1940 and the yellow-peppered salamander, *A. flavipiperatum* Dixon, 1963.

Ambystoma ordinarium is a polymorphic species native to the central part of the Trans-Mexican Volcanic Belt in the vicinity of Lake Patzcuaro in Michoacán state up to Tianguistenco, Western Mexico state (Anderson & Worthington, 1971; Anderson, 1975). It occurs at high elevation (1,400-3,000 m a.s.l.) in mountainous habitats (Anderson & Worthington, 1971; Weisrock et al., 2006;

Escalera-Vasquez et al., 2018). Both larvae and neotenic individuals inhabit high mountain brooks or spring pools at the headwaters of streams (Anderson & Worthington, 1971). Metamorphosed adults can be found hiding under debris in coniferous forests as far as 30 m from streams (Alvarado-Diaz et al., 2003).

By contrast, *A. flavipiperatum* Dixon, 1963 is a robust salamander that occurs at two sites around Guadalajara city, Santa Cruz, approximately 41.8 km south-west of Guadalajara at an elevation of 1,494 m a.s.l. (Dixon, 1963) which is the type-locality, and within the Sierra de Quila, Jalisco state, west-central Mexico between 1,494-2,400 m a.s.l (Santiago-Pérez et al., 2012; Raffaëlli, 2013; Rosas-Espinoza et al., 2013; Ahumada-Carrillo et al., 2014; Cortés-Vázquez et al., 2016; Cruz-Sáenz et al., 2017). These two *Ambystoma* species are classified Endangered according to IUCN and protected by Mexican laws (NOM-059-SEMARNAT-2010; IUCN, 2015, 2016; Cortés-Vázquez et al., 2016; AmphibiaWeb, 2019). To date, there have been few observations of this species in its natural habitat. Here we report new field observations and we discuss morphology, geographical distribution and conservation status.

Figure 1. A map of Mexico showing the main distribution of *A. ordinarium* and *A. flavipiperatum*. White area: *A. ordinarium*; red star: Rio Bello (this study); blue area: *A. ordinarium* sp.; Yellow area: *A. flavipiperatum* type locality; Orange area: Sierra de Quila (this study)

METHODS

Study sites

We conducted field work during the rainy season (July to August) in 2013 and in 2018 in the states of Jalisco and Michoacán, north-western Mexico (Fig. 1). A total of three specimens of *Ambystoma ordinarium* were found at Rio Bello (19°40'38.3" N, 101°8'47.5" W) at 2,120 m a.s.l. in the vicinity of Morelia city, Michoacán state, Mexico on 13 August 2018. One neotenic adult female, one neotenic adult male, one clutch of eggs and eleven larvae of *A. flavipiperatum* were observed during daytime at Sierra de Quila (20°17'56" N, 104°1'5.999" W) at 2,165 m a.s.l., Jalisco state, Mexico on 1 August 2018. By the Koppen classification the climate of both sites is rated 'subtropical highland'(Cwb).

Field survey

The Visual Encounter Survey (VES) was used to search for the axolotls, a method that is cost effective and does not disturb habitats of endangered or sensitive species (Heyer et al., 1994). Survey sites were selected based on historical published records and by studying local maps showing available water for the target species at the vicinity of forested areas (Anderson & Worthington, 1971; Anderson, 1975; Alvarado-Diaz et al., 2003; Weisrock et al., 2006; Rosas-Espinoza et al., 2013; Ahumada-Carrillo et al., 2014; Cortés-Vázquez et al., 2016; Cruz-Sáenz et al., 2017; Soto-Rojas et al., 2017; Escalera-Vasquez et al., 2018). Everyday field work was undertaken from about 09:00h to 12:30h. Observations of vegetation and habitats were made on field trips, both on sunny and on rainy days. We have surveyed almost all types of habitat including permanent and temporary streams but also ponds (including artificial reservoirs and irrigation canals), and surrounding terrestrial habitats, stumps, stones and leaf litter.

We photographed each taxon observed including plant and tree species for identification (Sony Nex-5; Sony Ltd., Japan). Coordinates, geographic and elevational data were collected in situ using a GPS (Garmin Montana 680; Garmin Ltd., Olahe, KS, USA) and located on maps. The temperature, pH and dissolved ions in water were measured in situ using a Expresstech @ LCD pH Medidor Digital (Expresstech, Kingpow Company Limited, Hong-Kong, China).

Morphology

Morphological measurements were made to the nearest 0.1 mm with a dial caliper (Louisware LSWCL1810, Louisware Ltd., USA). Data for comparisons were obtained from the original description provided by Taylor (1940) and Dixon (1963). When handling the axolotls we wore disposable vinyl gloves that had been rinsed with distilled or sterilised water. These gloves were changed between animals to prevent any cross-contamination. All animals were then released at the exact place of capture for conservation purposes following national and international ethical standards (NOM-059-SEMARNAT-2010; IUCN, 2015, 2016).

RESULTS

On 13 August 2018, *A. ordinarium* was recorded at Rio Bello (19°40'38.3" N, 101°8'47.5" W) at 2,120 m a.s.l. in the vicinity of Morelia city, Michoacán state, Mexico. We observed one neotenic adult male (Fig. 2A) in a small shallow stream located within a fragmented fir, oak and pine forest composed of

Table 1. Number of costal grooves and morphological measurements (mm) for *A. ordinarium* and *A. flavipiperatum*

Species	Gender	No. costal grooves (n)	TL	SVL	HW	HL	IOW	IEW	FL	HLL	GL
Ambystoma ordinarium	Male	13	131	58.7	15.4	18.2	5.5	4.5	12.6	18.9	11.8
A. flavipiperatum	Male	13	141	66.7	16.6	22.8	7.8	6.5	22.5	25.9	15.8
	Female	12	171	89.1	19.1	24.9	8.9	7.7	28.0	32.1	21.1

TL – total length, SVL – snout vent length, HW – head width, HL – head length, IOW- interorbital width, IEW- internarial width, FL – forelimb length, HLL – hindlimb length , GL –gill length

Figure 2. **A.** adult neotenic male of *A. ordinarium*, **B.** habitat of the target species at Rio Bello, Michoacán, Mexico
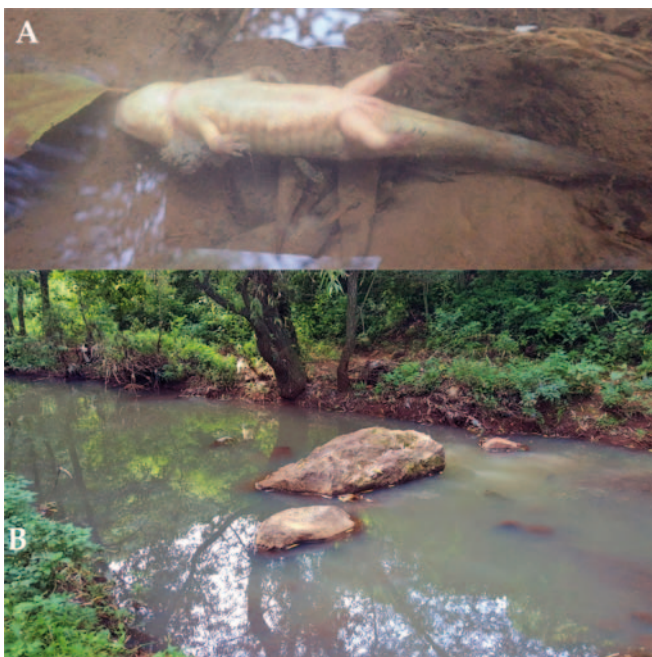
Figure 3. **A.** one dead adult female of *A. ordinarium*, **B.** picture showing degradation and flow changing of the main stream where the species was previously known to occur at Rio Bello. Dead specimens were found in the vicinity of this site.

Abies religiosa, *Pinus leiophylla*, *Pinus michoacana*, *Quercus castanea*, *Quercus crassifolia*, and partially some subtropical shrubs such as *Acacia farnesiana*, *Acacia pennatula*, *Eysenhardtia polystachya*, and *Ipomoea arborescens* (Fig. 2B). At the time the air temperature was 22.9-26.7 °C and water temperature 19.0-19.2 °C. The stream had a rocky substrate and the water quality was slightly alkaline (pH 7.92-8.06) with dissolved ions between 301-306 mV. The specimen was confirmed as male by the presence of a rounded cloaca. The number of costal grooves and physical dimensions of this specimen are shown in Table 1. Its general coloration was black with small yellow spots on the dorsal and ventral surfaces. Gills were prominent beside the head and had a reddish purple hue. We also photographed two dead females within the same stream 600 m below a road under construction (Fig. 3A & 5B). We noticed the presence of a dam nearby that may have affected the flow and quality of the stream water (Fig. 3B). This suggests that the development of Morelia city is having a negative impact on the habitat of *A. ordinarium*.

During daytime on 1 August 2018, we found *A. flavipiperatum* inhabiting a coniferous forest at Sierra de Quila (20°17'56" N, 104°1'5.999" W) at 2,165 m a.s.l., Guadalajara, Jalisco state, Mexico. The observations included one neotenic adult female (Fig. 4A), one neotenic adult male, one clutch of eggs and eleven larvae (Fig. 4B) all found in one slow-moving stream located through a riparian habitat (Fig. 5A) composed of *Alnus acuminata*, *Pinus douglasiana*, *Salix bonplandiana*, *Prunus serotina* ssp. capuli and ferns including mainly *Cheilanthes* spp., *Asplenium* spp. and surrounded by large pine-oak forest composed of *P. douglasiana*, *P. oocarpa* and *Quercus resinosa*. At the time the air temperature was 19.6-22.4 °C and water temperature 16.4-18.3 °C. The stream had a rocky-sandy including various rocks, fallen leaves, tree roots and decomposing debris in decomposition. The water quality was about neutral (pH 6.96-7.21) with dissolved ions between 401-410 mV. Adult individuals were neotenic specimens observed on the bottom of the largest parts of the stream at a water depth of 65-70 cm. The number of costal grooves and physical dimensions of the male and female specimens are shown in Table 1. The female had same coloration as the male but larger longitudinal white bands on the ventral surfaces. A total of 120-140 eggs were observed beside a female, attached to submerged tree roots on the bottom of this stream at a depth of 40-65 cm. The eggs ranged from 1.7 to 2.0 mm in diameter. The larvae were hidden under fallen leaves, within debris or under the banks of the river. When disturbed, they hid

quickly in dark refuges. Their total length was 17-62 mm, dorsally they were generally coloured dark-brown to black, while ventrally they tended to be clearer with a whitish appearance. Larger individuals (45-62 mm) had very small white to yellowish spots (Fig. 4B).
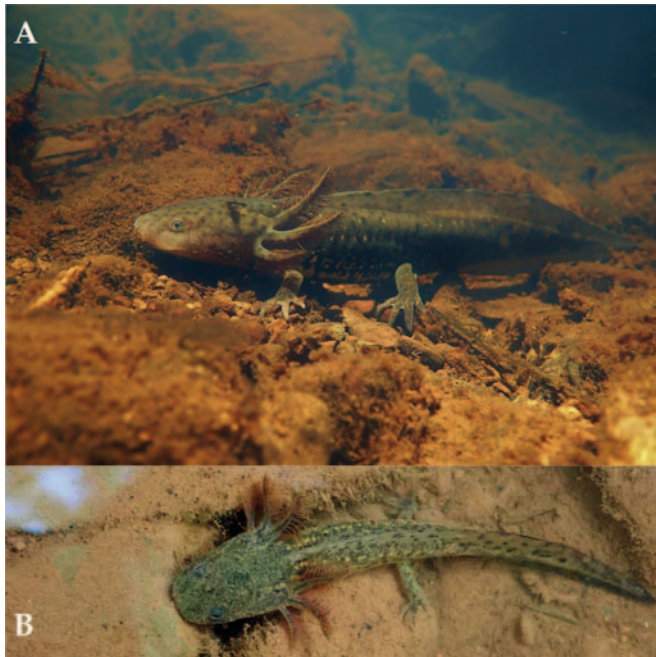

Figure 4. A. Adult neotenic female of *A. flavipiperatum* observed at Sierra de Quila, **B.** Larva of *A. flavipiperatum* at the same prospected site
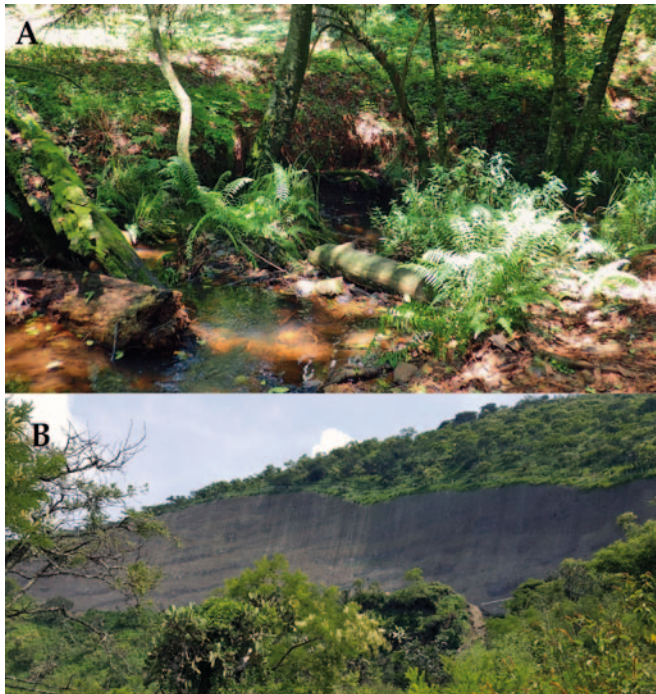

Figure 5. A. Microhabitat view of *A. flavipiperatum* at Sierra de Quila, **B.** Degradation of the main habitat of *A. ordinarium* at Rio Bello due to a road construction

DISCUSSION

Our observations confirm that both endemic species. *A. ordinarium* and *A. flavipiperatum* have the potential for facultative neoteny and that probably they are composed of small-numbered populations. Both inhabit small slow-moving streams surrounded by coniferous forests at Rio Bello and within the Sierra de Quila, altogether located within the Trans-Mexican Volcanic Belt, Mexico. However, the Santa Cruz type locality of *A. flavipiperatum* was a different habitat type, characterised by thorn forests including desert scrub vegetation with rocky soil and a multitude of rock outcroppings (Dixon, 1963).

Concerning morphology, our specimens of *A. ordinarium* conformed to the original description provided by Taylor (1940) and additional studies (Duellman, 1961; Anderson & Worthington, 1971). This salamander measures between 70 and 75 mm SVL at sexual maturity, reaching a maximum size of 86 mm SVL for terrestrial adults up to a maximum size of 191 in TL (Raffaëlli, 2013; AmphibiaWeb, 2019). *A. ordinarium* has a narrow head, and generally bears 16-24 tooth-rakers on the 3rd arch. Adults have a uniformly dark to black dorsum, but mottling may also be present with small yellow spots. Some adults retain the larval coloration, consisting of slight ventral, lateral, and dorsal rows of light silver-yellow specks from axilla to groin (Anderson & Worthington, 1971; Shaffer, 1984a,b). Dorsal patterns and colour may vary between specimens and populations (pers. obs.).

At Rio Bello, all specimens were found in water and were facultative neotenics with long reddish gills. According to Alavarado-Diaz et al. (2012), *A. ordinarium* inhabits high mountain brooks or spring pools at the headwaters of streams, with water temperatures remaining between 11.8 and 12.4 °C. Metamorphosed adults can be found hiding under debris in coniferous forests as far as 30 m from streams (Alavarado-Diaz et al., 2002).

Ambystoma flavipiperatum from Sierra de Quila is quite different from the original description by Dixon (1963). Dixon found four metamorphosed specimens in Santa Cruz, while we only observed neotenic individuals in the Sierra de Quila as previously reported (Santiago-Pérez et al., 2012; Rosas-Espinoza et al., 2013; Ahumada-Carrillo et al., 2014; Cortés-Vázquez et al., 2016; Cruz-Sáenz et al., 2017). However, some terrestrial adults are known to move through riparian zones in the vicinity of streams, especially in hidden burrows constructed by Buller's pocket gophers (*Pappogeomys bulleri*) probably seeking protection from dehydration and potential predators (Rosas-Espinoza et al., 2014). Thus, the species shows facultative neoteny at Sierra de Quila while metamorphosed individuals are fossorial as is well known in all other terrestrial ambystomatids (Petranka, 1998).

According to the original description of *A. flavipiperatum*, the male holotype has a snout-vent length of 99.5 mm and a total length of 175.0 mm while the three females ranged from 80.0-103.0 mm in snout-vent length and 137.0-188.0 in total length. The head is longer than wide, with the length being a quarter of the snout-vent length and the width being a fifth of the snout-vent length. The internarial width is slightly more than 1.5 times the length of the eye. However, the nostrils are only about one eye length from the eye. There is a shallow groove starting from the posterior of the eye and leading to the angle of the jaw (Dixon, 1963).

At Sierra de Quila, two neotenic specimens including one male and one female of *A. flavipiperatum* were previously reported (Rosas-Espinoza et al., 2013). They were smaller and measured 173.8 and 154.7 mm (TL) respectively, and had 12 costal grooves. Coloration was not reported. During our field survey, specimens observed differed by being shorter (141-171 mm in TL; 66.7-89.1 mm in SVL), having 12 to 13 costal grooves and a black general coloration on the dorsal parts with small white cream to yellowish spots. There were also two longitudinal whitish marks present on the ventral parts of the individuals (yellow in the type series; see Dixon, 1963). There are also many white-yellowish spots between the axilla and groin on both sides, and more yellow spots on the lateral sides of the tail. There were also a few large dark spots on the dorsal parts, head or limbs (not reported in Dixon, 1963). In conformity with the first description, adult females had longer tails than males while males had the cloacal region greatly enlarged and rounded (Raffaëlli, 2013).

Dixon reported eggs but not larvae in the original *A. flavipiperatum* description (see Dixon, 1963). He assumed that eggs are similar to those of *A. rosaceum* and that a female can lay 2500 to 3000 eggs in total while he only found 1451 eggs in ovaries of the largest paratype female. At Sierra de Quila, we observed 120-140 eggs arranged at the same place, but separated at the bottom of the stream, near a large female. Moreover, we found eleven larvae of this elusive species (Fig. 4B). Larval stages have not been reported until now. The larvae can be diagnosed by size 17-62 mm (TL) with a general greyish coloration on the dorsal parts while ventral parts tended to be clearer with a whitish appearance. Gills are long and reddish. Present on the dorsum of larger individuals (45-62 mm) were very small white to yellowish spots. These observations extend the original descriptions and also suggest that *A. flavipiperatum* at Sierra de Quila continue to breed at the beginning of August which is confirmed by the finding of eggs and larvae, but our results provide an incomplete diagnosis. New morphological studies are needed to evaluate the biological characteristics of different populations.

The ecology, biology and natural history of *A. ordinarium* and *A. flavipiperatum* are to date poorly known due to the small number of adult individuals observed in their natural habitat (Shaffer, 1989; Raffaëlli, 2013; Raffaëlli & Hernandez, 2019). Indeed, most of the *Ambystoma* species are endangered or highly endangered (Escalera-Vasquez et al., 2018; Raffaëlli and Hernandez, 2019). To date both species are both classified Endangered (EN) following the IUCN Red List and are placed under special protection by federal environmental laws (NOM-059-SEMARNAT-2010; IUCN, 2015, 2016).

Observation of two dead adult and neotenic females at Rio Bello reveals that *A. ordinarium* is facing a conservation threat at this site, probably due to a substantial increase in the flow of the main stream and a road construction that is destroying its natural habitat (Fig. 3B & 5B). In 1999, the species was abundant in several localities occupying a total area of 4,283 km² but surveys undertaken since 2004 indicated that all populations are very fragmented and declining, with the species disappearing in some localities (IUCN, 2015). In recent years, Soto-Rojas et al. (2017) detected only 16 streams inhabited by *A. ordinarium* despite there being 29 historical records in the last century. Moreover, most of the habitats analyzed contained populations severely affected by various abnormalities caused by water pollution (Soto-Rojas et al., 2017). The conservation outlook appears worse than it might seem as *A. ordinarium* is considered as a species complex containing at least one undescribed taxon within the western part of its known range, south of Lake Patzcuaro and known from just a few localities (Hime et al., 2016). Consequently, the eastern lineage from the type locality may actually have a range of only 120 x 20 km.

Ambystoma flavipiperatum is also threatened because its range and occurrence are very limited, showing 134 km² of occupancy comprising only two localities until now (Raffaëlli, 2013; IUCN, 2016; Amphibiaweb, 2019). Its major threats are habitat destruction and fragmentation from smallholder farming (Rosas-Espinoza et al., 2013; Ahumada-Carrillo et al., 2014; Cortés-Vázquez et al., 2016; Cruz-Sáenz et al., 2017). The species is also threatened by water pollution, road construction, human settlements, and introduction of predatory fish (Raffaëlli, 2013). Even though the Sierra de Quila is a protected area 'Área de Protección de Flora y Fauna Sierra de Quila', water pollution by visitors and climate change, which has affected rainfall patterns and intensity, has caused a reduction in suitable habitat (IUCN, 2016; Villers-Ruiz and Trejo-Vázquez, 2000). To date, a local committee in the Area de Protección de Flora y Fauna Sierra de Quila, is using *A. flavipiperatum* as an umbrella species to protect clean water (AmphibiaWeb, 2019). Moreover, at Santa Cruz, located 48 km south from the Guadalajara Metropolitan Area, the species has probably disappeared due to the construction of a shopping mall on the only available wetland and new assessments are needed (Rosas-Espinoza & Santiago-Pérez pers. com. 2018). Consequently, the population from Sierra de Quila is very important in terms of conservation and needs further assessments including genetic analysis to confirm its status and population viability. It is believed that both species can breed well in captivity (Raffaëlli, 2013) and it is possible that observations in captivity could generate new and important biological and ecological data that could be helpful in the design of conservation programmes (Hernandez, 2017).

Finally, another problem concerns the taxonomic status of many Mexican axolotls. Their taxonomy is uncertain because many species share mitochondrial genes due to recent introgression and also often show low genetic divergence between species (Shaffer & McKnight, 1996; Weisrock et al., 2006; Escalera-Vasquez et al., 2018). In addition, many isolated populations distributed within the western and northern part of the Trans-Mexican Volcanic Belt have not still not been assessed taxonomically or for their conservation status. Good examples of this are the southern lineage of *A. rosaceum* and populations of *Ambystoma velasci* from south-western Jalisco and southern Michoacán. In this alarming context, more surveys and analysis are needed to improve the knowledge base and conservation strategies of axolotls which today are on the verge of extinction in Mexico.

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Spitting behaviour in the Chinese cobra *Naja atra*

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ABSTRACT - This study describes the ability of a captive Chinese cobra (*Naja atra*) to spit venom and presents a video analysis of a strike and spit manoeuvre. However, among the many specimens of *N. atra* raised by the author only the female examined in this study regularly displayed spitting behaviour, and then only within the confines of its terrarium. Fang morphology of *N. atra* was investigated by microscopy and compared with that of a 'true' spitting cobra, *Naja sputatrix*. The results show that *N. atra* does not have specially modified fangs so that venom is ejected downwards from the fangs, not forwards. Consequently, to direct venom at a target requires a specific manoeuvre involving a forward lunge and head rotation. This arrangement is very similar to that described for *Naja kaouthia* and suggests that the evolution of spitting behaviour could precede the development of specialised fangs that refine the action.

INTRODUCTION

In Asia there are eleven cobra species belonging to the genus *Naja* (Wallach et al., 2009; Wüster, 1996). Most representatives of this group are 'spitters' having short tubular fangs at the front of the mouth with a peculiarity at the distal end of the fang that allows the snakes to spit venom forwards (Bogert, 1943; Triep et al., 2013; Wüster & Thorpe, 1992; Young et al., 2004). The venom duct within the fang is modified so that at the lower front side of the fang there is a reduced orifice with the inner posterior side of the duct transversally angled to the length of the tooth. This arrangement enables the cobra to release venom jets at approximatively the angle at which the head is inclined.

Among the 'non-spitting' Asian cobras, two species have long been rumoured to be able to spit. One of them is the monocled cobra (*Naja kaouthia*, Lesson 1831) of which spitting behaviour has been recently formally documented (Santra & Wüster, 2017). While the other is the Chinese cobra (*Naja atra*, Cantor, 1842), that was previously reported to be responsible for a case of conjunctival and corneal injury in a woman (Fung et al., 2010). In addition, prior literature has invoked *N. atra* as being able to spit its venom (Herklots, 1938; Reitinger & Lee, 1978), albeit rarely, and lacking the precision of the true spitting cobras (Karsen, et al., 1986; Wüster & Thorpe, 1992).

The subject of this study is an adult female Chinese cobra (Fig. 1) that was captive born in 2012 from a pair imported from Hong Kong in the early 2000s. This specimen is of the black banded phenotype (Fig. 1) which in recent years has manifested a special inclination to spit, the first photographic evidence is from February 2016.

MATERIALS AND METHODS

The female Chinese cobra (*N. atra*) used in the study was kept

Figure 1. Adult female *N. atra*, the protagonist of this study (with a congenital groove in the ventral scales seen close to where the snake's body reaches the substrate)

in accordance with Italian law. It was raised in a controlled environment and treated with special care after its spitting behaviour was manifest. Details of the snake's lepidosis are presented in Supplementary Material (see p. 25). The snake measured about 100 cm (total length) and weighed 292 g. It successfully bred and laid eggs in 2015, 2016 and 2018. To

capture the specimen's spitting behaviour, a digital camera (Nikon Coolpix P510) was positioned inside her terrarium and set on to records video at 120 frames per second. To optimise the results captured by the lens, dark cardboard panels and additional lights were applied inside and outside the terrarium which measured 120 cm long, 60 cm deep and 50 cm high. To visualise the traces of venom following a spit, sheets of A4 paper of different colours (red, yellow, blue or green) were used as targets although no one colour caught the attention of the cobra more than another. Before each filming session, the glass front of the terrarium was removed and then, as soon as the cobra's attention had been caught by my person (full visor protection and protective gloves were used), a paper target was placed at the same height as the terrarium directly in front of the cobra. The maximum distance set between the terrarium and a target was 40 cm.

For an examination of the fangs by microscope, a fang was collected directly from the mouth of one of the female's offspring (hatched 2016), sired by a male sibling from the same clutch. The fang was removed carefully from the live snake while no longer fixed to the maxillary bone. Microscope pictures of the fang were taken using a stereomicroscope (Nikon SMZ645). The sample was placed in a Petri dish containing distilled water, and the images were taken through the object when laid on a solid surface and also when suspended in distilled water. For comparative purposes, the same procedure was applied to the fangs of an adult female Indonesian spitting cobra *Naja sputatrix*, Boie, 1827, collected from a deceased adult female bred in captivity (for details of the snake's lepidosis see Supplementary Material). Fang measurements were taken using a digital calliper (accuracy ± 0.005 mm). Tinting of discharge orifices and venom canals in images of the fangs was possible due to the double illumination and the suspension of the fangs during microscopy. This technique conferred transparency to the fangs making the discharge orifices, venom canals and liquid within them clearly visible.

RESULTS

Video recording of striking and spitting
The data analysed and described here were collected from a single recording. However, numerous spitting events were captured (at total of 15 video clips were recorded over two days when the cobra exhibited the spitting behaviour in response to me coming too close to her terrarium), but only examples providing the clearest visual results were selected for analysis. The video selected had a duration of 16 minutes and 10 seconds, during which 33 spitting attempts were captured. Some of the examples of venom spitting were clearly visible to the naked eye, while others required the examination of single video frames. The time of the whole spitting action (time lapse calculated from the first frame in which the cobra moves forward to strike from its defensive posture to the beginning of the spit) varied from 0.76 to 1.16 seconds with an average of 0.97 seconds. The spitting time itself ranged from 0.03 to 0.13 seconds with an average of 0.07 seconds (n= 33). Observations were made of venom being spat from both fangs simultaneously and from a single

fang. The venom traces from a single fang were generally linear and following a spit from a distance of about 0-20 cm ranged from 5.8 cm to 10.5 cm wide. When two fangs were used the trace covered an area of up to 7.7 cm wide and 8.9 cm high (Fig. 2).
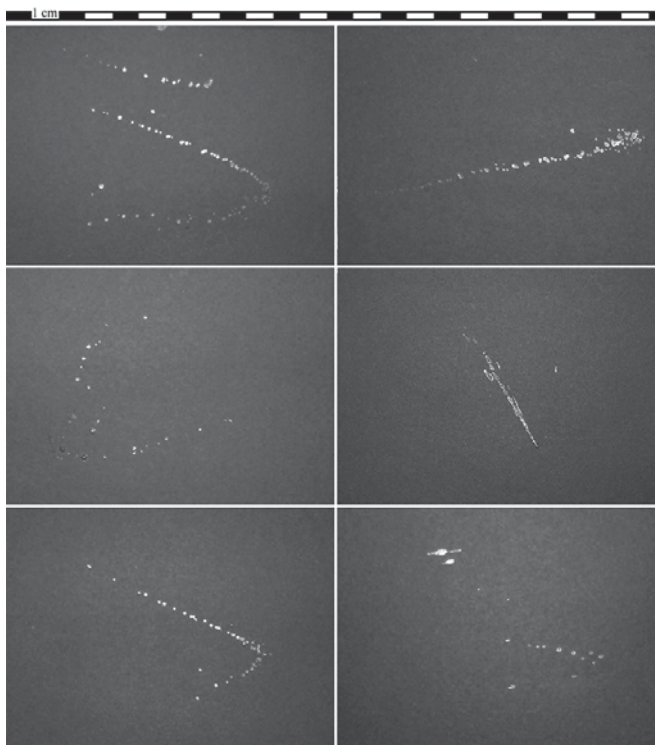
Figure 2. Venom traces on coloured paper from a series of consecutive spits by the female *N. atra*, presented chronologically from left to right, top to bottom. The first, third and sixth images are traces made by venom jets from two fangs, the others are from a single fang.

Fang analysis
Microscope pictures show the venom canal follows the length of the fang, leading straight to its tip (Fig. 3). The placement of the discharge orifice corresponds to 27.53 % of the total fang length (measurement from the fang tip to the basal orifice). This fang morphology is comparable to that of non-spitting cobras (Bogert, 1943). In contrast, the fang of the spitting species *N. sputatrix* shows the discharge orifice in the frontal wall of the fang at 9.18 % of its total length. Furthermore, the venom canal and its shape are clearly visible, as are the reduced orifice size and the angle leading to it (Fig. 3), which presents an inclination of about 110 °.

Spitting manoeuvre
The cobra started most of her defensive actions from the 'hooding' posture (Fig. 4a). From that position it throws itself in a frontal thrust against the target, opening her mouth during the manoeuvre (Fig. 4b). When the forward projecting movement results in the body reaching an inclination to the ground of about 45 °, the cobra pulls its head backwards, inclining the nape until the cranium axis is perpendicular to the ground (Fig. 4c). In that position, with the venom fangs aligned in parallel with the ground and the discharge orifice oriented towards the target, the snake releases a stream of
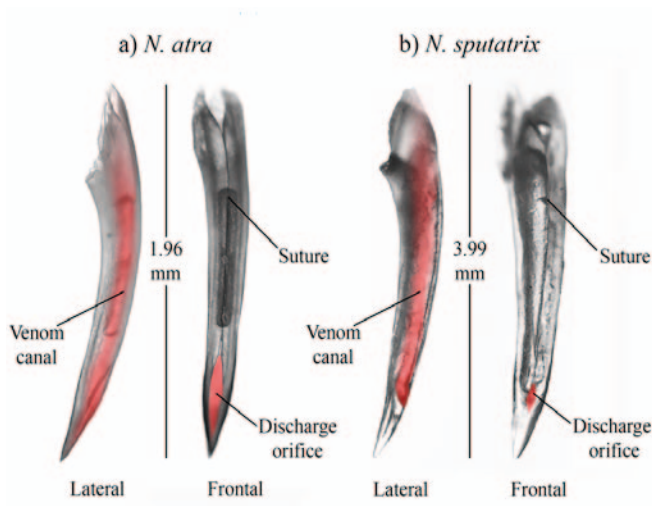
Figure 3. Lateral and frontal views of *N. atra* and *N. sputatrix* fangs, with venom canals and discharge orifices highlighted, following terminology of Bogert (1943), Wüster and Thorpe (1992)
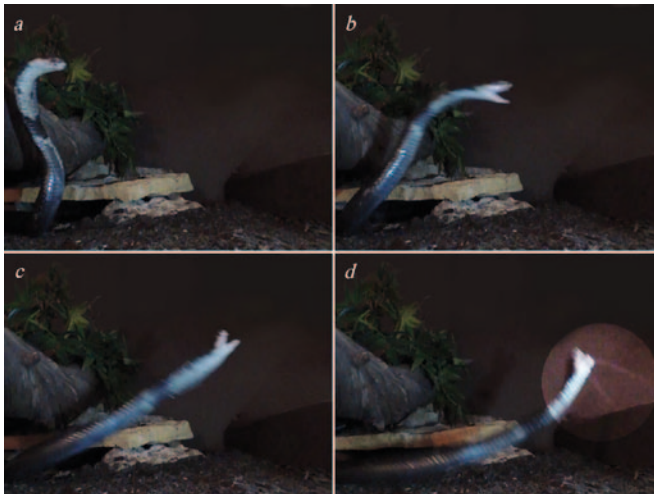

Figure 4. A spitting manoeuvre by the female *N. atra* that was completed in a total of 0.866 seconds (i.e. the time lapse between frames a. to d.)
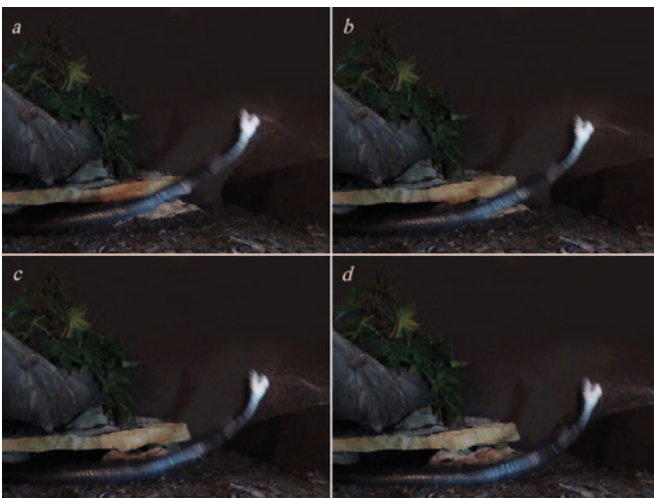

Figure 5. A venom spit by the female *N. atra* that was completed in a total of 0.083 seconds (i.e. the time lapse between frames a. to d.)

venom towards the target (Fig. 4d; Fig. 5). After the spitting event, the cobra keeps its head angled backwards (~90 °), until its body hits the ground. In very few cases, the specimen has been observed to spit directly from the hooding posture without the forward thrust.

DISCUSSION

This study confirms that the Chinese cobra (*N. atra*) can spit venom. However, among the many specimens raised by the author, and 18 offspring bred in captivity of the same bloodline, the female examined in this study was the only one to spit regularly. In addition, the observed defensive technique was shown only when the cobra was inside her terrarium. Once extracted from the terrarium, the cobra completely lost her defensive temperament. Nonetheless, venom spitting is performed by this individual almost every time a figure is perceived close to the front of the terrarium, and this behaviour has been observed repeatedly within a short period. While the quantity of venom ejected from each fang was variable, the shapes and characteristics of venom imprint are similar to those produced by 'true' spitting cobras featuring modified fangs (Young & O'Shea, 2005; Young et al., 2009).

As shown from the microscope pictures, the fang of the Chinese cobra presents a linear canal and a large discharge orifice. The morphology of the venom duct does not allow the specimen to spit frontally, as do the typical spitting species of the same genus. Thus, differences in the fang structure between *N. atra* and the spitting *N. sputatrix* are evident (Fig. 3). The discharge orifice of the fang of the Indonesian spitting cobra exhibits smaller dimensions and is located on an upper position on the frontal side of the tooth, distant from its tip. Comparing the size of the fang orifice in the two species as a proportion of total fang length, that of *N. atra* accounts for 33.3 % which is three times bigger than that of *N. sputatrix*. In Figure 3, the angle of the venom canal of *N. sputatrix* is easily recognisable, which, together with the reduced dimensions of its outlet, enables the cobra to spit forward and for notable distances.

Given the fang morphology of the Chinese cobra, in order to spit it must incline its head backwards to about 90 °. The act of spitting itself is executed in a very brief time, consisting of few hundredths of a second, while the whole manoeuvre of the spitting behaviour typically takes place in less than one second. However, compared to the 'true' spitters of the same genus, the venom jets seem to be able to reach only a relatively short distance. Moreover, the nature of this way of spitting is less precise than that of 'true' spitting species (this was also observed and reported in Karsen, et al., 1986). Reclining the head backwards to perform a horizontal spit, the cobra successfully directs the venom jets straight to the target, but it aim seems to lack precision in the vertical axis. In all the spitting recorded, the curvature of the venom spit is downward with respect to the fang inclination (Fig. 4 & 5). This same characteristic has been described for *N. kaouthia* by Santra and Wüster (2017) and was predicted by Bogert (1943). The fang structure and striking manoeuvre that accompanies spitting described here for *N. atra* are

both very similar to those described for *N. kaouthia* (Santra & Wüster, 2017; Rasmussen et al., 1995). This suggests that the evolution of spitting behaviour could precede the development of specialised fangs that refine the action.

In conclusion, the spitting ability of the specimen examined here occurs despite the fang morphology and discharge orifice being similar to those of non-spitting cobras, and to enable venom spitting, a spitting manoeuvre is employed. Further investigation is required to establish the nature and frequency of this behaviour in the Chinese cobra in general, with particular attention as to whether such behaviour occurs in the wild. This research also raises a more fundamental question, specifically whether venom spitting can be an individually learned defence behaviour?

ACKNOWLEDGEMENTS

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Disruption of reproductive behaviour of black caiman, *Melanosuchus niger* in the Santo Antônio hydroeletric dam, Madeira River, Brazilian Amazon

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ABSTRACT - A female black caiman (*Melanosuchus niger*) was monitored by radio transmitter for three years in the area of Santo Antônio hydroelectric dam in Amazonia. Her nest was inundated during the formation of the reservoir and she abandoned the area and moved to a stream. It is recommended that the formation of new reservoirs is avoided in the three months from October to December to allow caimans to hatch from their eggs before inundation.

INTRODUCTION

The black caiman (*Melanosuchus niger*), one of the largest caiman species in South America, faces several threats to its conservation (Da Silveira & Thorbjarnason, 1999). Hydroeletric dams (HD) have been cited as a potential threat to *M. niger* in Brazil (Marioni et al., 2013), for example large individuals (SVL> 2.00 m) may die while attempting to cross the reservoir of the Santo Antônio HD (Campos, 2015). Females *M. niger* select more isolated environments when they reach reproductive age, a snout-vent length (SVL) of 100 to 120 cm or 15 to 20 years of age (Villamarin et al., 2011; Da Silveira et al., 2013), and in Amazonia lay eggs at the end of the dry season, between September and December. Like other crocodilians, *M. niger* females guard their nests and young hatchlings (Herron et al., 1990). In this study, I examine how rising water levels caused by closure of the Santo Antônio HD affected *M. niger* reproduction on an island in the Madeira River, in the Amazon.

MATERIAL AND METHODS

On 24 June 2011, a reproductive female was captured at night in the area of the Santo Antônio HD reservoir in Rondônia, Brazil, near the Ilha dos Búfalos. A radio-transmitter (Telonics, MOD 400) was fitted to her tail, and tracked with a TR-4 receiver and 2H antenna (TELONICS). The female was monitored from 24 June 2011 to 17 December 2013. The reservoir was filled on 24 November 2011, causing the Madeira River mean water level to rise from 8.8 to 13.95 m, reaching a level of 19.86 m at the peak of the rainy season in April 2012 (Campos et al., 2017).

From 28 October to 02 November 2011, searching for *M. niger* nests was undertaken on foot by three people on the Ilha dos Búfalos in the Madeira River (9°9'55.14''N 64°31'17.47' W). The Ilha dos Búfalos covers an area of 14 km² with meanders of the Madeira River and dense forest. The nests were opened and the eggs counted and weighed.

One egg was collected to measure the total embryo length to give an estimate of its age (Vieira et al., 2011) and at this time a second female was captured next to her nest and measured. Nests and young hatchlings on the Ilha dos Búfalos were monitored between 15 and 17 December 2011, and those in a rainforest stream on the left bank of Madeira River were monitored in November and December 2012 and 2013.

RESULTS

Twelve *M. niger* nests were found on the Ilha dos Búfalos, one of which was built by the female monitored by radio transmitter (Fig.1A and B). The number of eggs per nest varied from 28 to 48 (mean = 42.7, SD ± 6.57, N=10), their mean length and width were 88.51 mm (SD ± 2.77) and 58.87 mm (SD ± 1.45) respectively, and their mass varied from 124 to 161 g (mean=146.3, SD ± 11.07). The embryos varied in estimated age from 1 to 45 days, (N = 10), and peak egg laying occurred in October. The radio monitored female had a SVL of 152.3 and laid 45 eggs the second female captured next to her nest had a SVL of 150 cm and laid 43 eggs.

The radio monitored reproductive female arrived at Ilha dos Búfalos on 08 Jun 2011 and traveled 3 km to the breeding site, where she laid her eggs sometime between 22 and 27 October 2011. She remained near the nest until it was flooded, which probably occurred on 24 November 2011, when the water level of the Madeira River rose 5.5 m. The female then left the flooded nest and departed from the island on 27 December 2011. She moved to the left bank of the Madeira River and entered a stream, where she remained until 19 December 2013 (Fig. 1B). This female apparently did not reproduce in the breeding seasons of 2012 and 2013 as night time searches for nests in her surroundings and for young hatchlings in her proximity were unsuccessful. The greatest distance this female caiman traveled in the period before the dam was closed was 3 km, in the second year it was 2 km and in the third year it was only 120 m.
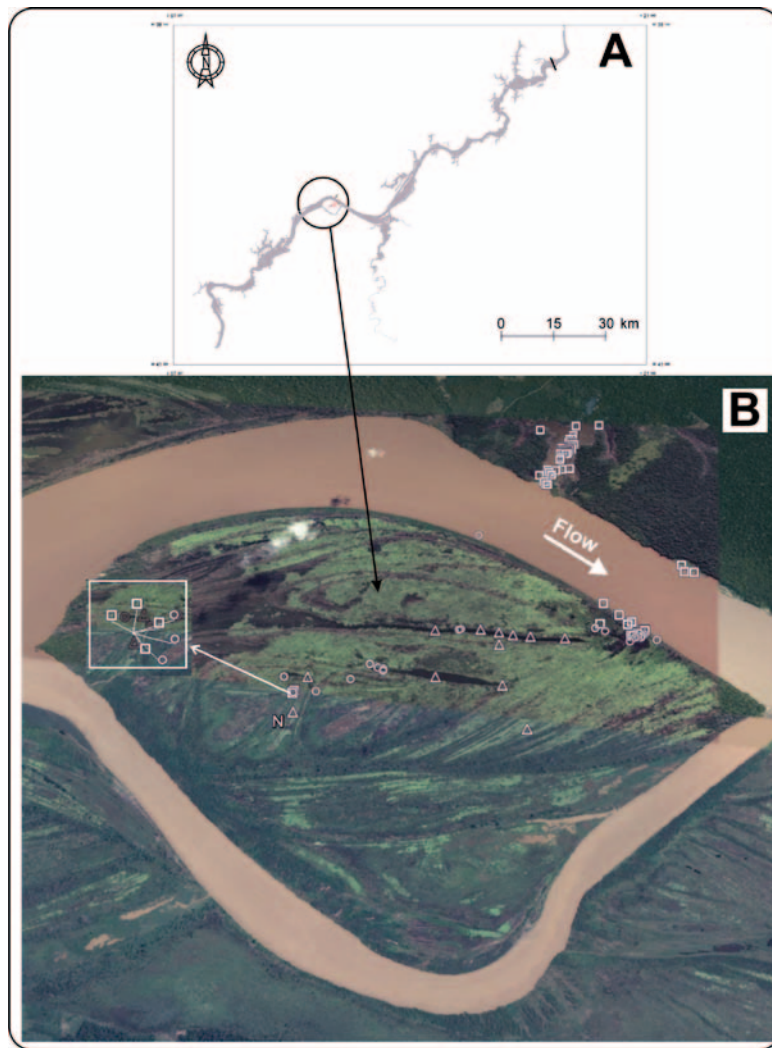
Figure 1. A. Area of the Santo Antônio HD reservoir, highlighting the Ilha dos Búfalos; **B.** Distribution of the 12 nests of *M. niger* (triangles), locations occupied by the female with the radio-transmitter before the formation of the reservoir (circles), the female beside her nest (N), locations occupied by the female after flooding (squares). She eventually moved from the Ilha dos Búfalos and entered a stream on the left bank of the River Madeira.

Of the 12 nests found on Ilha dos Búfalos, two were subject to predation, five were flooded when the reservoir was filled, while in the remaining five the caimans hatched successfully.

DISCUSSION

The closure of the Santo Antônio hydroelectric dam gates altered the reproductive behaviour of the *M. niger* female, since her nest was flooded on 24 November 2011 when the water level rose rapidly in the region of Ilha dos Búfalos. It is known that *M. niger* females select environments to reproduce according to the hydrological condition of the site, and an essential condition is for the water level to remain stable until hatching (Villamarin et al., 2011). The female stayed close to her nest until 30 November 2011, after which she left the island. The Ilha dos Búfalos was an area used by 12 breeding females at the end of the dry season of 2011, and was considered an environment conducive to *M. niger* reproduction prior to the construction of the hydroelectric plant. The female moved similar distances before and after the first year of flooding, i.e., 3 km and then 2 km, but in the

second year after the reservoir was filled, she remained more sedentary in the new environment that emerged, since the river had a constantly high level and the surrounding forests were flooded and dead.

The high density of *M. niger* nests (0.86 nests/km²) on Ilha dos Búfalos indicates that many female caimans used it as their breeding site, but breeding success was reduced after the water level rose to fill the reservoir. Given that the female *M. niger* monitored in this study lost her nest to flooding and did not reproduce in the two subsequent years, it would be better if hydroelectric dam gates in the Amazon region with a similar hydrological regime to that of the Madeira River were closed only after the young *M. niger* have hatched from their eggs. Closure should start in December by which time most caiman will have hatched from their eggs.

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Notes on Wallace's Racer *Wallaceophis gujaratensis* (Serpentes, Colubrinae): a recently described species endemic to Gujarat, India

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Observations in 2007 on a snake from Bhavnagar (Gujarat) with two black longitudinal stripes led eventually to naming of a new genus and species, *Wallaceophis gujaratensis* (Mirza et al., 2016), shown in Figure 1. The genus name honours Alfred Russel Wallace for co-discovering the theory of evolution by natural selection. This species was described on the basis of three specimens all from Gujarat, India (Mirza et al., 2016). This note presents some natural history information and new distribution records from Gujarat, collected subsequent to the description of the species.

Whilst monitoring birds of prey in Surendranagar district, Saurashtra region in the period 2013 to 2015, we detected *W. gujaratensis* in the food samples of Short-toed snake eagle (*Circaetus gallicus*). The breeding pair of Short-toed eagle under observation offered 23 different species of vertebrates, including amphibians, reptiles, birds and mammals to their hatchlings. Dead snakes of various sizes were offered and in all three years of study *W. gujaratensis* was included.

We were able to examine five specimens of *W. gujaratensis* from five different localities in Gujarat (Table 1). All five were provided by local rescuers and thoroughly examined. After taking photographs for documentation, the specimens were released back into scrub habitats from near their original rescue sites, except for two voucher specimens deposited in NCBS (National Centre for Biological Science) Museum, Bangalore, India. In addition to these specimens, we received a photograph of a further *W. gujaratensis* from Bayad, Aravalli but the specimen was not available for examination. However, we have included this locality in the updated distribution map of the species (Fig. 2) based on the photograph.

Data on locality, habitat type, scales and body measurements are shown in Table 1. Ventral scales were counted following the method proposed by Dowling (1951) other characters were recorded following Patel et al. (2015). Body measurements were made with string and a ruler to the nearest mm. The number of dorsal scale rows was counted at approximately one head length behind the head, mid-body, and one head length before the vent, respectively. Sub-caudal counts reported here do not include the terminal scute. Values for symmetric head characters are given in right/left order. Sex was determined by checking the presence of hemipenes, whenever possible.

Figure 1. Dorso-lateral aspect of Wallace's Racer (*W. gujaratensis*) from Madhavpur Ghed, Porbandar District, Gujarat
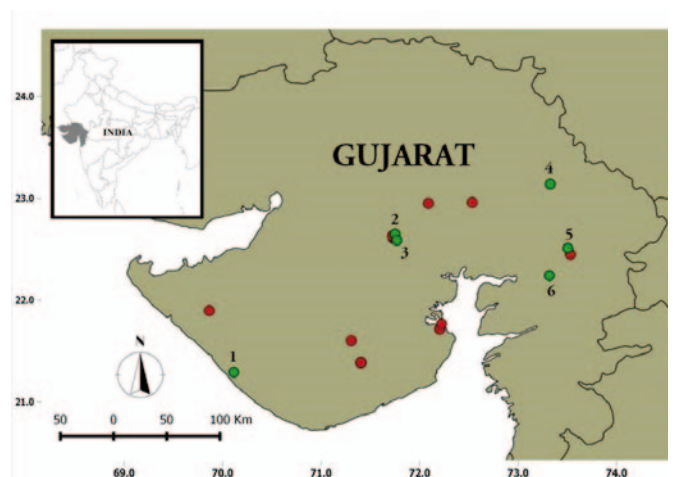
Figure 2. Map showing distribution range of Wallace's Racer (*W. gujaratensis*). Red circles represent localities reported in literature. Green circles represent new records as follows: 1. Madhavpur Ghed, Porbandar district; 2. Kharwa, Surendranagar district; 3. Wadhwan, Surendranagar distric;t 4. Bayad, Aravalli district; 5. Halol, Panchmahal district; 6. Khatumba, Vadodara district

With one exception, the colour and markings of all five specimens matched those of the species description (Mirza et al., 2016). The exception was that belly marking were not present for the specimens of Halol and Khatumba. Both were large adult females whose ventral belly markings had faded and were totally indistinct (Fig. 3A). But in two

Table 1. Measurements (cm), scale counts and collection details of Wallace's Racer (*W. gujaratensis*), Gujarat, India

Specimen No.	1 (NCBS AQ496)	2 (NCBS AQ497)	3	4	5
Locality	Halol, Panchmahal District	Wadhwan, Surendranagar District	Khatumba, Waghodiya Road, Vadodara District	Kharva, Wadhwan, Surendranagar District	Madhavpur Ghed, Porbandar District
Geo-coordinates	22°29'48.88"N; 73°28'20.59"E	22°42'23.02"N; 71°40'25.28"E	22°17'9.12"N; 73°15'25.69"E	22°38'38.21"N; 71°41'45.51"E	21°17'57.44'' N; 70°1'30.50'' E
Altitude (meters)	102	51	35	54	9
Date of rescue	11 March 2013	15 March 2016	15 February 2018	20 September 2018	7 October 2018
Habitat / vegetation	Industrial Area / Cultivated garden	House / No vegetation	New Urban Settlement / Agricultural	New Human Settlement / Scrubland	Rural / Agricultural
TL	93.0	68.3	81.0	14.5	45.5
SVL	82.0	60.0	70.5	9.0	37.8
Tal	11.0	8.30	10.5	5.5	7.7
Dorsal scale row Anterior: middle; posterior	25:23:17	24:23:21	25: 23:17	25:23:17	25:23:18
Ventrals	233	239	231	220	214
Anal	Single	Single	Single	Single	Single
Sub-caudals (divided)	52	54	59	52	-
Supra-labials	8/8 (4th ,5th touch the eye)	8/8 (4th ,5th touch the eye)	8/8 (4th ,5th touch the eye)	8/8 (4th ,5th touch the eye)	8/8 (4th ,5th touch the eye)
Infra-labials	10/11 (6th biggest)	10/10 (6th biggest)	9 (6th biggest)	9 (6th biggest)	-
Loreal	Single	Single	Single	Single	Single
Pre-ocular	1	1	1	1	1
Post-ocular	2	2	2	2	2
Temporal	1+2	1+2	1+2	1+2	1+2
Sex	Female	Not determined	Female	Not determined	Not determined

smaller specimens collected from Amareli and Kharva; the ventral markings were distinct (see Fig. 3B of Mirza et al., 2016). It would appear that the belly markings fade with age. The snout to vent body length (SVL) of the species was documented to range from 25.3 to 93.0 cm (n = 9) (Mirza et al., 2016). In our collection the shortest SVL was 14.5 cm. This was from a specimen probably only one month old found in September. This suggests that it hatched from the egg in August which coincides with the typical July to September wet season breeding of snakes in India (Smith, 1943).

Here, we add further precise colour markings of body scale rows along with the size of body scales, not mentioned in Mirza et al. (2016); lateral most two scale rows being large and light yellow towards each side (2 rows); dorso-lateral six scale rows smaller than the lateral most scales but larger than the mid-dorsal scale rows with dark brown colour, upper and lower most scales of these rows are partially coloured (half portion of the scale row is dark brown and other half is wheat coloured) on each side (1+4+1 rows); mid-dorsal scale rows smaller than the dorso-laterals and lateral scale rows with light cream or wheat colour (9 rows). The total body scale rows 25 at mid-body part (2+6+9+6+2=25), mid-dorsal rows are reduced towards the anterior body part (as 24 rows) and same as in posterior body part (as 21 or 17 rows).

The new locality records show that the species is actually quite widespread in Gujarat and can be found close to human habitation. Despite being a reasonably common snake species of Saurashtra and Central Gujarat, the species
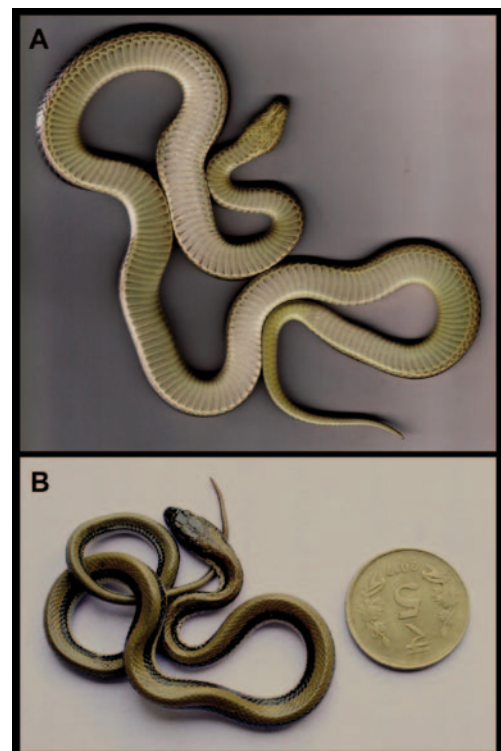
Figure 3. A. Large adult Wallace's Racer (*W. gujaratensis*) from Kalol, Panchmahal District, showing each ventral scale with indistinct brown spot on each anterior corner **B.** The dorsal aspect of smallest ever recorded specimen of Wallace's Racer (*W. gujaratensis*) from Kharva, Surendranagar District, Gujarat

was only described very recently. It is possible that earlier explorers might have misidentified *W. gujaratensis* as *Coelognathus helena*. However, Gujarat largely remains unexplored herpetologically so that it is also possible that this species simply evaded the attention of earlier workers.

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Ultraviolet-A 'blacklight' fails to serve as a useful survey tool for great crested newt (*Triturus cristatus*) in ponds

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Great crested newts (*Triturus cristatus*) are offered the highest level of protection throughout their range, owing to their listing as an Annex II species of conservation concern within the Habitats Directive (Council Directive 92/43/EEC). In Scotland, this is translated in to law by the Conservation (Natural Habitats, &c.) Regulations 1994 (as amended) which imposes a duty for the statutory nature conservation organisation, Scottish Natural Heritage, to maintain the favourable conservation status of this, and other Annex II species. Great crested newts are offered the same level of protection in England and Wales, though there are legislative differences.

Perhaps the way in which this statutory obligation is most commonly encountered is within an ecological consultancy environment. Wherein the presence of great crested newts within proximity to development proposals requires surveys by a licensed ecologist to inform mitigation requirements that can maintain or promote favourable conservation status of the species (English Nature, 2001).

One of the most efficient methods for detecting great crested newts is the illumination of a pond by torchlight (Griffiths et al., 1996 & Kröpfli et al., 2010). However, torchlight surveys using white light often result in movement of great crested newts which could impact upon the reliability of counts by promotion of double counts (e.g. if the newt moves from one side of the pond to another and is double-counted) or by reducing the ability to count (e.g. if startled newts move too quickly and an accurate count cannot be undertaken). The use of ultraviolet-a light may reduce these disturbance effects and may improve the detection of male great crested newts since the white tail flash may reflect UV light.

To test this hypothesis, the author visited a pond in North Lanarkshire, Scotland on the evening of the 15 April 2017 at approximately 21:30 h. The pond surface was c.450m^2 and between 200 mm and 500 mm deep within 1 m of the shoreline. Surveyors wore yellow-lensed protective glasses and used a WolfWill WW-UVL-0019 LED blacklight torch, peak light emission frequency of 395nm (UV-A), to illuminate the pond following the methodology of Griffiths et al. (1996). Immediately after this, the pond was illuminated using the same basic methodology but instead with a standard LED spotlamp (Clulite Clubman CB3 1,000,000 candlepower). Newts were identified using key ID features visible in torchlight (McInerny & Minting, 2016) and the number of newts counted under both lighting conditions was compared. The sample with the ultraviolet light did not reveal any newts as particulate matter within the water column was illuminated by the ultraviolet light and thus penetration of the water column by the light was reduced. However, the pass with the standard LED spotlamp was successful as an unquantified number of great crested newts of both sexes as well as smooth and palmate newts were observed.

In this instance, ultraviolet light did not reveal any newts as particulate matter within the water column refracted UV-A light more than it did white light. However, this failure may have been due to pond-specific conditions and it may be worth testing the UV-A torch in a variety of ponds and in a variety of environmental conditions. Additionally, there may be value in extending the testing to UV-B and infrared torches to reduce the disturbance to great crested newts from torchlight sampling and achieve more accurate counts (e.g. Buchanan, 1993; Baker & Richardson, 2006).

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The advertisement call of *Pristimantis erythropleura* (Boulenger, 1896) (Craugastoridae) from a population in the central Andes of Colombia

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Pristimantis erythropleura is a frog distributed between 980 and 2600 m a.s.l. in the Western and Central Andes of Colombia (Lynch, 1992; Ruiz-Carranza et al., 1996; Acosta-Galvis, 2000). Individuals of this species are nocturnal and usually found on shrubs, trunks, and pastures between 10 and 200 cm above ground; they are especially abundant in disturbed forested habitats (Rincón-Franco & Castro-Herrera, 1998; Lynch, 1996; Montes et al., 2004; Duarte-Marín et al., 2018). Although some aspects of the natural history of *P. erythopleura* are known or inferred according to its phylogenetically relationships (e.g. reproduction with direct development), we lack a quantitative description of the auditory signals they use in social interactions. Herein, we describe the advertisement call of this species.

We recorded two calling individuals of *P. erythropleura* at 19 March and 04 April 2017 in the Natural Reserve "La Patasola" (4˚ 40'58.8'' N, 75˚ 32'60'' W; elevation 2300 m a.s.l.) municipality of Salento, department of Quindío, Central Andes of Colombia. The advertisement calls were digitalised using a Sennheiser ME62 / K6 unidirectional microphone connected to a Zoom H4n Handy Recorder. The body size (snout-vent length, SVL) and temperature of the frog was obtained with a digital caliper and an infrared thermometer, respectively; the individuals were not collected. We digitised recordings with a 16-bit resolution and a sampling frequency of 44.1 kHz. Oscillograms and spectrograms were analyzed with a 256-point Fourier Transformation window and the Blackman algorithm. For the call analysis, we use the software RAVEN Pro 1.4 (Bioacoustics Research Program, 2011), and for the call description we followed the terminology described in Köhler et al., (2017). Our unit for the descriptive analysis was the individual recorded. Digital copies of the calls (in WAV format) were deposited at the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia.

Males were calling in a pasture adjacent to a secondary forest, on the upper side of fern leaves (*Pteridium aquillinum*) at 15 to 30 cm above ground. The mean air temperature was 18.3 ± 1.83 (17.0-19.6) °C and relative humidity was 78.5 ± 2.1 (77 − 80) %; these measurements were taken from the two individuals independently. The advertisement call of *P. erythropleura* consisted of a note comprising 9-10 pulses (Fig. 1); the whole note lasted 47.1 ± 0.2 (47.0-47.2) ms, at a mean pulse duration of 3.2 ± 0.3 (3.0-3.5) ms, and the mean
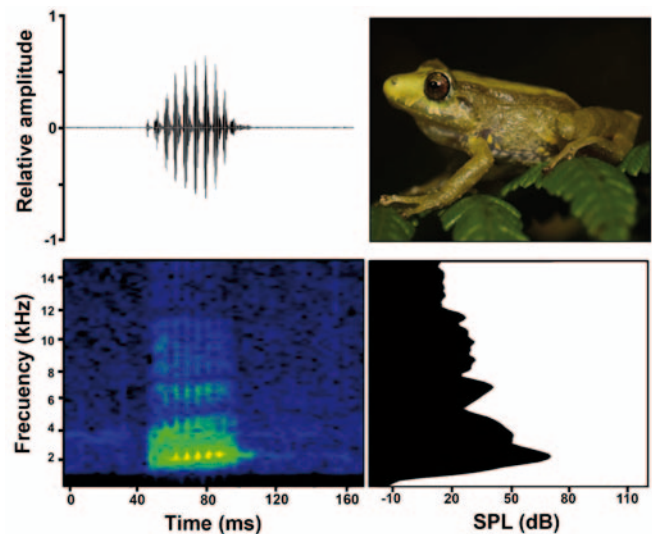
Figure 1. The advertisement call of a male *P. erythropleura* (top right): oscillogram (relative amplitude), spectrogram (frequency) and power spectrum (SPL- sound pressure level). The call consists of nine pulses and corresponds to male 1 (see Table 1).

interpulse duration of 1.9 ± 0.3 (1.7-2.2) ms. The dominant frequency of the call was 2067 Hz. There were two harmonic bands with dominant frequencies of 3402 ± 860 (3273 - 3445) Hz and 6416 ± 865 (6373 - 6546) Hz.

Our recording is only the second description of an advertisement call for the *P. ridens* group, which includes a total of 33 species (sensu Padial et al., 2014). The only other species of this group for which the advertisement call has been described is *P. caryophyllaceus* (Barbour, 1968), which has a similar advertisement call consisting of a single pulsed note with a duration of 40-50 ms (Batista et al., 2014). The dominant frequency in *P. erythropleura* is lower than in *P. caryophyllaceus* (2566 - 3010) Hz and may reflect their differences in body size. More call descriptions are needed to establish the patterns of call structure within *P. ridens* species group.

ACKNOWLEDGEMENTS

Table 1. Advertisement call features for each individual *P. erythropleura* recorded at municipality of Salento, Central Andes of Colombia. Values are reported as mean ± standard deviation and range. IAvH-CSA Codes assigned at the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia, are shown. Individuals were not collected.

Individual	Voucher	Number of calls recorded	Snoutvent length (mm)	Body temp (°C)	Pulse duration (ms)	Interpulse duration (ms)	Number of pulses/ call	Dominant frequen-cy (Hz)	Dominant frequency of harmonics (Hz)	
									First	Second
1	IAvH-CSA 18275	5	24.9	14.9	3.0 ± 1.0 (1-4)	2.2 ± 0.2 (1-3)	9.1 ± 0.2 (9-10)	2067.0	3273.0 ± 55.0 (3210-3305)	6373.0 ± 230.0 (6105-6404)
2	IAvH-CSA 18276	4	26.1	11.6	3.5 ± 0.8 (3-5)	1.7 ± 0.1 (1-3)	9.4 ± 0.3 (9-10)	2067.0	3445.0 ± 110.1 (3237-3510)	6546.0 ± 99.3.0 (6508-6612)

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A tale of two tails: a rare occurrence of tail bifurcation in the red-backed salamander (*Plethodon cinereus*)

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Tail bifurcation, in which the tail duplicates after mechanical damage, is a rare event in the wild that has been reported in some Squamata (Kornilev et al., 2018) and Urodela (reviewed in Henle et al., 2012; Romano et al., 2017). This condition can be replicated experimentally by inflicting irregular damage to the tail including partial spinal cord removal (Dawson, 1932). Despite plethodontids being the most abundant vertebrates in eastern North America (Burton & Likens, 1975), tail bifurcation has only been reported in plethodontids twice in the last 113 years, both times in the common and abundant *Plethodon cinereus* (Blatchley, 1906; Lynn, 1950). On 31 May 2008 at 15:35 h, I found an adult female *P. cinereus* with a bifurcated tail (Fig. 1) under a rock near Spring Road at Mountain Lake Biological Station, Giles County, Virginia, USA (37° 22'12.97" N, 80° 31'14.92" W) during collection of this species for an experiment (Liebgold & Dibble, 2011). The salamander was brought into the laboratory, measured (snout-vent length = 38.88 mm, longest tail length = 29.83 mm, tail length past the point of bifurcation: main tail = 22.68 mm; supernumerary tail = 12.85 mm), and then released at the point of capture within 24 h. Lynn (1950) described an individual of *P. cinereus* with a dorsal-ventral tail bifurcation and found red pigment was present only on the dorsal tail. In contrast, the individual presented here had a lateral tail bifurcation, with red dorsal pigment present on both tails (Fig. 1). Both tails had regenerating tips, which indicates that tail autotomy had occurred within the few weeks prior to collection. This demonstrates that, after tail bifurcation, autotomy and regrowth can occur in both the main and supernumerary tails.

Little information is available about the frequency of tail bifurcation in amphibians due to the scarcity of data on the number of normal individuals in any particular population. However, in a detailed review, Henle et al. (2012) quoted two examples of bifurcation rate, *Triturus cartifex* 1:48 (2.08 %) and *Ambystoma talpoideum* 1:17,935 (0.006 %); on the two previous occasions when tail bifurcation was reported in *P. cinereus* there was no information on frequency (Indiana-Blatchley, 1906; Massachusetts- Lynn, 1950). At Mountain Lake Biological Station and in the surrounding Jefferson National Forest, Virginia, USA, *P. cinereus* is abundant, with densities of 0.97-6.30/m². From 2005-2009, a colleague (T. Ransom) and I collected 1942 different individuals of *P. cinereus* for studies that were published from 2006-2017 (including the collection during which this individual was

Figure 1. Female *P. cinereus* with a bifurcated tail captured on 31 May 2008 at Mountain Lake Biological Station, Virginia, USA

found but not used: Liebgold & Dibble, 2011). Individuals were marked with visible implant elastomer (Northwest Marine Technology Inc., Shaw Island, WA) so they were not used in multiple experiments or individuals were collected from areas that were not revisited. None of the other 1941 individuals collected had bifurcated tails. Our estimate of the frequency of tail bifurcation in *P. cinereus* in this area was therefore 1:1942 (0.05 %).

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Hamerkop (*Scopus umbretta*) predation on an Augrabies flat lizard (*Platysaurus broadleyi*)

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On 27 August 2018 at Augrabies Falls National Park (28.5942° S, 20.3381° E) we observed an adult Hamerkop (*Scopus umbretta*) foraging adjacent to the main waterfall along a rock shelf midway down the cliff-side. At 15:37 h we saw the bird capturing and swallowing an Augrabies flat lizard (*Platysaurus broadleyi*; Fig. 1). This observation is noteworthy as to date the primary predators of Augrabies flat lizards are known to be raptors (e.g. Rock Kestrel, *Falco rupicolus*, and Booted Eagles, *Aquila pennatus*) and sand snakes (*Psammophis* sp.), with other potential predators being mongooses and large arthropods, such as spiders and centipedes (Whiting, 2002; A. Jenkins pers. comm.). Hamerkop, to the best of our knowledge, have not been documented previously to consume reptiles, and as such there have been no previous observations of them eating this specific lizard species. The known diet of Hamerkop consists of frogs, particularly of the genus *Xenopus*, insects, and fish (Skead, 1953; Liversidge, 1963; Dean & MacDonald, 1981; Kahl, 1987; Yohannes, et al., 2014).

On the same day as this observation, we had seen hundreds of Augrabies flat lizards living and foraging on the granite rocks and cliffs on either side of the Orange River near the falls. Consequently, for an opportunistic predator these lizards would be a widely-abundant local food source that is easily exploited. Furthermore, this may represent an important ecological link within Augrabies Falls National Park that may have management implications. Previously, attempts have been made to control the blackfly (*Simulium* spp.) population along the Orange River and within the park through the use of aquatic larvicides (Palmer et al., 1996). Early control measures in 1960's involved the application of DDT, however after widespread negative ecological impacts later applications used the soil bacterium *Bacillus thuringiensis* var. *israelensis* as a blackfly larvicide (Palmer et al., 1996). These control programs were undertaken not for human health concerns but rather due to the lost revenue and taxes related to the impact biting flies have on livestock growth and production (Palmer et al., 1996), as well as the general annoyance these swarms have on local human populations. However, the dense blackfly population supports the substantial Augrabies flat lizard population (Branch & Whiting, 1997). If future efforts are made to reduce blackfly populations along the Orange River this may have

Figure 1. Two Augrabies flat lizards (*P. broadleyi*) on the granite outcrops alongside the Orange River in Augrabies Falls National Park, Northern Cape, South Africa. The lizard on the left is a female, and the lizard on the right is a male. These lizards congregate around the Orange River at Augrabies Falls to consume blackflies (*Simulium* spp.), which are also visible within the photo.

Figure 2. A hamerkop (*S. umbretta*) foraging in the water at Augrabies Falls National Park, Northern Cape, South Africa.

a ripple effect on the biodiversity of the area. For instance, reducing the food resources of Augrabies flat lizards could, in turn, reduce resources for both specific and opportunistic predators of this lizard species, like Hamerkops.

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Arboreal behaviour in the Stuart's burrowing snake
Adelphicos veraepacis (Dipsadinae) in Baja Verapaz, Guatemala

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*A*delphicos veraepacis* Stuart, 1941 is a little known snake species that is endemic to the mountain regions of Guatemala, occurring only in Sierra de las Minas, Cuilco Mountains, Sierra de los Cuchumatanes and Sierra de Xucaneb (Acevedo et al., 2014). It inhabits cloud, rain and pine forests from 1,200 to 2,200 meters asl. Snakes in this genus are encountered infrequently due to their predominantly fossorial and terrestrial lifestyle. Consequently, there are few published observations of their ecology or natural history. According to the IUCN Red List, *A. veraepacis* is classified as vulnerable due to its restricted distribution and the associated threat of habitat loss. The cloud forests from the Verapaces region, where this snake was observed, are currently threatened owing to the increased cultivation of leatherleaf (*Chamaedaphne calyculata*) for export to Japan and Europe, pine plantations and intentional fires (Acevedo et al., 2014).

During the second week of September 2014, a field trip was made to Ranchitos del Quetzal, Purulha, Baja Verapaz (15.215489°N, 90.219249°W, 1,682 m asl). During a night time visual encounter survey at 21:30 h, a female *A. veraepacis* (Fig.1) was found approximately 3 m up a tree in a cloud forest habitat. Previous accounts of this genus have noted the behaviour of *Adelphicos* to be highly fossorial and terrestrial (Acevedo et al., 2014). However, arboreal behaviour has been recorded in a closely related species, *A. quadrivirgatum*. In this case, clutches of eggs were found 1.5 m above the ground in a termite nest (Pérez-Higareda & Smith, 1989). Our observation was made in September but the egg laying season in *A. veraepacis* is reported to be from May to June (Campbell, 1998). While we have no evidence, it remains possible that the reproduction of this species is not seasonally restricted, and that the observed female specimen had laid eggs. Alternatively, facultative use of arboreal habitats while foraging has been described for numerous terrestrial and presumably fossorial snakes (Keller & Heske 2000; Brown et al., 2018). Snakes of the genus *Adelphicos* are known to prey on earthworms and other invertebrates (Dos Santos et al., 2017; Stafford & Meyer, 2000), so arboreal foraging remains a possibility.

Figure 1. Photograph of the female *A. veraepacis* the day after it was observed 3 m up in a tree in the Ranchitos del Quetzal lodge, Baja Verapaz, Guatemala

Herpetology trip. We thank Tom Brown for reviewing and correcting the writing of this note and the staff from the Ranchitos del Quetzal lodge for always allowing us to conduct our research and field trips in the area.

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Predation of *Scinax ruber* (Anura: Hylidae) tadpoles by a fishing spider of the genus *Thaumisia* (Araneae: Pisauridae) in south-east Peru

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The red-snouted tree frog, *Scinax ruber*, is a medium sized hylid frog that is widely distributed throughout the Amazon basin where it mainly inhabits cleared areas of rainforest, as well as agricultural lands (Solis et al., 2010). This species breeds year-round, with the females depositing eggs on the vegetation overhanging temporary ponds where the tadpoles later develop (Solís et al., 2010). Temporary water sources are known to harbour fewer predators than permanent water sources (Skelly & Werner, 1990; Skelly, 1997; Wild, 1996) but semi-aquatic and terrestrial predators may be unaffected by the lifespan of the water source. This is the case with nursey web or semi-aquatic spiders (Pisauridae), as there are several reports of such spiders predating anurans (Bernarde et al., 1999; Mendes Luiz et al., 2013; Bovo et al., 2014; von May et al., 2019).

At 21.39 h on 5 October 2018, an *S. ruber* tadpole was observed held between the chelicerae and pedipalps of a *Thaumasia* sp. (Pisauridae) spider on the lip of a water-filled barrel (Fig. 1). The spider appeared to be chewing the *S. ruber* tadpole, as the authors saw it moving its mouthparts while holding it. The sighting occurred during a night time survey in the biogarden area of the Manu Learning Centre (12.78917°S, 71.39111°W, WGS 84, 460 m elev.), a research station situated in the buffer zone of the Manu National Park in south-east Peru. The specimens were observed and photographed but not collected. To give a tentative identification of the predated tadpole, others from the same location were identified using the guide of Schulze et al. (2015). Predation events of *Scinax* adults, tadpoles (Machado & Mendes, 2014; Pinto-Silva & Bruno, 2018) and other hylids (Santos-Silva et al., 2013; Mendes Luiz et al., 2013; von May et al., 2019) by Thaumasia have been reported previously but to the best of our knowledge predation of *S. ruber* tadpoles by Thaumasia has not been documented. This observation provides an interesting addition to prey-predator interactions between anurans and spiders.

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Figure 1. *Thaumasia* (Pisauridae) holding a *S. ruber* tadpole in the Manu Learning Centre, Peru

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Kyphosis in a free-living *Marisora brachypoda* (Squamata: Scincidae) from Utila Island, Honduras

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The skink *Marisora brachypoda* (Taylor, 1956) is known from low to moderate elevations in Central America. In Honduras, the species is widespread in open habitats across much of the mainland and is reported on Utila Island (McCranie, 2018). Utila Island is part of the Honduran Department of the Bay Islands, being the westernmost island of the three major Bay Islands (Utila, Roatan, Guanaja) off the Caribbean coast of Honduras (McCraine, 2018). Notably, populations of Marisora on the islands of Roatan and Guanaja represent a cryptic endemic species, *M. roatanae* (Hedges & Conn, 2012), while populations occurring on Utila belong to *M. brachypoda* (McCranie & Orellano, 2014).

On multiple occasions over the course of April and May of 2018, we observed an adult male *M. brachypoda* with a visible vertical curvature of the spine, basking regularly on a tree trunk ca. 2.2 m high outside the Kanahau Utila Research and Conservation Facility (KURCF) (16.119383° N, 86.884989° W, WGS 84). Despite its visible deformity, the skink appeared to move and behave in a normal way, being extremely quick and agile whilst moving up and down the tree. On 25 May 2018, 13:00 h, we managed to capture and inspect the individual while collecting morphological data (Snout vent length: 70.4 mm; tail length: 64 mm, weight: 7.3 g). Upon external visual inspection, the kyphosis started in the mid-section of its body, continuing all the way to the end of its tail (Fig. 1.). The individual also presented signs of caudal autotomy and subsequent regeneration, which can be observed in Figure 1. To our knowledge, this is the first evidence of kyphosis in the genus *Marisora*.

Malformations such as the kyphosis seen in this individual have been reported in other free-living populations of lizards (Gering, 2009; Garin-Barrio et al., 2011; Pérez-Delgadillo et al., 2015; Ortiz-Medina & Valdez-Villavicencio, 2016; Valdez-Villavicencio et al., 2016), as well as captive populations of skinks (Cooper et al., 1982). Bellairs (1981) discussed the aetiology and pathogenesis of various developmental abnormalities of reptiles, and stated that they are of either genetic or environmental origin. In reptiles, spinal malformations can arise owing to incorrect conditions during the incubation of eggs, i.e. excessively low or high temperatures and low relative humidity; though notably, "toxins and pollutants" (e.g. insecticides) might also be responsible for malformations observed in wild reptiles (Bellairs, 1981). Additional studies have also shown that inbreeding of reptile populations with low genetic diversity

Figure 1. Lateral view of the adult male *M. brachypoda* with kyphosis

can cause malformations (Madsen et al., 1992; Olsson et al., 1994; 1996), and that spinal malformations can be caused by metabolic bone diseases (Frye, 1991).

In this case, as our diagnosis is purely external, we cannot safely confirm the cause of the deformity in *M. brachypoda*. While we were unable to collect and radiograph the specimen, ideally, such practice should be followed as it allows for more detailed investigation of skeletal abnormalities. Nonetheless, our observations demonstrate that individuals can survive under natural circumstance with such a developmental anomaly.

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Observations on the diving behaviour and defensive strategies of the endemic, semi-aquatic lizard *Potamites erythrocularis* (Squamata: Gymnophtalmidae)

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Lizards of the genus *Potamites* (Doan & Castoe, 2005) are semi-aquatic and are known to inhabit riparian habitats and may even be found beneath rocks in streams (Chávez-Arrisbaplata et al., 2015). We have found no information describing their adaptations to a semi-aquatic lifestyle, such as their diving behaviour or apnea abilities, but were fortunate to observe these for *Potamites erythrocularis*, a recently described species from the highlands of Manu National Park in south-east Peru (Chavez & Catenazzi, 2014).

On the 14 of April 2019 at 12.52 h, we observed a male *P. erythrocularis* (Fig. 1) (SVL=7 cm, tail length=7.1 cm, weight 9 g) submerged in a stream (12.802573°S, 71.403664°W, WGS 84, 525 m elev.) in the regenerated forest reserve of the Manu Learning Centre (MLC), a research station situated in the buffer zone of the Manu National Park in south-east Peru. The lizard was submerged 5 cm underwater with its eyes closed, remaining motionless while positioned between two rocks. After being submerged for ca. 40 seconds, it resurfaced to breathe for approximately 1 to 2 seconds, with only its nostrils breaking the surface of the water. This behaviour was repeated for a total of 10 minutes, remaining underwater for up to 1.5 minutes and only resurfacing for a few seconds to breathe in the manner already described. Following this observation, we decided to catch the lizard to collect morphological data. Upon capture, the lizard began thrashing its body and bit the first authors' hand. Shortly after, it started performing a spinning manoeuver that made it extremely hard to hold. This movement was similar to the "death roll" behaviour exhibited by crocodilians when subduing prey (Fish et al., 2007). Upon further inspection, we observed evidence of tail regrowth (Fig. 2). The individual also produced a cloacal discharge.

Diving has been reported in several families of lizards including the Gymnophtalmidae, with Vitt et al. (1998) documenting this behaviour in another species of *Potamites* (previously known as *Neusticurus*). It has been suggested that several aspects of reptile physiology, such as lower metabolic rates and larger lung volume compared to mammals, provide lizards with advantages and adaptations to survive in an aquatic habitat if needed (Heatwole, 1977). The ability to submerge underwater for extended periods of time improves the chances of the lizards catching aquatic prey and of evading terrestrial and diurnal predators (Hare & Miller, 2009). Martins (1996) states that semi-aquatic lizards face strong

Figure 1. An adult *P. erythrocularis*, a semi aquatic species of Gymnophtalmidae that is endemic to the south-east region of Peru

Figure 2. Tail autotomy and regeneration in *P. erythrocularis*

predation pressure from carnivorous fish and will therefore develop defensive tactics to avoid being preyed upon by both aquatic and terrestrial predators. This would potentially explain the wide array of defensive strategies we observed in *P. erythrocularis*, which include body rotation, body thrashing, tail autotomy, biting, and cloacal discharge. Rotating the body has been reported in crocodilians for dismembering prey (Fish et al., 2007), and in several species of Amazonian snakes as a defensive mechanism (Martins, 1994), yet it is not commonly documented amongst lizards. At the moment when the individual started to display this behaviour, it made it extremely difficult for the first author to hold on to it, which could prove to be useful while trying to escape a predator's

grip. Snakes and lizards are also known to use random and unpredictable movements to confuse their potential predators in the form of body thrashing (Martins, 1996).

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The common toad (*Bufo bufo*) - dark coloration

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Colour anomalies in amphibians may act as bioindicators of disrupted environments (Henle et al., 2017a). Melanism (abnormally black coloration) is an anomaly that may cover part or all of the body. Its proximal cause is an over-representation of eumelanin, usually due to an excess of melanophores (Henle et al., 2017a) and a reduction of xantophores and iridophores, which may have a genetic basis linked to xanthine dehydrogenase (Bagnara et al., 1978). Melanism can be caused by UV-B radiation but natural, ambient, radiation of this type was proven to play no part in the genesis of melanism in the Common Toad (*Bufo bufo*) (Langhelle et al., 1999). In amphibians, melanism can be innate (hence heritable, as in *Salamandra salamandra*; Henle et al., 2017a), or be developed as a physiological reaction to specific conditions (as in *S. keyserlingii*; Henle et al., 2017b). Innate melanism can be a result of anthropogenic mutagenesis. Axanthism, on the other hand, is caused by a lack of particular pigment cells - iridophores, xantophores and erythrophores (Jablonski et al., 2014). This colour anomaly, rarer than albinism or melanism, is characterised by blue/grey or dark coloration with black eyes and has been observed in *Bufotes viridis* (Ibid.). Full axanthism can be mistaken for melanism but axanthic specimens tend to have discernable patterns and be lighter in colour as they differ from melanistic specimens in having no overproduction of melanin (Ibid.). It is thought that both genetic and environmental factors contribute to axanthism (Ibid.).

The common toad (*B. bufo*) is a fairly common amphibian in Poland (Juszczyk, 1986) that tolerates disturbed environments (Speybroeck et al., 2016; Kaczmarski et al., 2016). This species is common throughout almost all Europe (Speybroeck et al., 2016) and as a result it has been one of the best-studied tailless amphibians. Axanthism has been described in *B. bufo* (Dubois, 1969) but in the case of melanism only black spots on adult (Mattes, 2013) and juvenile (Kliemt, 2017) have been reported and it has been assumed that these were caused by a fungal disease.

On the 20th of June 2011 a number of freshly metamorphosed *B. bufo* were seen dispersing from the banks of a body of water in an active sand and gravel quarry next to fields on the outskirts of Gorzów Wielkopolski (Western Poland)- 52° 46'50.6" N, 15° 14'29.1" E. A minority (>10) of them were very dark- their skin and eye colour was black and this coloration did not change over half an hour. Their

Figure 1. Freshly metamorphosed common toads, normally coloured (left) and unusually dark (right)

behaviour was not different from that of normally-coloured toadlets and no pathological changes were seen. Histological samples were not obtained but photos were taken. A year later (11th June 2012), at the same location, another group of unusually dark freshly metamorphosed common toads was observed and photos of specimens were once again taken. In the following years, after the quarry had been shut down, no other abnormally dark specimens were observed at that site.

It is suggested that the quarry may have been using chemicals that contaminated the water and caused mutations in the common toad eggs and/or adults. The disappearance of these black toads may be due to the fact that this coloration is not adaptive. Melanistic reptiles may gain thermal advantages from their coloration but this would not appear to be an advantage for toads as they do not bask in sunshine, preferring to remain at relatively low temperatures. Melanism or axanthism could be advantageous to toads that live on black backgrounds as this would provide camouflage but the habitat of this toad population did not appear to offer black backgrounds and so instead the black toads may have been at a disadvantage being more easily seen by predators.

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