



# The Herpetological Bulletin

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# THE HERPETOLOGICAL BULLETIN

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**Front Cover:** Adult female of *Salamandra algira splendens* observed near caves at Al Hocéima, Rif Mountains, northern Morocco, 2018. See article on page 19. Photograph by Axel Hernandez.

# The behaviour of recently hatched Tobago glass frog tadpoles

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**ABSTRACT** - Egg clutches of the Tobago glass frog *Hyalinobatrachium orientale tobagoense* were collected from streams around the north-east of Tobago and hatched into tanks of native stream water. Previously unreported behaviour of newly hatched tadpoles was observed. Observations were made through a series of tests for substrate preference (gravel, rocks or leaves), shelter or open water preference, and surfacing behaviour under different depths and turbulence levels. Tests found that tadpoles showed a preference for stream substrates over plastic tank floor, with the highest percentage of tadpoles found in gravel. Tadpoles preferred sheltered areas of the tank, and surfaced significantly less when water was turbulent, with depth having no significant effect on surfacing behaviour.

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## INTRODUCTION

The Tobago glass frog, *Hyalinobatrachium orientale tobagoense* is a subspecies endemic to the island of Tobago (Jowers et al., 2014). They reproduce by laying a clutch of gelatinous eggs on the underside of broad leaves overhanging streams, a mode of reproduction which is characteristic of all *Hyalinobatrachium* species (Hoffmann, 2010). The embryos develop on the leaf until hatching when they drop into the stream below where they develop further, eventually emerging as metamorphs. Nokhbatolfighahai et al. (2015) and Downie et al. (2015) reported on embryonic development, hatching, and tadpole growth and morphology in *H. o. tobagoense*. Initially lacking pigmentation, the tadpoles develop pigment over much of the body, darker on the dorsal side, but lacking on the tail fins and ventral body. The tadpoles' eyes are dorsal and very small, and they have very long tails, 64% of total length. Growth, at least under well provided laboratory conditions, is very slow: after six weeks, tadpoles had tripled in mass, increased in length by a little under 40%, but were still at Gosner (1960) stage 25, with no sign of limb buds. Hoffmann (2010) also reported very slow growth and development in two glass frog species which took well over 200 days to reach metamorphosis.

By contrast, several species of pond-dwelling tadpoles in Trinidad can reach metamorphosis in less than 20 days (Downie, 2013). The elongated tadpoles of glass frogs are adapted to a fossorial lifestyle, presumably to escape the rigours of currents and predators in streams (Haddad & Prado, 2005; Hoffmann, 2010). They hide superficially in sediment and among rocks, gravel and fallen leaves, and escape with bursts of fast swimming when disturbed. Given their secretive nature, it is not surprising that the behaviour of these tadpoles is poorly known. The tadpoles grown by Downie et al. (2015) were never observed in the water column and were 'only retrieved after removal of all the rocks and gravel'. However, in preliminary experiments, recently hatched Tobago glass frog tadpoles were observed darting quickly to the water surface and back down again.

In this paper, we report on glass frog tadpole surfacing behaviour in relation to water depth and turbulence. We also look at tadpole substrate preference when faced with a choice between three kinds of natural substrate and an empty plastic surface.

## MATERIALS AND METHODS

All research and collections were conducted under a Research Permit granted by the Tobago House of Assembly, Department of Natural Resources and the Environment, to carry out research across north-east Tobago from June 09 2014 until August 31 2019, on *H. orientale* and other local amphibian species. Fifty six *H. o. tobagoense* egg clutches were collected from transects along five small forest streams in the north-east of Tobago over eight weeks from June to August 2016. The section of leaf on which the clutch lay was cut and stuck clutch side down in a petri dish, with damp cotton paper below it. Once brought back to the laboratory, clutches were stapled through the leaf clutch side down, to a cover on the lid of a clear plastic aquarium tank (29 cm × 18 cm × 16.5 cm) with a petri dish filled with native stream water sitting directly below to collect the tadpoles upon hatching. The tanks were kept in dark closed cupboards at room temperature (27–28 °C). Ten of the clutches were used in pilot studies, and observational tests carried out on the remaining forty six as explained below. The tests were carried out in a room at 27–28 °C with no artificial lighting, during daylight hours.

Once 10 tadpoles out of a clutch had hatched into the bottom of the petri dish, they were transferred to one of two clear plastic tanks (29 cm × 18 cm × 16.5 cm). These tanks contained native stream water, collected on the day of hatching, to a depth of 12 cm, except when the experiment required a different depth. The water was not aerated for any tests apart from the turbulence test. The tank was prepared for the test before the 10 tadpoles were transferred, and the tadpoles were left to acclimatise to these experimental conditions for 24 hours, with observations beginning at 08.00 h the next morning. All tadpoles and any remaining clutch material or undeveloped embryos



were released back to the stream they were collected from after the observations.

For surfacing behaviour, the number of times the surface of the water in the tank was breached by any of the tadpoles was counted for a ten-minute period. After a five minute interval to minimise observer attention drift, this was repeated six times, so 60 minutes of tadpole behaviour was recorded per clutch over 90 minutes. For these observations, we used an empty tank substrate. These observations were made at two different water depths, 6 cm and 12 cm, and under two conditions: calm water at 12 cm and turbulent water at the same depth. Turbulence was created by aerating the water using pipes connected to two Resun air pumps AC-9602 producing an airflow rate of 120L/H. The tubes attached to the pumps were inserted to the tank and held underwater, 3 cm from the bottom at either end. The pumps created a steady flow of water within the tank, with air bubbles rising to the surface. The tests were carried out on six groups of 10 tadpoles, each from different clutches.

For substrate preference, the positions of the tadpoles were recorded at 10 minute intervals for one hour. Each test was repeated six times, each repeat on a different ten tadpoles from a new clutch. To test which substrate tadpoles preferred out of gravel, leaves and rocks from their native streams, half of the floor of the tank was covered with one of the different substrates, while the other half was left empty. At 10 minute intervals, the number of tadpoles in open water was recorded, and the number of tadpoles positioned in or on the substrate was deduced from that number. This method was necessary as the tadpoles are highly transparent and difficult to see against a substrate, and to disturb the substrate to find them would disturb the tadpoles and likely cause them to move to another part of the tank. For this reason also, it was not possible to give the tadpoles a four way choice of substrates at one time. Each test was carried out on six groups of 10 tadpoles, each from different clutches.

To test whether tadpoles chose to position themselves under shelter, half the bottom of the observational tank was covered using 6 cm high opaque half pipes. At 10 minute intervals over one hour, the number of tadpoles in the open was counted, allowing the number under shelter to be determined as the remainder. The test was carried out on six groups of 10 tadpoles, each from different clutches.

Data were analysed using R software, version 1.0.44 (2016-11-30) with RStudio. ANOVAs and Confidence Intervals were used to test for significance in tadpole behavioural tests. Each model was run simply with just the explanatory variable in question and compared using the LogLik function with more complex models including other explanatory variables to test which model fit the data best. For tests with random effects the lme4 package was used.

## RESULTS

### Substrate Type

The most preferred substrate was gravel (95% CI= 0.952 to 0.999; n=6), then rocks (95% CI= 0.006 to 0.6264; n=6) and then leaves (95% CI= 0.0566 to 0.9601; n=6). When gravel, leaves and rocks are grouped as one explanatory variable, tadpoles were found significantly more on one of these substrates than on the empty tank floor (95% CI= 0.9534 to 0.9986). The overall percentage of tadpoles

found in gravel was 97.8%, in rocks was 96.7% and in leaves was 86.9%.

### Shelter Preference

Tadpoles were found significantly more under shelter than in open water (95% CI= 0.5742 to 0.6739; n=6). The mean percentage of tadpoles found under shelter was 62.5%. The majority of the sheltered tadpoles were located within the narrow gap between the two shelter components. This was recorded as an observation at the time of the experiment, but no precise figures were recorded.

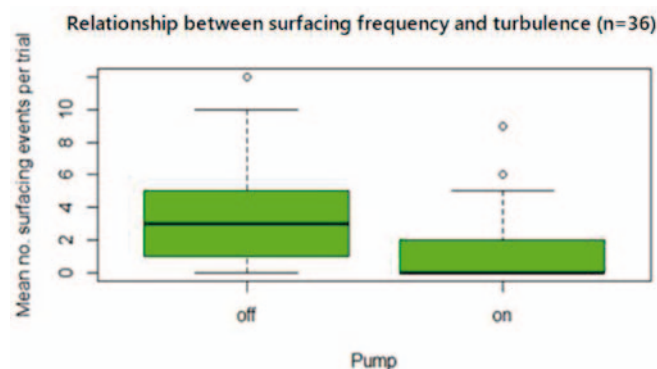
### Surfacing Behaviour: Turbulence Tests

A “surface” was recorded when a tadpole swam from the bottom of the tank to the surface of the water. The tadpoles swam fast, taking 1-2 seconds to get from bottom to surface. The majority of tadpoles would immediately dive down after surfacing, but it was observed on occasion that some would “bob” along the surface before diving back down.

The frequency of tadpole surfacing events decreased significantly when the pumps were on in the tank (mean = 1.36 +/- 1.51SD number of surfacings per trial with pump on; mean = 3.33 +/- 1.77 SD number of surfacings per trial with pump off;  $F_{1,51} = 12.7294$ ,  $p = 0.0007$ ,  $R^2 = 0.46735$ ; n=6).

### Surfacing Behaviour: Depth Tests

Depth was found not to have a significant effect on tadpole surfacing frequency ( $F_{1,55} = 0.3901$ ,  $p = 0.5345$ ,  $R^2 = 0.2319$ ; n=6). The mean number of surfaces per group of 10 tadpoles, per 10 minute observation period, was 2.86 +/- 2.2SD at a depth of 6 cm, and 3.53 +/- 2.3SD at 12 cm. (Fig. 1)



**Figure 1.** The differences in tadpole surfacing frequency with pump on versus pump off. ‘Trial’ here means 10 minute observation period. Clear circles represent outlying data points. Upper and lower horizontal lines represent minimum and maximum values. The median value is represented by the bold black line, within the green box which shows the interquartile range.

## DISCUSSION

Because of the small transparent nature of *H. orientale* tadpoles, we used binary choice tests for substrate preference, establishing a strong preference for rocks, gravel or leaves over an empty plastic substrate, with some evidence of a preference for rocks or gravel over leaves. It would be inappropriate to assess the significance in

differences between the three natural substrates, given that the choices offered were binary. Hoffman (2010) reported that most *Hyalinobatrachium* species hide between rocks, stones and coarse river gravel, according with our results. As is commonly the case (Altig et al., 2007), the *H. orientale* tadpole diet is unknown, but their preferred habitat along with their rasping mouthparts (Downie et al., 2015a) suggests biofilm grazing.

It was interesting that, although there was a preference for shelter over lack of shelter, this was not as strong as in the substrate tests. This may have been because of the empty plastic substrate available for both choices. It was noticeable that most of the sheltered tadpoles were not located simply under the shelter, but in the narrow gap between two shelter components, suggesting that the strong preference is for a narrow space. This also suggests that the shelter provided for the tests may not have been perceived as shelter by the tadpoles. Hoffman (2010) describes centrolenid tadpoles as using their flexible bodies and tails to sequester themselves into narrow cracks between stones and rocks, similar to the narrow crevice between the two half-pipes.

Some species of tadpoles swim to the water surface to fill their buccal cavity with air, then quickly swim back down again (Feder & Catherine, 1984). Tadpoles are sensitive to environmental conditions, and alter their behaviour in response to stressors; for example, between a bare, lit environment, compared to shade or partial shade (Michaels & Preziosi, 2015). This response can be manifested by decreasing activity or hiding (Semlitsch & Reyer, 1992). It was initially a surprise that *H. orientale* tadpoles surfaced at all, given their secretive nature and habitat, running water, where oxygenation should be high. However, given that surfacing was observed we expected that *H. orientale* tadpoles would surface less in deeper or turbulent water, as energy expenditure and risks of predation or being swept downstream would be higher. Surfacing frequency, however, was not different at depths of 6 cm compared to depths of 12 cm. Perhaps a bigger depth variation might show a difference, but in the shallow streams where these tadpoles live, the depth variation we used is relevant to real life conditions.

We expected that tadpoles would surface less when the pump was on and creating turbulence in the tank, as it would simulate a faster flowing stream which could sweep tadpoles downstream. This result was observed during the behavioural tests. In streams, *H. orientale* tadpoles may use a form of flow sensing, similar to *Xenopus laevis* tadpoles which use their lateral line organs to sense their environment (Simmons et al., 2014), and alter their behaviour, in this case surfacing, accordingly. The heterogeneous environments which *H. orientale* inhabit are heavily affected by abiotic factors, such as increased turbulence, flow and depth of stream water from rainfall, and these may impose selective pressures on *H. orientale* tadpole behaviours which are not yet well understood. These pressures may result in the behavioural flexibility seen in the surfacing frequency tests, and these tests provide a baseline of previously undescribed behaviours for tadpoles of this species. It is possible that the difference in surfacing between calm and turbulent water reflected differences in oxygenation. We consider it unlikely that the fresh stream water used in our experiments became sufficiently de-oxygenated to make a difference in the calm water test. We did not have access to an accurate oxygen

meter to test this possibility.

Tadpole behaviour is not well studied across all species, and even a basic knowledge of diets is still lacking. Our observations were restricted to newly hatched tadpoles; it would be of value to investigate how behaviour changes as individuals grow larger as they approach metamorphosis. Considerable work is needed on enigmatic groups like Centrolenidae (McDiarmid & Altig, 1999), and there are very few data available on the natural history of *H. orientale* (Lehtinen & Georgiadis, 2012). Understanding the trophic status of tadpoles and their ecological roles is especially important in light of the current and rapid global amphibian decline. Knowledge of their natural histories and trophic interactions are increasingly required for effective conservation and predictions of the consequences of their possible losses, and to determine their functional roles within the ecological communities they inhabit (Altig et al., 2007). The behaviours and preferences described in this report are previously undocumented for the tadpoles *H. o. tobagoense* and, to the best of our knowledge, for other *Hyalinobatrachium* species. It is hoped that these insights may shed some light on the stream microhabitats which are crucial to glass frog development.

## ACKNOWLEDGEMENTS

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# Seeing the heat with inexpensive thermography: natural history observations on the northern viper (*Vipera berus*) and grass snake (*Natrix helvetica*)

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**ABSTRACT** - The advent of thermal imaging (TI) cameras that attach to smartphones has dramatically reduced the cost of thermography. TI cameras are now within easy reach of naturalists and this has given an opportunity to field test one model, the FLIR ONE, on grass snakes and northern vipers. The potential of the camera to provide useful insights into snake thermal ecology is demonstrated in six short case studies; a further three case studies highlight some practical constraints. The main constraint was that although the camera contributed very precise temperature measurements, to 0.1°C, individual measurements did not always correspond to independently measured values. Nevertheless, the temperature differences between subjects were well maintained, even following automatic recalibration of the camera. Consequently, the temperature differences between subjects in the same sequence of thermographs are reliable and the case studies demonstrate that these can be very informative. For example, they show the patchy heat distribution across the artificial refuges used in reptile monitoring; the thermal imprint of a grass snake resting below a refuge; and the warming of a female viper basking on an ant hill, where the rate of warming seemed to differ between the front and back end of the snake. The camera was fun to use and there is still great potential for thermography to reveal the thermal secrets of reptiles in natural environments and as an adjunct to captive husbandry.

## INTRODUCTION

Measurement of the infrared (IR) radiation emitted from reptiles is a convenient and non-invasive way to estimate body surface temperatures. Such measurements could be made using either IR thermometer guns or thermal imaging (TI) cameras. Most previous field studies have used IR thermometers as they are relatively inexpensive, usually costing less than fifty pounds. In contrast, until recently TI cameras have cost thousands of pounds. With the advent of TI cameras that attach to smartphones, the cost barriers have been lowered to a few hundred pounds so their use is now within easy reach of many naturalists.

TI cameras have a two dimensional array of detector elements that create a recognisable thermal image (thermograph). In contrast, an IR gun is equipped with a single detector element that senses IR radiation emitted from a very small spot on the surface being tested, equivalent to one pixel in a thermograph. Consequently, thermographs show a great deal more information about the temperature of both the animal and its immediate environment. IR guns must be used relatively close to the subject of study as they are constrained by distance to spot size ratios that typically range from 8:1 to 12:1. This ratio is an optical characteristic of the system and at 8:1 the thermometer will sample the temperature from a 1 cm diameter target when it is 8 cm away. If IR gun is moved further away then a greater area is sampled, potentially including temperatures from more than just the subject of study. TI cameras generally have somewhat greater distance to spot size ratios and so can be used at greater distances from the subject. However, manufacturers do warn that the accuracy of the temperature readings is reduced with increasing distance from the subject as infrared radiation may be attenuated by atmospheric absorption.

Now that TI cameras are available at relatively low cost, an opportunity was taken to test one model, the FLIR ONE, to see what natural history observations could be collected on the northern viper (*Vipera berus*) and grass snake (*Natrix helvetica*). Nine case studies were developed to show the use of the camera and the interpretation of thermographs in different field situations. The first four case studies deal with artificial refuges and snakes beneath them, two are devoted to snakes in the open, and finally there are three focusing on some of the constraints when using the FLIR ONE.

## METHODS

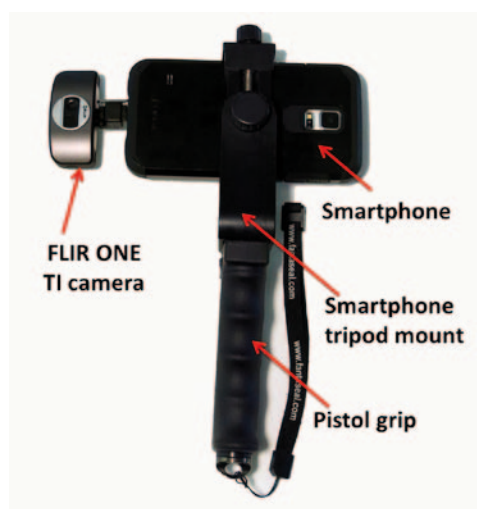
General methods are presented in this section while methods specific to a particular case study are dealt under the relevant heading.

### TI camera

The TI camera used in this study was the 'FLIR ONE TM' version 2 (FLIR Systems, Inc., USA). This was attached to the micro USB port of a Samsung Galaxy Note 5 android smartphone. For ease of use in the field the smartphone was clamped to a tripod mount and camera pistol grip (Fig. 1).

The FLIR ONE comprises a thermal imaging camera (FLIR Lepton 3) and a light camera (VGA 640 x 480). On taking a picture both cameras are activated and the resulting images are combined by MSX TM technology. The advantage of this approach is that the combined image is much more recognisable than a thermal image alone. To create this contrast, it is the negative of the light image that is combined with the thermal image; this creates an interesting effect as the bold patterns of snakes will appear





**Figure 1.** FLIR ONE thermal imaging camera attached to a smartphone that is held in a tripod mount bolted to a camera pistol grip

in negative (i.e. dark where it is normally light and vice versa). An app in the smartphone is required to operate the FLIR ONE. Two are available, one from FLIR can be downloaded free of charge or the other, the Thermal Camera plus which is FLIR approved, can be purchased. Both were used during this study. For manipulating the thermographs on the smartphone, or more easily on a computer, FLIR Tools can be downloaded for free. In FLIR Tools it is possible to toggle between the light and thermal images or adjust the balance between the two. Also when using FLIR Tools it is possible to display 'spot temperatures' (in effect similar to IR gun readings) by adding cross hair sights. At the intersection of the cross hairs there is a circle that shows the area from which a temperature estimate is being taken and gives a rough indication of the distance to spot size ratio; believed to be close to 40:1. In the current tests, the smallest targets reported had diameters typically of 1.5 cm to 3 cm and were photographed at a distance of 50 to 70 cm to avoid distance to spot size ratio issues. Spot temperatures were selected carefully in FLIR Tools by using the cursor keys to move the cross hairs over the chosen subject area to the location returning the highest temperature. Besides spot temperatures, in FLIR Tools it is possible to delimit areas by drawing ellipses or boxes on the thermograph for which average (mean), maximum, and minimum temperatures are displayed.

The Lepton 3 camera has a 160 x 120 detector array. Its scene temperature range is stated as -20°C to 120°C and operating temperature range 0°C to 35°C. It provides thermographs that resolve temperature differences as small as 0.1°C. The manufacturer suggests an accuracy of temperature measurement of  $\pm 2^\circ\text{C}$  or 2%. The accuracy of the temperature measurement is dependent on the unit's calibration and from time to time the FLIR ONE will recalibrate automatically. The camera will appear to freeze for a moment while it does this as the Lepton's mechanical shutter is closed to create a dark frame to calibrate against. IR radiation is invisible to the human eye but not to the camera's sensors, which record variations in IR intensity and interprets these as different temperatures. The thermograph is formed by allocating different colours to different temperatures. Both in the apps and in FLIR tools a variety of colour palettes can be selected to give colour

ranges that most suite the purpose of the study. It is also possible to select a 'saturation' palette in which only those parts of the image either above or below a temperature limit are coloured. Unless otherwise stated, images are displayed in 'Rainbow HC' palette.

### Emissivity adjustment

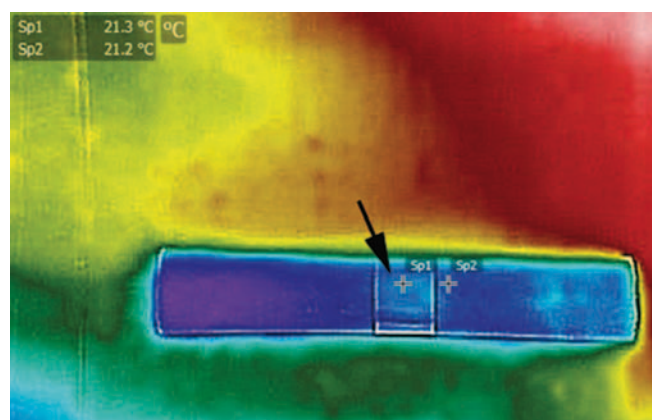
The emissivity value of a subject expresses the proportion of radiation emitted, other IR might be transmitted or reflected; a perfect emitter (black body) has an emissivity of 1. The more accurately the emissivity of a subject is known the more accurately its temperature can be estimated. If emissivity is set too high for the subject then the observed temperature in the thermograph will be too low and vice versa. The FLIR ONE is set at a value of 0.95, an appropriate value for reptiles (Tracy, 1982; Tattershall et al., 2004). However, if subjects do not have an emissivity of 0.95 then this default is adjustable during processing in FLIR Tools.

### IR thermometer gun

Comparisons were made between thermograph temperatures and those recorded by an IR gun (Foxnovo DT8380); the same unit as used by Hodges & Seabrook (2016a). In brief, this IR gun had a measurement range of -50°C to +380°C, a distance to spot size ratio of 8:1, and a resolution of 0.1°C. Emissivity is fixed at 0.95. A clear plastic tube, 1.8 cm long and 1.8 cm wide, mounted on the front of IR gun acted as spacer from the subject. All measurements of snakes were made at the mid-body. A calibration curve for the IR gun had been prepared using a viper cadaver and laboratory calibration thermometer (Hodges & Seabrook, 2016a).

### Observations under refuges

Many of the temperature measurements were made of vipers or grass snakes under refuges of galvanised corrugated-iron sheets (0.5 mm thick and 0.5 g/cm<sup>2</sup>) camouflaged by spraying their upper surface with brown paint (Espresso, satin finish, Rust-oleum), referred to as tins, or roofing felt (Garage felt, green slate finish, Homebase, #242805, 2 mm thick and 0.3g/cm<sup>2</sup>). They were both cut to the same dimensions (50 cm by 65 cm) and pairs, one of each type, were placed together in sunny locations. The operational temperatures to which the snakes were exposed under the



**Figure 2.** Testing the emissivity of the physical model by the application of a strip of black insulating tape (arrow) of 0.96 emissivity. The thermograph was taken at the default emissivity of 0.95. The tape (Sp1 = 21.3°C) was fractionally hotter than the adjacent areas of the model (Sp2 = 21.2°C) indicating an emissivity of 0.95 for the model surface.



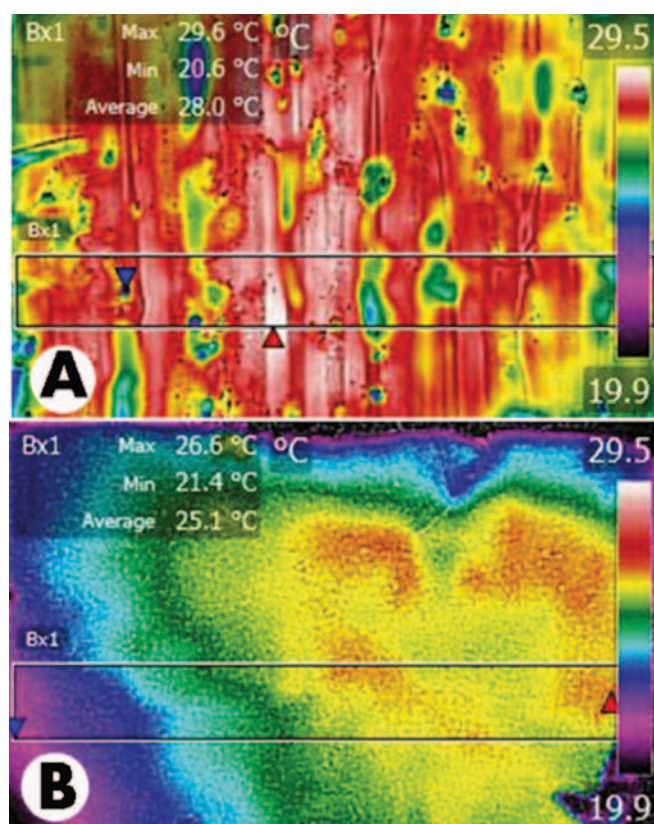
refuge tins were estimated using physical models (Hodges & Seabrook, 2016a). These consisted of copper pipe (ID 20 mm, wall 1mm thick, length 150 mm) sprayed with grey paint (Surface primer, matt, Rust-oleum). Such models have similar thermal properties to small snakes (Peterson et al., 1993). By indicating the temperatures available to snakes below the refuges they serve as null models for quantifying the extent of thermoregulation. To determine the emissivity of the tin and roofing felt refuges, and the physical models, a check was made by testing them against black tape, Scotch Brand 33 black vinyl electrical tape with known emissivity of 0.96 (FLIR, 2015). The estimated emissivity values for tins, roofing felt and physical models (Fig. 2) were approximately 0.95. Consequently, no corrections for emissivity were required.

## RESULTS

### Observations of refuges and snakes beneath them

#### *Case study 1 - The heat distribution on refuge surfaces (30 May 2017, 09:34 h)*

Thermographs were taken of 15 pairs of adjacent tin and felt refuges in full sun with the camera held 70 cm away from, and normal to, the subject. In all cases the refuge surfaces showed a patchwork of temperatures, those in Figure 3 are typical and look like a rather nice piece of modern art. The box function in FLIR Tools was used to check temperature ranges across the refuges which varied by 8.0°C for the tin and by 5.2°C for the felt.



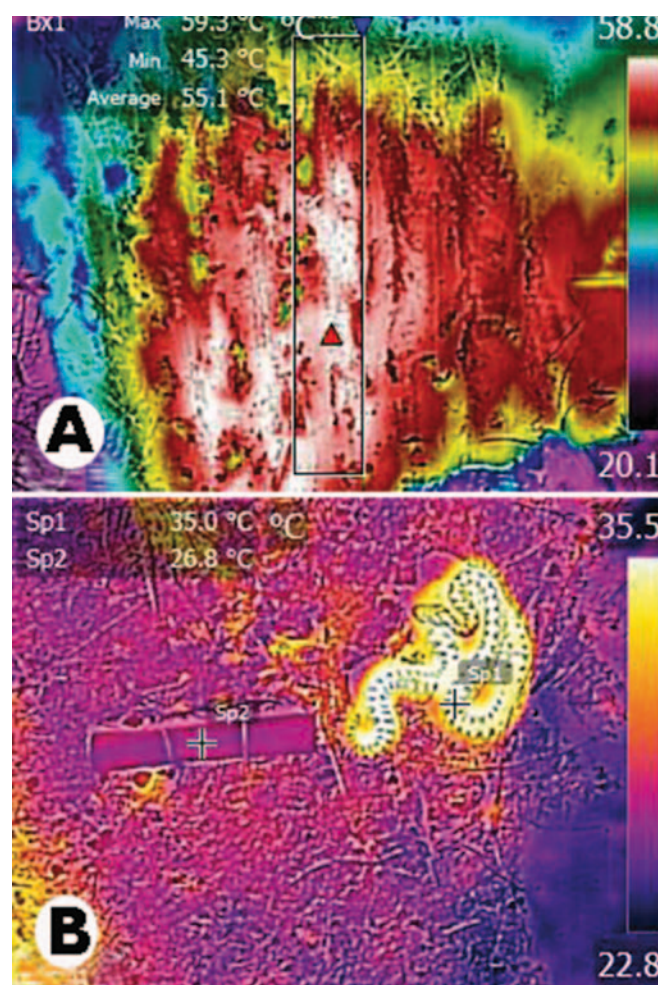
**Figure 3.** **A.** Thermograph of a tin refuge, **B.** Thermograph of an adjacent felt refuge. The boxes on each refuge, drawn in FLIR Tools, return the average (mean) temperature as well as maximum and minimum (indicated by a red and blue triangle respectively)

The pattern of temperatures also varied between tin and felt with tins tending to be more diverse (broken up). This is understandable as being corrugated both the angle of the tin to the sun and contact with the ground below is more variable than for a flat piece of roofing felt. The variations in refuge temperature are clearly an advantage to any reptile attempting to thermoregulate as there are different temperatures to choose from. This is demonstrated in the next case study.

#### *Case study 2: Female viper and physical model under a refuge (20th August 2017, 10:48 h)*

A gravid female viper was observed sheltering under a tin, beneath which there was also a physical model. During a period of two hours the refuge was in full sunlight, after which, at a distance of about 60 cm and normal to the subject, a thermal image was taken of the tin. The tin was then lifted and a further image taken of the physical model and viper.

The refuge showed a typically varied pattern of temperatures and across a central section ranged from 45.3°C to 59.3°C with a mean of 55.1°C (Fig. 4A). Under the refuge the physical model and viper showed quite different temperatures from both the tin and from



**Figure 4.** **A.** Thermograph of tin refuge with a box showing maximum (red triangle), minimum (blue triangle) and the average (mean) temperatures, **B.** Beneath the same refuge an adult female viper and physical model. This image is in 'iron palette' to emphasis the fact that it has a different temperature span (22.8°–35.5°C) from 4A. Note the viper markings are displayed a negative image (see 'Methods' for explanation).



each other (Fig. 4B). The model at 26.8°C was similar to the temperature of the ground below the refuge while at the mid-body the viper temperature was very much higher at 35.0°C. However, the viper was not actually as hot as suggested in the thermograph as measurement at the mid-body using the calibrated IR thermometer gun indicated a temperature of 29.4°C. Differences between the thermograph temperatures and those returned by the IR gun are dealt with in Case study 8.

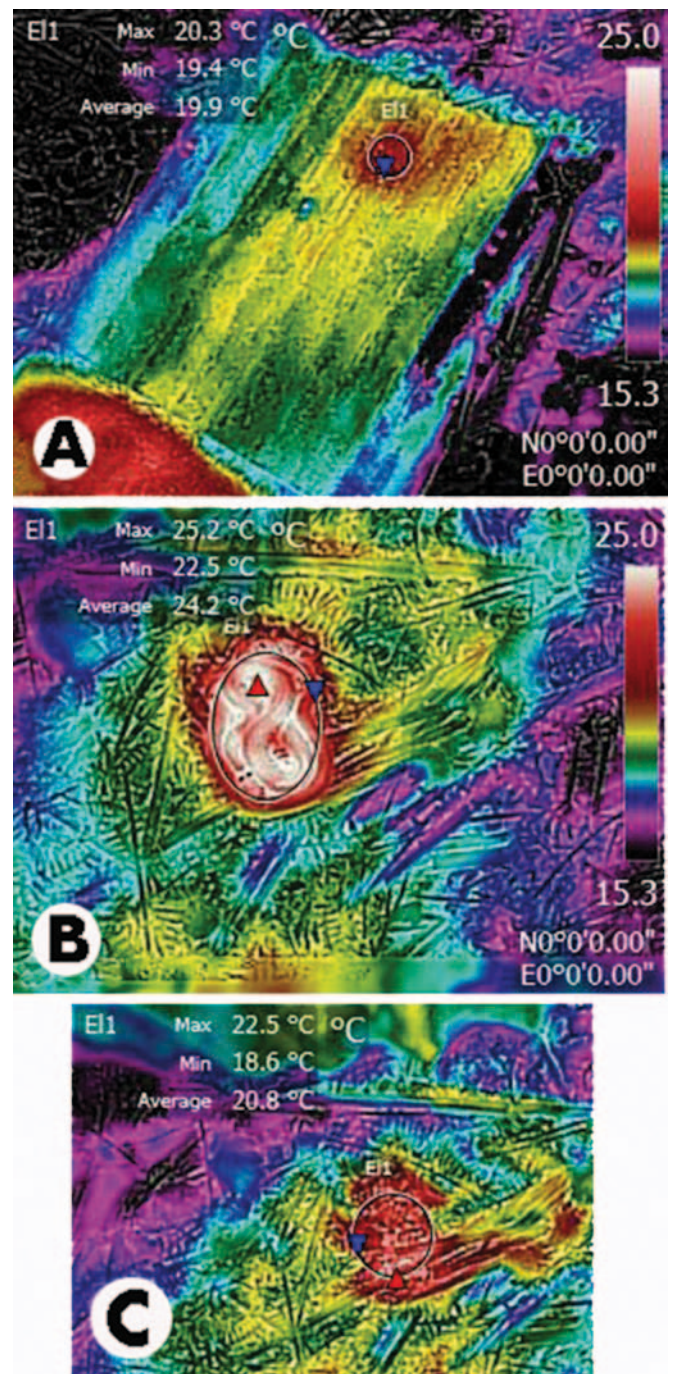
The physical model is in a fixed position and its temperature is determined by its position below the refuge; different locations would probably have returned different temperatures. In contrast, the viper is free to move around to locate the most thermally beneficial position and alter its body posture to either increase or slow down the rate at which heat is gained or lost. Consequently, the viper was much warmer than the model. At 29.4°C the viper is still some way below its upper thermal set point of around 32°C (Hodges & Seabrook, 2016a) and so is not in danger of overheating despite the high temperature of the refuge above it. Perhaps the relatively low temperature of the ground prevented it reaching the upper thermal set point.

### **Case study 3 - Thermal imprint of a grass snake (18th May 2017, 12:52 h)**

During an overcast period, a thermal image was taken at 70 cm and normal to a tin refuge. The surface of the tin had a distinct small warm patch with mean temperature of 19.9°C (Fig. 5A). The tin was lifted and two further images were taken at 50 cm. The first was taken ten seconds after lifting the tin and immediately below the tin's warm patch was a grass snake; it had a mean body temperature of 24.2°C (Fig. 5B). The second was taken five seconds after the snake had departed and was a warm patch on the dry bracken where the snake had been resting; it had a mean temperature 20.8°C (Fig. 5C).

It is interesting to note that the precise position of the warm patch on the tin corresponded with the position of the grass snake below and likewise the dried bracken below the snake was also warmer than that surrounding it. It would appear that the warm patches on the tin and vegetation were the thermal imprints of the grass snake. The tin would have warmed up in earlier sunshine but when the sky became overcast would have started to cool down. The rate of cooling of the tin would have been faster than the grass snake below because corrugated iron has a lower specific heat (about 0.45 cal/g-°C) than a grass snake which is mostly water (1 cal/g-°C). Consequently, the thermal inertia of the grass snake appears to have maintained a warm patch on the tin. It is perhaps unexpected to find a reptile warming the refuge above it. In overcast conditions warm patches on tins might be reasonable indicators of reptiles below although this is unlikely to be a practical monitoring procedure.

Grass snakes have an upper thermal set point of about 31°C (Gaywood, 1990), consequently the specimen in this example (mean body temperature 24.2°C) would probably seek a warmer position if one was available (see Case study 4). Until it was disturbed, the grass snake had maintained its position despite losing heat. The thermograph shows the snake to be at the warmest point in a thermal gradient. It is not known what behavioural cue, e.g. reaching a specific low body temperature or rate of temperature decline, would eventually have led it to move away from the tin to seek warmth elsewhere.



**Figure 5. A.** Tin refuge with a small warm patch (mean 19.9°C), **B.** A grass snake coiled below the warm patch of the tin (mean 23.6°C), **C.** Five seconds after the grass snake had departed showing a warm patch on dried bracken (mean 20.8°C). Ellipses have been drawn on images to return average (mean), maximum (red triangle) and minimum (blue triangle) temperatures.

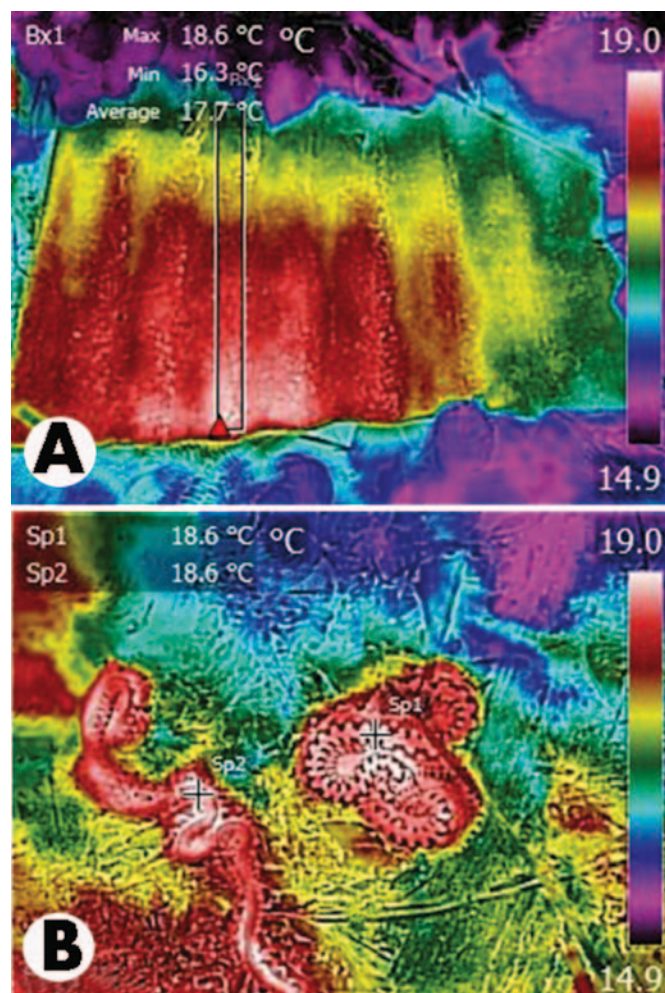
The next case study considers an observation of a viper and a grass snake below the same tin refuge.

### **Case study 4: Male viper and a grass snake under the same refuge (31st July 2017, 11:08 h)**

A refuge tin in dappled sunlight (Fig. 6A) was found to be sheltering both a grass snake and a viper (Fig. 6B). Thermographs of both tin and snakes were taken at 60 cm and normal to the subject. The snakes were directly below the warmest part of the refuge which the thermograph showed was at 18.6°C (Fig. 6A). Interestingly, the hottest



spot temperature for each of the two snakes was also 18.6°C (Fig. 6B). Measurement of the viper temperature at the mid-body using the IR thermometer gun indicated 18.4°C. The very close correspondence between the mid-body temperature as measured by thermography and IR gun is typical when body temperatures are in the region of 18°C (see Case study 8).



**Figure 6.** A. Thermograph of tin refuge with a box showing average (mean), maximum (red triangle) and minimum (blue triangle) temperatures. B. Grass snake and adult male viper beneath the same refuge both with maximum spot temperatures of 18.6°C

The grass snake is shown as uncoiled (Fig. 6B) but prior to lifting the refuge it had been coiled up close to the viper. Clearly, under the prevailing conditions both snakes had attained the same, or at least very similar, body temperatures. The variations in temperature shown across the surface of the refuge suggests that there was a choice of different thermal conditions but the snakes had chosen the highest available to them. However, the situation was clearly thermally limiting since the snakes would normally allow their bodies to reach their upper thermal set point of around 32°C for the viper (Hodges & Seabrook, 2016a) and 31°C for the grass snake (Gaywood, 1990). Although both snakes attained the same temperature the rate at which this was achieved may not have been the same due to differences in their abilities to thermoregulate, the northern viper having a more sophisticated behavioural

repertoire (Spellerberg, 1976; Gaywood, 1990; Gaywood & Spellerberg, 1995).

The case studies so far have focused on northern vipers and grass snakes under refuges. The next two deal with observations in the open.

### Observations in the open

It is difficult to take thermal images of grass snakes in the open as they tend to move off rather quickly whereas vipers are more tolerant, especially if you move slowly, stay down wind and avoid casting a shadow over them. In long-term monitoring on chalk grassland there are quite big annual variations in the proportion of northern vipers encounters made in the open, which from 2008 to 2015, ranged from 20% to 60% (Hodges & Seabrook, 2016b).

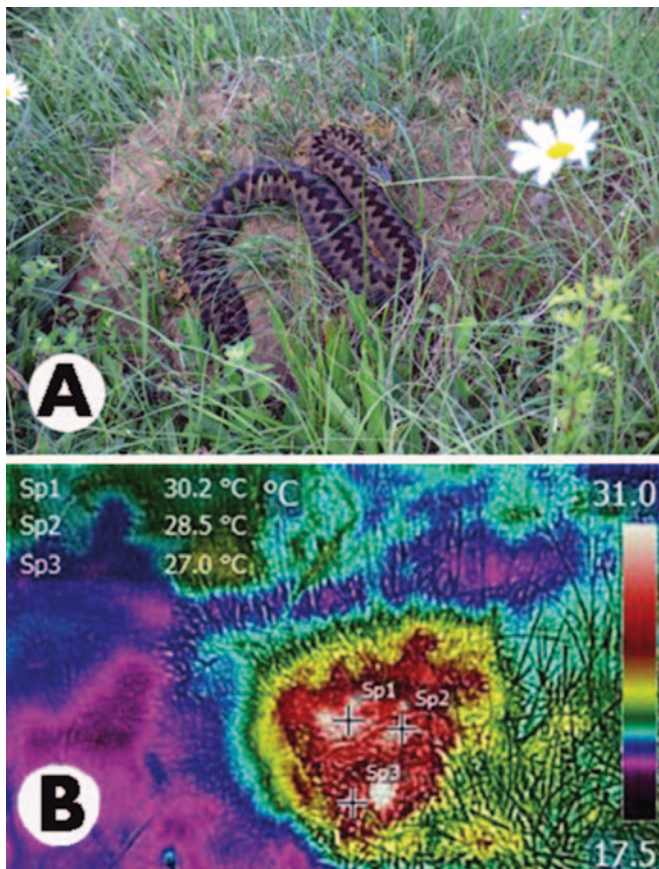
#### Case study 5: Female viper warming on an ant hill (27 May, 2017, 07:48 – 08:06 h)

On a west facing slope, a gravid female viper was observed using the top of an ant hill to warm up in the early morning (Fig. 7A). The ant hill on top was sparsely covered with vegetation so that the substrate was mostly fine soil. This situation offered an opportunity to take a series of shots of the viper as she warmed up in the morning. To do this the TI camera was mounted on a tripod at 75 cm from the surface of the ant hill and adjusted to be normal to the location where the snake habitually sunned herself. The camera and photographer were in place early in the morning before the sun fell on the ant hill, located so that they would not cast a shadow.

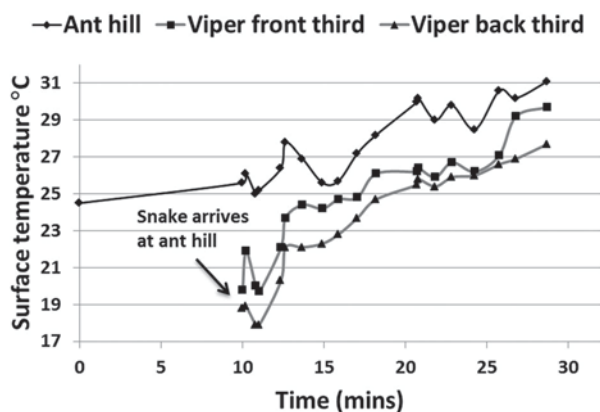
The female viper emerged from the undergrowth at 07:48 h, with a body temperature that appeared to be at about the same temperature as the vegetation at the base of the ant hill, front third and back third of the snake were 19.8° and 18.8°C respectively (Fig. 8). The top of the ant hill already had patches that were much warmer, a box estimate across the surface gave a mean temperature of 24.8°C (max. 30.4°, min 19.6°). By 78 sec after emergence the snake had draped its body across the top of the ant hill (similar to Fig. 7A). The body was flattened and the tail more obscured by vegetation than the rest of the body. Over the period of 18 minutes both viper and ant hill surface warmed up but the ant hill was always a little hotter than the viper (Fig. 7B). The front third of the viper remained warmer than the back third (Fig. 8), so that over the whole observation period the mean body temperature values were 24.7° C and 23.2°C respectively. The rate of warming of the front and back thirds were 0.54°C and 0.48°C/min respectively while in the same period the ant hill surface warmed at only 0.29°C/min but was still warmer than the snake at the end of the observation period due to its head start. The reason for the difference in warming rate between the front and back thirds of the snake could be that the tail was in more dappled sunlight than the rest of the body and/or subtle variations in orientation to the sun between back and front. In any case, it would seem that the blood circulation system did not even-out the difference. It has been shown, at least in the case of the garter snake (*Thamnophis sirtalis*), that when cold the snake reduces blood flow to the tail and significantly increases it to the heads and vice versa when hot (Amiel et al., 2011). As a sophisticated thermoregulator, it seems likely that northern viper may do the same.

So far all the observations on vipers have been on the usual colour morphs. However, colour can affect the rate





**Figure 7.** **A.** Light photograph of a gravid female viper basking on top of an ant hill, **B.** Thermograph of the same viper 25 min 45 sec after the start of thermography basking on the same ant hill (spot temperatures Sp1 – top of ant hill, Sp2 front third of viper, Sp3 back third of viper)



**Figure 8.** Temperatures of an ant hill surface and of the front and back thirds a female viper basking on top of the ant hill (as in Fig. 7)

of heat loss or heat uptake from snakes. The next case study offers an example of this from a black (melanistic) viper.

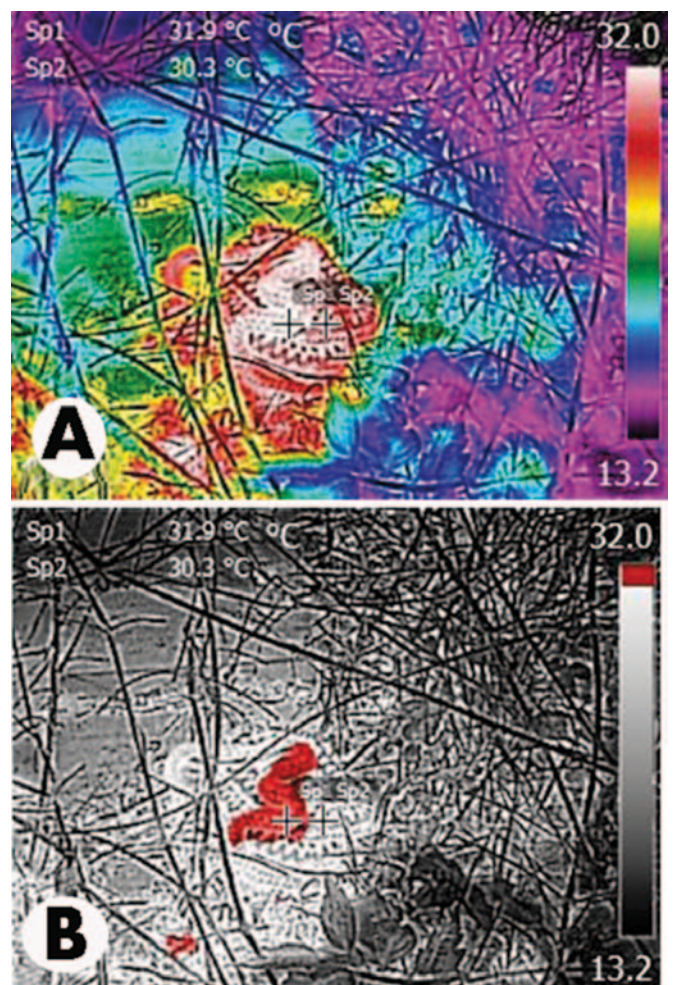
**Case study 6 – Temperature difference between a melanistic and normal morph viper (18 April, 11:28 h)**

In April, a black male viper was observed 'mate-guarding' a normal morph female in the open (Fig. 9); male vipers often remain with females for several days post copulation.

The pair was observed basking together undisturbed for 20 minutes after which a series of thermal images was taken; the closest (Fig. 10) was from 70 cm and at an angle of 45°. The black male viper appears to be 1.6°C warmer than the female (Fig. 10A). This difference can be highlighted using the saturation palette of the TI camera (Fig. 10B).



**Figure 9.** Light camera image of a melanistic male viper mate-guarding a normal coloured female



**Figure 10.** **A.** Thermograph of a melanistic male viper lying on a normal coloured female (shown in Fig. 9), the male is warmer (31.9°C) than the female (30.3°C), **B.** The same thermograph as 10A, but demonstrating the use of the saturation palette which colours only the hottest areas in red (selected to be above 31.1°C)



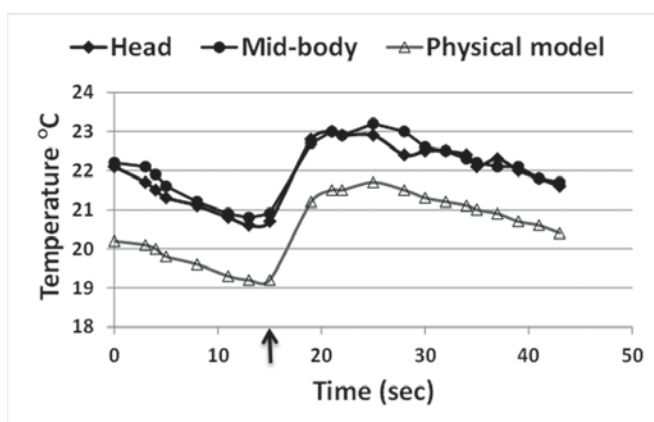
Differences in heating rates between melanistic and normal colour morphs are expected as the black colour would make the animal more efficient at absorbing the wavelengths of light visible to humans. It has been suggested that this results in significant fitness benefits to black vipers which grow faster (Andren & Nilson, 1981; Trullas et al., 2007). However, there is also a disadvantage as black male vipers suffer a greater predation rate (Andren & Nilson, 1981). The faster warming of black vipers has been demonstrated under experimental conditions but was not detected in studies under natural conditions (Forsman, 1995). Unfortunately, the thermograph in our study (Fig. 10) does not contribute to our understanding of the heating rate in the field for two reasons. First, it could be argued that the female was not warming more slowly due her normal colour but due to a larger body volume that would result in greater thermal inertia. Second, being on top of the female, the male was probably being insulated from the cold ground below.

The next three case studies demonstrate some of the constraints when using a TI camera.

#### *Observations on the constraints of using the TI camera*

##### *Case study 7 – TI camera re-calibration*

An important feature of the TI camera is that from time to time it will recalibrate itself. This is explained in more detail in the Methods section. Recalibration occurred during a sequence of shots of a viper beneath a tin refuge and next to a physical model. The camera was about 50 cm away and normal to the subject. This gave an opportunity to examine the temperatures before and after recalibration. During the first 15 sec after lifting the refuge, the temperature of the snake at its head and mid-body and the physical model declined by about 1°C at a mean rate of 0.076°/sec. This was to be expected as heat escaped from beneath the refuge (Fig. 11). The camera then started recalibration which was soon completed so that the next shot was taken at 19 sec. The temperatures of the snake head and mid-body and the physical model were all increased by 2°C in the first shot following recalibration, i.e. more or less retained the differences from each other but gave higher absolute values. Thereafter, the temperatures continued to decline but at a slightly lower rate than before, 0.04°/sec. It seems that the period of most rapid heat loss from



**Figure 11.** TI camera temperature measurements of the head and mid-body of an adult female viper and a physical model below a tin refuge taken over a period of 43 sec. At 16 sec the camera made an automatic recalibration (arrow). The apparent temperatures after recalibration were all increased by about the same amount

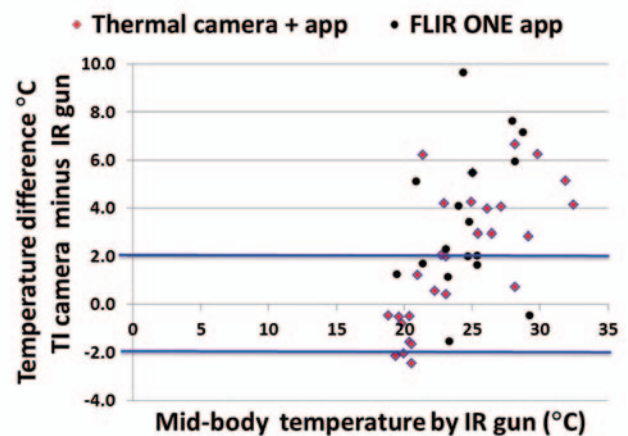
under the refuge was apparently passing and even after 43 sec the temperature was still above the lowest value before recalibration.

This experience with re-calibration shows that the absolute values returned by the thermograph cannot be relied upon but that the relative values correspond well. In other words, when looking for differences between subjects then the thermograph gives a consistent result but the actual temperature of the subject is uncertain.

##### *Case study 8 – Accuracy of the temperature in a thermograph*

Unlike the IR gun, the TI camera was not calibrated for viper body temperature measurement. During the course of 2017, many opportunities were taken to test viper body temperature at the mid-body using the TI camera with a paired IR temperature gun measurement made either before or after the thermograph. Nearly all measurements were taken of vipers found below refuges and the camera was operated using either of the two smartphone apps that are available for the purpose.

There was no systematic difference in the temperatures recorded using the two different mobile phone apps (Fig. 12). However, if the viper surface temperature at the mid-body was below 20°C by IR gun then nearly all corresponding thermograph temperatures were lower than that. Conversely, if the mid-body temperature was above 20°C by IR gun then the corresponding thermograph temperatures were higher (Fig. 12). Only 31% (14) of the temperatures observations fell within the accuracy range quoted by the manufacturers  $\pm 2^\circ\text{C}$  (Fig. 12). The wide variation between IR gun and TI camera suggests there would be little value in preparing a calibration for the TI camera based on this data.

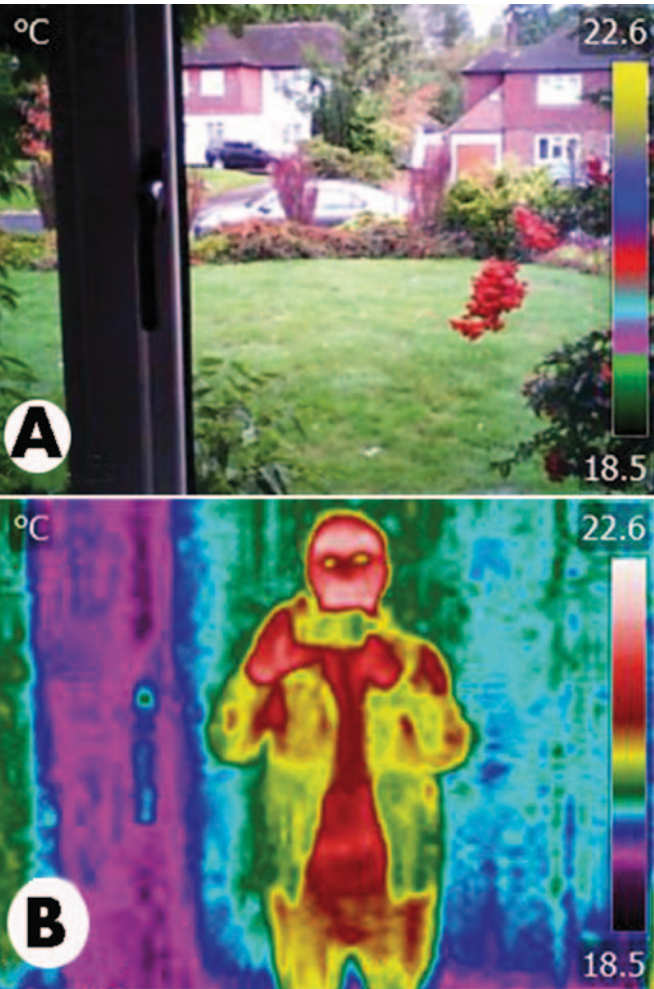


**Figure 12.** Differences between body surface temperatures (°C) of vipers estimated by TI camera and by IR gun, plotted against the body temperature estimated by IR gun. The data were gathered using two different smartphone apps. Values within blue lines show variation expected by manufacturer's specification ( $\pm 2^\circ\text{C}$ ). N = 45

##### *Case study 9 - Effect of reflections on body temperature in a thermograph*

Reflected infra-red radiation could interfere with the temperatures observed in a thermograph. An example of this is a thermograph of a pane of glass. Glass is a good reflector of infra-red but transmits visible light and as a consequence we can see a scene through a glass window (Fig. 13A). But when viewed as a thermograph, which

detects the reflected infra-red radiation, the window shows the temperature of the photographer not a scene through the window (Fig. 13B). Likewise there is the potential for snakes' scales to reflect sunshine so that thermographs taken in sunshine and those in shade may differ in the amount of reflected radiation. The degree to which reflection is a problem could be affected by the angle at which the thermograph is taken; at some angles reflection may be more problematic than at others.



**Figure 13. A.** Light photograph taken through a window, **B.** The corresponding thermograph (not combined with light image) showing the reflected thermal image of the photographer who is wearing glasses so has cool eyes

**Table 1.** Temperature estimates at the mid-body a female viper from thermographs taken five seconds apart in sun and then in shade at four different angles, to examine whether there might be sunshine reflected from scales

Camera angle	In sun	In shade	Difference
90°	28.3	28.7	+0.5
70°	27.1	27.0	-0.1
45°	27.2	26.4	-0.8
30°	26.4	26.1	-0.3

An opportunity was taken to observe whether direct sun on a female viper would affect the temperature estimates of the snake. A thermograph was taken 60 cm from the subject and at a camera angle of 90°. Immediately after, a shadow

was cast across the snake and the thermograph retaken. This process was repeated with camera angles of about 70°, 45° and 30°. The temperature differences recorded in this process were small (Table 1) and suggest that at least for northern vipers sunshine on scales is of limited practical significance. This is perhaps not surprising as viper does not have particularly shiny scales.

CONCLUSION

The FLIR ONE is an inexpensive thermal imaging camera and consequently has a relatively low resolution. Despite this, it has been used to make interesting natural history observation on northern vipers and grass snakes. The case studies have given graphic illustrations of both known and previously unknown temperature effects and put temperature measurement into a broader context. Earlier studies on the thermal ecology of British snakes would have been much enriched had this technology been available in the 1990s (Gaywood, 1990; Vanner, 1990).

The TI camera clearly has one serious limitation; the temperatures shown in thermographs are not often very close to the actual (absolute) values obtained under field conditions with a calibrated IR temperature gun. It is possible that better agreement may have been possible in more controlled conditions, although the lack of agreement has also been the experience of medical researchers using sophisticated TI cameras (Heuvel et al., 2003; Andrade Fernandes et al., 2014; Bach et al., 2015). However, this need not be a serious issue if the interest is in relative temperatures, i.e. temperature differences between subjects. Temperature differences in thermographs have been the basis of previous controlled laboratory studies with reptiles, for example rattlesnake digestion (Tattersall et al., 2004). Such comparisons would still be valid even if the FLIR ONE recalibrates itself in the midst of a series of shots since temperature differences appear to be maintained. The user also has to be aware that the thermograph temperatures may well be impacted by reflections or draughts that could easily raise or lower temperatures and that adjustment is require when comparing subjects of different emissivity (FLIR, 2015). Likewise there are techniques to determine reflectance with suitable crumpled pieces of aluminium foil (American National Standard, 1998) so that reflectance defaults can also be adjusted in FLIR Tools. Another issue is that the temperature of mammals can be affected by stress; consequently taking thermal images of them should be done in a way that minimises disturbance of the subject (Cilulko et al., 2013). It would be interesting to know if the same applies to ectotherms such as reptiles.

The individual animals included in this study were adult or large sub-adults so that they were still of a reasonable size in thermographs taken from 50 cm to 70 cm. Consequently, the distance to spot size ratio issue does not appear to have affected the results. Small immature specimens remain to be tested and this would require the TI camera be brought much closer to the subject to be within the distance spot size ratio. There is no reason to believe that the results in these conditions would be any less valid and for macro shots there is a control in the FLIR ONE app allowing manual adjustment for parallax that would otherwise result in misalignment of the light and thermal images.

An understanding of thermal ecology is essential to the interpretation of reptile monitoring data (Gaywood &



Spellerberg, 1995). Thermal imaging has been a very useful addition to a long-term monitoring programme for northern viper and has the potential to make significant contributions to our understanding of vipers, both at refuges and in the open. When applied to other reptiles, thermal imaging will undoubtedly provide new and interesting insights into behaviour and thermal ecology. When applied to captive husbandry it may also offer a means of assessing and adjusting living conditions. Finally, as thermal imaging makes headway in the consumer market, naturalists may soon have access to even more sophisticated cameras at affordable prices.

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# Life history traits in the northern ring-necked snake, *Diadophis punctatus edwardsii* (Merrem, 1820), in West Virginia

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**ABSTRACT** - To describe the life history traits of the northern ring-necked snake (*Diadophis punctatus edwardsii*), we examined 187 museum specimens collected in West Virginia during 1926–2000. This facilitated geographical comparisons across the species range and will serve as a baseline to detect future changes in life history traits that could result from climate change. Our findings revealed a unimodal distribution of captures during January–October. Such a distribution could arise from unequal sampling effort, nevertheless such a distribution is typical of those species in the mid-Atlantic and north-eastern region of the United States. The male gonadal cycle conformed to the temperate pattern, whereas that of females tended towards a tropical pattern. Females oviposit during April–July, which began earlier than those of surrounding northern states; however, the June peak in captures of gravid females was similar to that found elsewhere in its geographic range. Mean clutch size (4.3 eggs) was similar to those reported from elsewhere in the eastern USA, and mean adult body size of males (26.4 cm SVL) and females (28.9 cm SVL) typified those of northern populations. Age at sexual maturity was similar to that of conspecifics from Pennsylvania. For many females, first clutches occurred at an older age than those in Florida. Our findings corroborated the relative stability of some life history traits as well as geographic variation in other traits. These may be subject to change in response to contemporary and future region-wide changes in climate.

## INTRODUCTION

Twenty-two species of snakes are native to West Virginia, in the USA (Green & Pauley, 1987). The northern ring-necked snake, *Diadophis punctatus edwardsii* (Merrem, 1820), is geographically widespread in West Virginia. Despite the ubiquity of this species, little attention has been paid to its ecology in the state. Access to West Virginia specimens from the holdings of the Carnegie Museum of Natural History and the herpetological collection of Marshall University provided us with the opportunity to examine certain life history traits of the northern ring-necked snake. We recognise that the opportunistic nature of this collection limits assessment of seasonal activity patterns in West Virginia but it enabled us to document seasonal activity and reproduction for comparison with data from other latitudes and to discuss the role of these data as a baseline for future comparisons in relation to climate change.

## MATERIALS AND METHODS

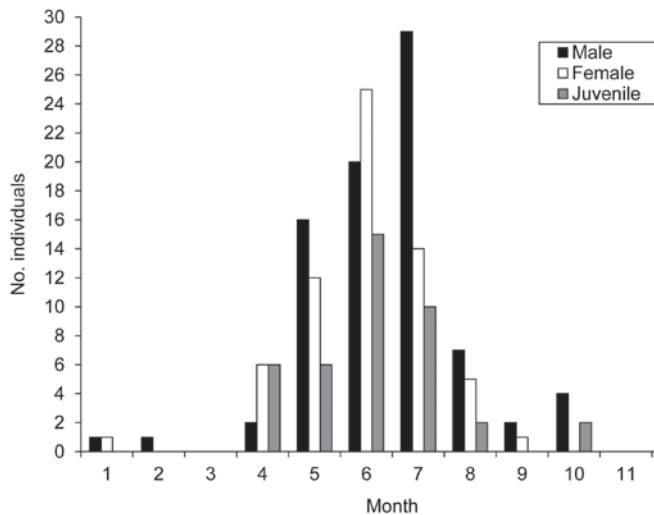
We examined specimens that were captured during 1926–2000 and deposited in Marshall University Museum of Natural History and the Carnegie Museum of Natural History. We recognise that the opportunistic nature of this collection limits assessment of seasonal activity patterns in West Virginia. Body length of each specimen was measured in cm snout-vent length (SVL). Sex was determined by

internal examination of the gonads through dissection. Enlarged testes indicated sexual maturity in males. The length and width were measured and recorded in mm. These measurements were equated into a percentage of the SVL. This percentage was then used as an indicator of fertility and plotted by month on graphs. Methods by Trauth et al. (1994) served as guidelines for size ranges associated with categories of ovarian follicles and oviductal eggs and embryos. Follicles < 2 mm were deemed immature. Vitellogenic ovarian follicles were generally  $\geq 3$  mm.

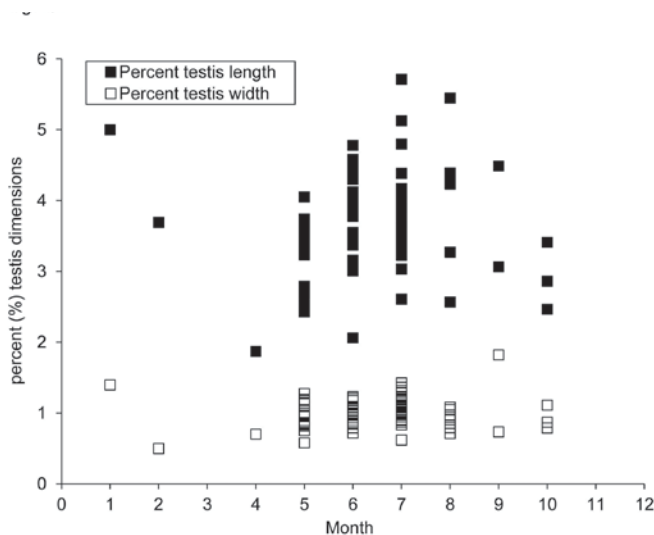
The largest ovarian follicle among those follicles  $\geq 2.0$  mm, yellow in colour, were recognised as vitellogenic and used to provide a measure of monthly follicular growth. The largest oviductal eggs or embryos were measured for a monthly estimate of respective growth rates. Clutch size was estimated by counts of enlarged follicles, oviductal eggs or counts of young. Mean values were followed by + 1 standard deviation. F-tests were performed to test for statistical differences in variances around the means. Two-tailed t-tests were used to assess statistical differences between means, and regressions were calculated to quantify relationships in reproductive characteristics. Statistical analyses were undertaken in Excel, and statistical significance was recognised at  $p < 0.05$ .

## RESULTS

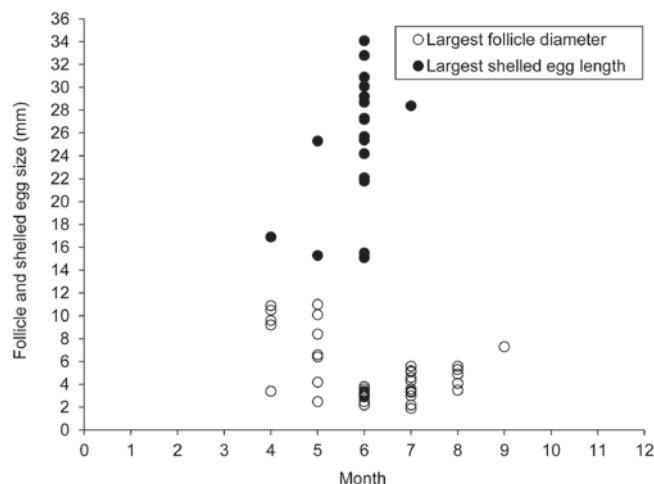
We examined 187 specimens of the northern ring-necked snake from collections made during 1926–2000 in West



**Figure 1.** Monthly incidence of capture of 82 male, 64 female and 41 juvenile northern ring-necked snakes (*D. punctatus edwardsii*) from West Virginia



**Figure 2.** Monthly distribution of testis size as a percentage of male snout-vent length (SVL) of 71 northern ring-necked snakes (*D. punctatus edwardsii*) from West Virginia



**Figure 3.** Monthly distribution of follicle ( $n = 40$ ) and ovum size ( $n = 19$ ) in 59 northern ring-necked snakes (*D. punctatus edwardsii*) from West Virginia

Virginia. Individuals were collected during January–October (Fig. 1). As determined by incidence of capture, a unimodal pattern to seasonal activity was evident in this sample and peaked in June (Fig. 1). A unimodal pattern to seasonal activity was also apparent in each sex- and size-class, with numerical peaks in June (females, juveniles, all individuals combined) and July (males) (Fig. 1).

Monthly distribution of testis size revealed a unimodal peak in both length and width in July (Fig. 2). Females bearing shelled eggs were detected during April–July (Fig. 3). Most gravid females were found in June, concomitant with the smallest ovarian follicles (2.0–3.8 mm) (Fig. 3). Thereafter, largest follicle sizes increased to 7.3 mm in September (Fig. 3).

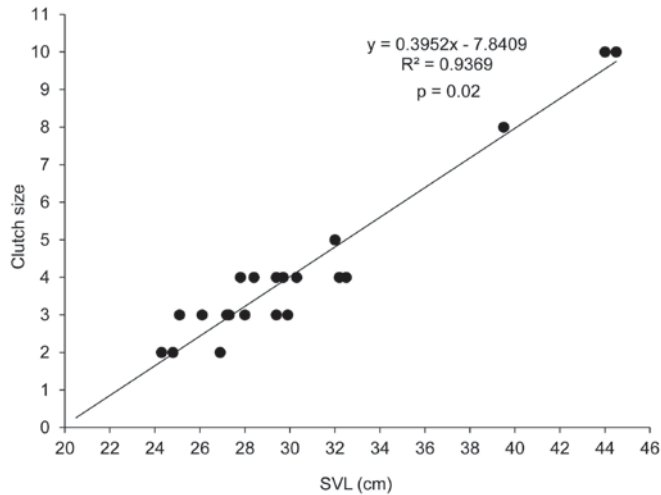
Clutch size as estimated from counts of vitellogenic ovarian follicles (9.2–11.0 mm) averaged 5.2 young ( $+ 2.6$ ; range = 3–9;  $n = 6$ ). Clutch size estimated from counts of shelled eggs averaged 4.1 ( $+ 2.3$ ; range = 2–10;  $n = 22$ ). Neither variances ( $F = 1.308$ ,  $p = 0.30$ ) nor means ( $t = 0.984$ ,  $df = 26$ ,  $p = 0.33$ ) of these two clutch size estimates differed significantly. Combining data from both counts yielded a mean clutch size estimate of 4.3 ( $+ 2.4$ ; range = 2–10;  $n = 28$ ). Clutch size significantly co-varied with female body size (Fig. 4). Shelled egg length ranged 12.5–32.8 mm (mean =  $24.0 + 5.0$ ;  $n = 37$ ). Shelled egg width ranged 4.2–9.0 mm (mean =  $6.0 + 1.3$ ;  $n = 37$ ). Largest shelled egg width, but not length, significantly co-varied with both female body size (Fig. 5) and clutch size (Fig. 6).

Mean adult body size of males (mean =  $26.4 + 4.6$  mm SVL; range = 15.3–36.2;  $n = 81$ ) was significantly smaller ( $F = 0.940$ ,  $p = 0.40$ ;  $t = -3.252$ ,  $df = 144$ ,  $p = 0.001$ ) than that of females (mean =  $28.9 + 4.8$  mm SVL; range = 20.5–44.5;  $n = 65$ ). The male: female mean body size ratio of this sample was 0.91. Using presumed body-size cohorts from a monthly distribution of body sizes (Fig. 7), the smallest individuals ( $< 10.0$  cm SVL) captured in the fall could have reached sexual maturity, approximately 19–20 months of age. The smallest egg-bearing female (24.3 cm SVL) could have produced her first clutch a few months before her second birthday, and all females would be primiparous by 2.5 years of age (Fig. 7).

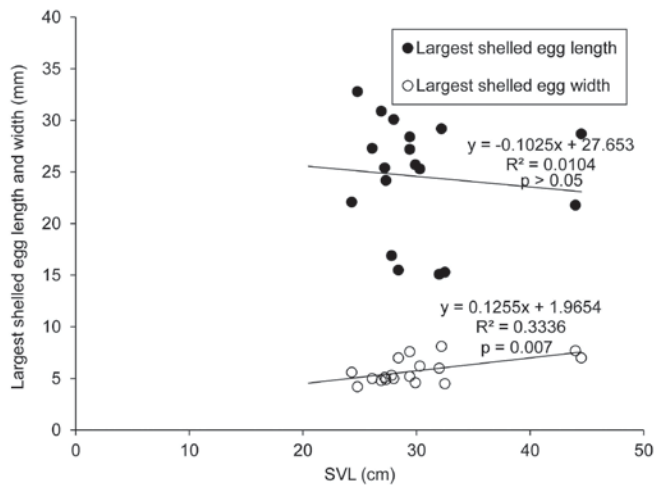
## DISCUSSION

Several life history traits of the northern ring-necked snake from West Virginia were examined within the context of patterns evident across its geographic range. In the East, seasonal activity of the ring-necked snake was shown to be unimodal, the peak of which was variable among locations. For example, the peak in seasonal activity was spring or summer in Florida (Meshaka & Layne, 2015). Peak surface activity was highest in June in Pennsylvania generally (Meshaka & Wilkerson, 2008) and in south-western Pennsylvania (Meshaka, 2010). However, in south-central Pennsylvania, the peak occurred in May (Meshaka & Delis, 2014). The incidence of capture was highest in June in our study. Although also unimodal, we leave open the possibility that the observed pattern in our study is an artifact of sampling given the limitations of uneven survey

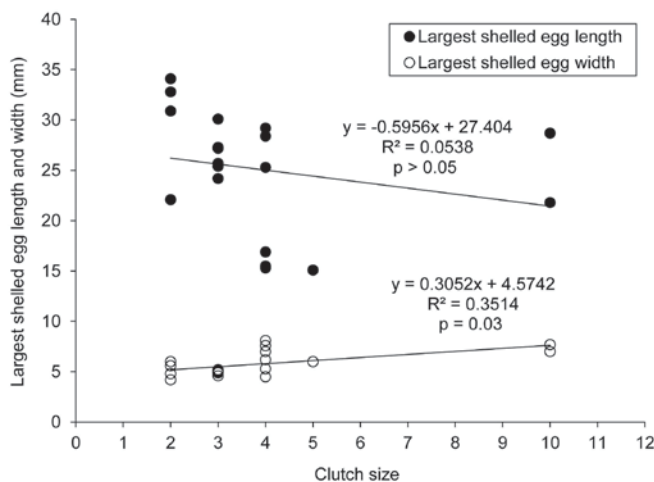




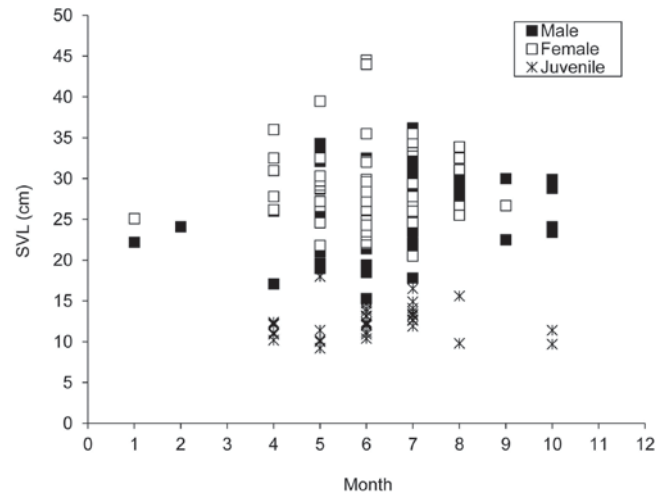
**Figure 4.** Relationship between clutch size and female body size of 21 northern ring-necked snakes (*D. punctatus edwardsii*) from West Virginia



**Figure 5.** Relationship between largest shelled egg length and width and female body size of 19 northern ring-necked snakes (*D. punctatus edwardsii*) from West Virginia



**Figure 6.** Relationship between largest shelled egg length and width and clutch size of 20 northern ring-necked snakes (*D. punctatus edwardsii*) from West Virginia



**Figure 7.** Monthly distribution of body size of 81 male, 65 female and 41 juvenile northern ring-necked snakes (*D. punctatus edwardsii*) from West Virginia

effort in the dataset. In Connecticut, collection success was greatest in May (Klemens, 1993). Farther west, the seasonal activity pattern of the prairie ring-necked snake (*D. p. arnyi* Kennicott, 1859) in north-eastern Kansas was bimodal, with spring and fall peaks (Fitch, 1975).

The duration of the activity season in the ring-necked snake was longer in the south than in the north (Meshaka & Layne, 2015). In southern Florida, individuals were active year-round, especially in the spring, and were typically active during April–October farther north (Meshaka & Layne, 2015). In our study, most individuals were collected during April–October, with a few found in January and February, which is similar to an active season of April–October in Pennsylvania (Meshaka and Wilkerson, 2008), May–mid-October in southern New England (Klemens, 1993), and mid-April–mid-October in New York (Wright & Wright, 1957). North-eastern Kansas populations were active during late-March–early-November (Fitch, 1975).

The male gonadal cycle of the northern ring-necked snake in West Virginia conformed to that of other north temperate populations of colubrid snakes, whereby spermatogenesis occurred in the summer (Saint Girons, 1982). The same pattern was reported in northern ring-necked snakes from New Jersey (Price, 1975) and Pennsylvania (Meshaka & Wilkerson, 2008).

On the other hand, vitellogenesis in West Virginia northern ring-necked snakes adhered to a tropical pattern to vitellogenesis (Aldridge et al., 1995) rather than the type II spring vitellogenesis pattern, typical of north temperate colubrids (Aldridge, 1979; Aldridge et al., 2009). The same pattern was evident in females from Pennsylvania (Meshaka & Wilkerson, 2008). Interestingly, Fitch (1975) described what would be interpreted as spring vitellogenesis in a north-eastern Kansas population.

The egg-laying season was found to be longest in Florida (May–September) and typically to occur during June–July farther north (see Meshaka & Layne, 2015). Oviposition occurred during 16 June–21 July in Virginia (Mitchell, 1994) and during April–July in our study. In New Jersey, ovarian mass was greatest in June before ovulation, and

egg-laying was expected during July–August (Price, 1975). Farther north, eggs were laid during June–July in Pennsylvania (Hulse et al., 2001; Meshaka & Wilkerson, 2008) and in July in northern Michigan (Blanchard, 1942). The late-June–early-July egg-laying season in north-eastern Kansas (Fitch, 1975) was similar to much of the Mid-Atlantic and North-east.

Clutch characteristics varied little with respect to clutch size. A mean of 3–4 eggs/per clutch was typical in the East (Meshaka & Wilkerson, 2008; Meshaka & Layne, 2015). Data from our study did not conflict with this trend. A mean clutch size of 3.4 eggs in north-eastern Kansas prairie ring-necked snake populations (Fitch, 1999) also conformed to this pattern. Likewise, our data did not conflict with the finding of a positive relationship between clutch size and female body size reported in Pennsylvania (Hulse et al., 2001; Meshaka & Wilkerson, 2008) and north-eastern Kansas populations (Fitch, 1975).

Mean body sizes of adult ring-necked snakes were found to be larger in northern and north-eastern Kansas populations (Meshaka & Layne, 2015). Data from our study corroborate that trend, as does the weak sexual dimorphism in body size that favoured slightly larger female body size (Meshaka & Layne, 2015). We note, however, the importance of variation in mean body size associated with habitats (Fitch, 2004).

Meshaka & Layne (2015) noted delayed maturity in northern populations of this species. Monthly body size distributions in our study were suggestive of sexual maturity in females by their second spring. If so, some females could lay their first clutch of eggs before they reach two years of age, and all females could mate in the fall just after they have passed two years of age. A similar observation was made in Pennsylvania (Meshaka & Wilkerson, 2008). First clutches in north-eastern Kansas were produced by females in their third year (Fitch, 1999), when presumably all females in our study would be ready to lay eggs for the first time.

Despite its ubiquity and ease of capture, the northern ring-necked snake remains a poorly-studied subject of ecology in the East. Our findings corroborated the relative stability of some life history traits in this species as well as geographic variation in other traits. In turn, understanding these patterns can provide a measure of predictability in the likelihoods of responses by the northern ring-necked snake in other places yet to be studied. These data are, in turn, useful in understanding factors that influence life history traits and in applied terms, for species management. The effect of climate on aspects of ophidian ecology as indicated in our findings was a matter of spatial comparisons to test life history patterns. Our dataset ended in 2000. Since that time, human-mediated changes to climate pattern have been shown to be severe and to affect ecology, geographic range, and even existence of amphibian and reptile species. For example, climate change affects phenology and population structure of amphibians (Blaustein et al., 2001 & 2010, Corn, 2005). Identified as a threat to reptiles (Gibbons et al., 2000), Aubert and Shine (2010) reveal the challenge of thermal plasticity in the face of climate change-related

year-to-year variation in temperature. More broadly yet, south-east Asian amphibian and reptile species will within 50 years meet or exceed most limits in their ability to adapt to effects of climate change with respect to temperature-dependent sex determination, higher metabolic rates, and less bio-available water (Bickford et al., 2010).

We proffer that even as the spatial analysis of our study corroborates predictable effects of climate on selected life history traits, it also serves as a baseline dataset for measuring future changes in the ecology of West Virginia populations of the northern ring-necked snake in advance of quantifiably different weather patterns and overall warming of their environment.

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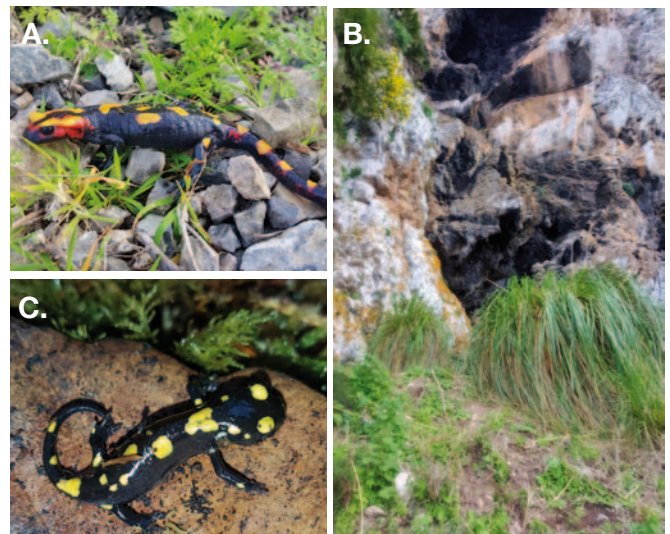
# *Salamandra algira splendens* and *Pleurodeles waltl* in Moroccan caves; new distributional records

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The Mediterranean Basin is a global hotspot of biodiversity, exhibiting high levels of endemism and is considered an important centre of diversification for Amphibians (Bons & Geniez, 1996; Schleich et al., 1996). Two urodeles species are distributed throughout Morocco: *Pleurodeles waltl* and *Salamandra algira* (Escoriza & Ben Hassine, 2015). They both are threatened by loss of their habitat and pollution (Hernandez, 2017). *Pleurodeles waltl* occurs also in Spain and Portugal. In Morocco, the species is mainly found within the Atlantic lowlands ranging from Tangier southwards to Essaouira, where it generally occupies temporary ponds and flooded fields (Bons & Geniez, 1996; Schleich et al., 1996; Beukema et al., 2013). Several fragmented populations are also found in the western Rif at Chefchaouen, and in eutrophic lakes of the Middle-Atlas Mountains such as Daït Ifrah and Daït Aoua near Ifrane (Hernandez, 2017). *Salamandra algira* is the only species of the genus *Salamandra* present in North Africa (Bons & Geniez, 1996; Schleich et al., 1996). Its distribution comprises northern Morocco up to north-eastern Algeria (Escoriza & Ben Hassine, 2015). Its presence in Tunisia is questionable (Ben Hassine & Nouira, 2012; Bogaerts et al., 2013). Several subspecies and genotypes are known to occur in isolated populations confined to humid to sub-humid mountainous areas containing forests; *S. algira tingitana* in the Tingitana Peninsula (northern Morocco) including Ceuta (Spain), *S. algira splendens* in the north-eastern Middle Atlas and Rif mountains (northern Morocco), and *S. algira spelaea* in the Beni Snassen mountains (north-eastern Morocco), being restricted the nominotypical form to northern Algeria (Raffaëlli, 2013; Ben Hassine et al., 2016). Recently, new localities were reported for *S. algira splendens* that improve our understanding of its biogeography through Morocco; the easternmost population in central Rif Mountains was recently found in the Bökkoyas massif at Al Hocéma around caves (Hernandez & Escoriza, 2017; See Fig. 1. A, B, C); and the southernmost record was discovered in Jbel Sidi Ali, central Middle Atlas, at 190 km south of the current distribution range (Hernandez, 2018).

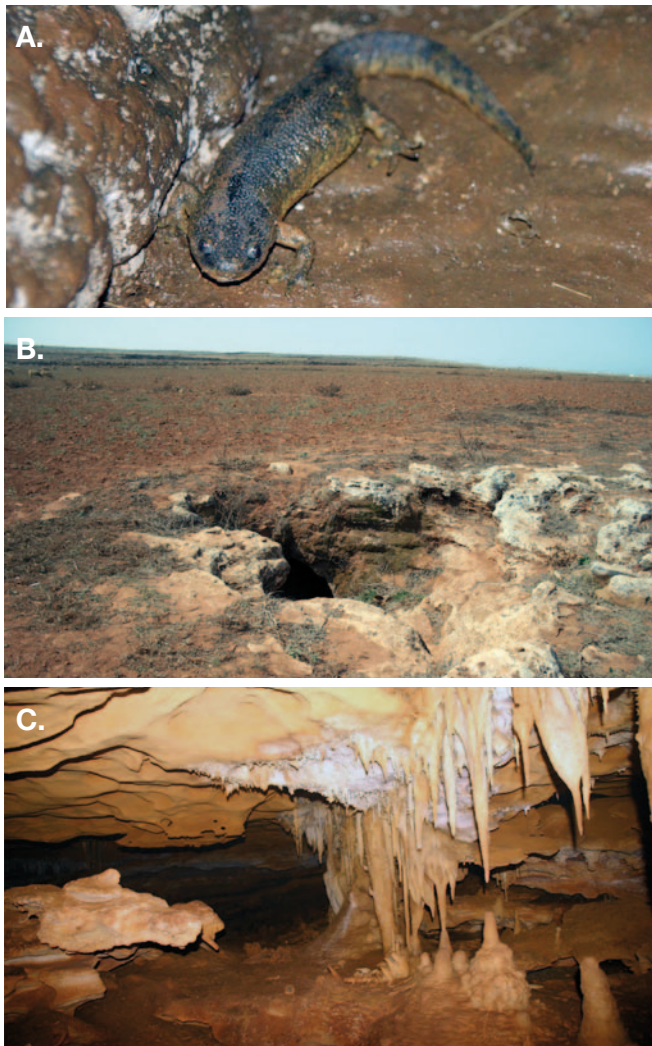
Some authors discussed the presence of these urodeles in Moroccan caves where habitats are poorly vegetated and where the species live under xeric and hard arid conditions (Escoriza & Comas, 2007; Beukema et al., 2013; Hernandez & Escoriza, 2017; Hernandez, 2017, 2018). In this short communication new localities for *S.*



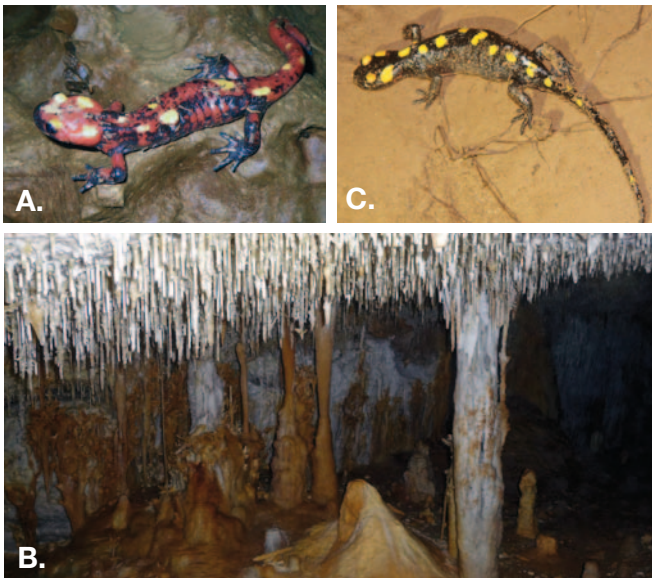
**Figure 1.** **A.** Adult female of *S. algira splendens* found under rocks near to the Mediterranean sea at 214 m a.s.l in Al Hocéma, Rif Mts, Morocco. **B.** Cave used as a refuge for this population. **C.** Young juvenile measuring 18 mm found near the cave. This population is suspected to be viviparous and occurs under xeric conditions.

*algira splendens* and *Pleurodeles waltl* found in Moroccan caves are described. During field work (12 February to 15 April 2017), six adult *S. algira splendens* were found at Tabehirte, Grotte de Chaâra, Taza region, north-eastern Middle Atlas (33°57'24.336" N, 4°14'44.957" O) which is located 1213 m above sea level. The adults were found in a large chamber of the cave that included a permanent stream (See Fig. 2. B; two individuals are shown on Fig. 2. A, C). On the Atlantic coast, one adult female of *P. waltl* was found at Cap Beddouza, grotte de Ghar-Goran, north of Safi (32°33'22.278"N, 9°15'5.774"O) located 34 m a.s.l (Fig. 3. B, C). The specimen was observed on the ground characterised by clay soil (Fig. 3. A).

These observations indicate use of caves for urodeles as already reported for Morocco in *S. algira* (Escoriza & Comas, 2007; Beukema et al., 2013; Hernandez & Escoriza, 2017); but also for *P. waltl* in Portugal (Herrero & Hinkley, 2014). Moreover, Chaâra cave is near Ikfou Ouan cave where specimens were already observed during speleological expeditions (Camus & Lamouroux, 1981; Ayoub pers. obs.). This habitat use seems to be a widespread phenomenon that may be linked to specific biogeographical factors of regions characterised by Mediterranean climates



**Figure 2.** **A.** Adult female of *P. waltl* found at Cap Beddouza, grotte de Ghar-Goran, north of Safi. **B.** General view of the cave from the outside. **C.** The large chamber in the cave where the specimen was recorded. Pictures: Ayoub Nehili.



**Figure 3.** **A.** Adult male of *S. algira splendens* showing red discolouration marks on the dorsal part discovered at Grotte de Chaâra, Taza region, north-eastern Middle Atlas. **B.** Habitat view in the cave. **C.** Another adult female specimen found in the same habitat. Pictures: Ayoub Nehili.

with dry and warm summer periods (Manenti et al., 2017; Balogová et al., 2017; Hernandez, 2017). The environment of caves with groundwater and stable humidity play an essential role as refugia for urodeles (Hernandez, 2017). These habitats are also used as winter shelters, hiding places during the active season, feeding habitats and also as regular breeding sites in some cases (Herrero & Hinckley, 2014; Manenti et al., 2017; Balogová et al., 2017). However, the finding of just one specimen of *P. waltl* at Cap Beddouza, grotte de Ghar-Goran during the reproductive season of the species could be also explained by the fact that the specimen fall and become trapped in this cave. New studies are needed to understand the new cryptic and threatened populations within Morocco. Thus, the occurrence of salamanders in Moroccan caves can be an important refuge. This particular use need further assessments to improve our knowledge on their behaviour and distribution especially in poorly surveyed areas localised under xeric and hard arid conditions.

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# Digital kitchen-spoon scales: another instrument in the field herpetologist's tool-box?

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Getting an accurate and reliable weight of amphibians (and other vertebrate classes) when in the field is an important consideration for a number of reasons, including measuring the health and overall well-being of individuals (Orton et al., 2014). Weight can also be used as an indication of the age-class an individual belongs to (Brown, 1990), for individual recognition or species determination. A number of different methods have been used previously to weigh amphibians such as spring balances (Jennings & Hayes, 1985), sensitive dynamometers (Pereira & Maneyro, 2016) and digital scales (Orizaola & Laurila, 2009). However, there has been a shift towards the use of digital techniques due to their more accurate mass measurements (Deichmann et al., 2008). In this paper we present data that indicates that with the right application digital kitchen-spoon scales (often used in household applications) can be used in the weighing of amphibians. Previous to testing scales were compared to check their performance using a number of British sterling coins, each with its own known weight and a 10 g calibration weight. The scales did not differ when tested in ideal conditions (on a level kitchen worktop) and so the experimental trial proceeded.

In mid-May 2017, twenty-five smooth newts (*Lissotriton vulgaris*), 16 males and 9 females, were captured using dip-netting techniques from a site in Cambridgeshire, UK (TL399625), and placed in a temporary aquarium. For the first treatment, each newt was individually sexed before being weighed on both a set of digital scales (Metro Electronic MH-Series, 0.1 g accuracy up to 200 g) and then using a digital spoon (Technoline KW-120, 0.1 g accuracy up to 300 g). Both pieces of measuring equipment were bought from a local Maplin store and tared off periodically (when required) between the weighing of each newt. For the second treatment, the same procedure was followed but both the scales and the spoon were tared off and wiped dry of any water droplets before weighing each newt again (Fig. 1). After weighing twice all newts were released at the point of capture.

In the first treatment, there was no significant difference between the weights of the newts weighed (paired t-test,  $t = 0.231$ ,  $df = 24$ ,  $p = 0.41$ ). The mean weight of each newt when weighed with the scales was 2.57 g (SD = 0.489) and 2.43 g (SD = 0.489) with the spoon. Likewise in the second treatment, the results were also not significant (paired t-test,  $t = 0.39$ ,  $df = 24$ ,  $p = 0.35$ ). Again there was a small difference, the mean weight of each newt weighed with the



**Figure 1.** A male smooth newt being weighed with a digital kitchen-spoon

scales was 2.17 g (SD = 0.469) and 2.12 g (SD = 0.469) with the spoon. As expected for a sample using the same individuals with each replicate, standard deviations were similar. Comparing both methods to weigh newts in the field showed very little difference despite the precautions taken, this can easily be attributed to the degrees of error that each of the two pieces of digital equipment operates to, although this wasn't observed when we tested the calibration of both scales. There are clear differences in the mean weights between the two methods, showing the effects of excess water droplet building in potentially aiding to misrepresent weights. The small differences between the digital scales and the measuring spoon (which ranged from +0.1 g to -0.2 g) may also be explained by the movement of the newts when being sampled or the effects of outside disturbance such as the wind, as sampling was carried out in the field. The error of up to 0.3 g ranges between 8.75-20% of the overall weight of the newts sampled and may be due to the technique rather than the equipment.

Using the methods described above we have shown experimentally that digital spoons can be used to reliably weigh amphibians when in the field. Digital spoons have some additional advantages over scales but also limitations. Digital spoons are perfect for weighing smaller amphibians such as newts (e.g. *L. vulgaris* or *L. helveticus*) and smaller anuran species (e.g. *Alytes obstetricans*) and similar spoons have been used previously (e.g. Spitzen-van der Sluijs et al., 2017). Like digital scales, the best results are achieved when the spoons are placed on a level plane. Potentially

small plastic containers placed on the digital scales may be as effective and may be more adaptable but spoons are a slim-lined self-contained unit that were originally designed to make measuring ingredients in the kitchen easier.

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## New records of the turtle *Trachemys venusta callirostris* in the Central Andes of Colombia

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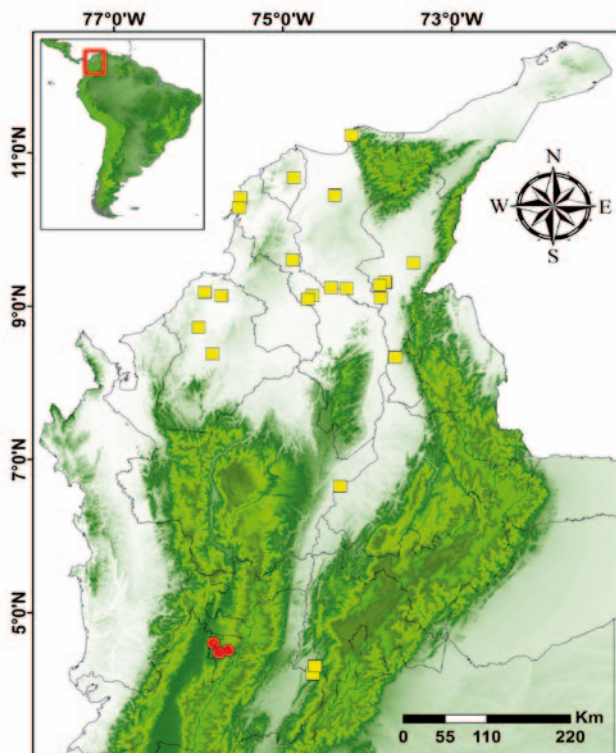
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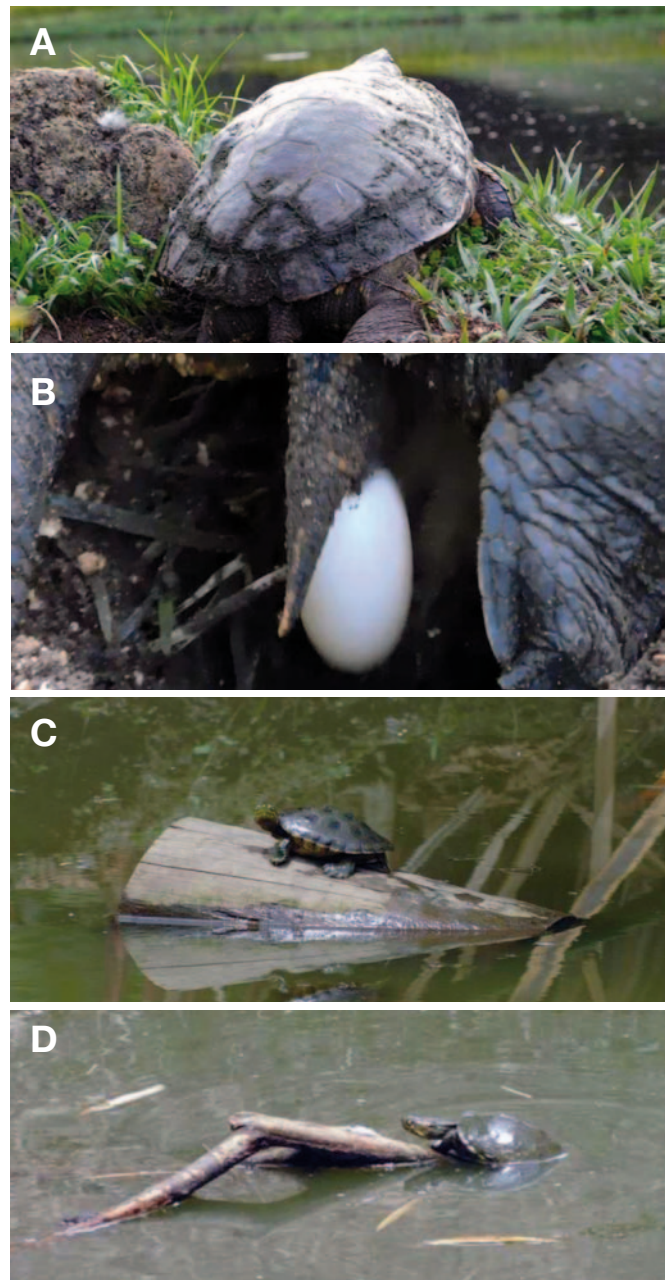
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The turtle, *Trachemys venusta callirostris* (sensu Fritz et al., 2012, and Vargas-Ramírez et al., 2017) is of medium size with a straight carapace length (SCL) between 19-25 cm (Páez et al., 2012) although some individuals can reach up to 35 cm (Rueda-Almonacid et al., 2007; Páez et al., 2012). Diagnostic morphological characters of *T. v. callirostris* are: 1) yellow longitudinal stripes along the neck and a wide red, orange or yellow-orange postorbital band that does not come into contact with the eye; 2) the neck, limbs, vertebral, costal and marginal shields have long yellow lines, and 3) the presence of large rounded blotches of black colour bordered by yellowish areolas (Pritchard & Trebbau, 1984; Rueda-Almonacid et al., 2007; Bock et al., 2010). Currently, this species is known



**Figure 1.** Distribution of *T. v. callirostris* in Colombia. Yellow squares: historical records (Rueda-Almonacid et al., 2007; Páez et al., 2012; Restrepo et al., 2014, and SibColombia <https://www.sibcolombia.net>); Red circles: records reported in this short-note



**Figure 2.** A) Female of *T. v. callirostris* nesting in an open area near an artificial lake; B) Egg laying; and C) and D) Juveniles in the municipality of Armenia and Quimbaya, respectively



in Colombia from the Caribbean and Magdalena basins (Fig. 1; Rueda-Almonacid et al., 2007; Páez et al., 2012; Restrepo et al., 2014).

During field trips between March 2017 and March 2018, we recorded the presence of *T. v. callirostris* in two artificial lakes at the Department of Quindío, Central Andes of Colombia (Figs. 2A, 2C and 2D); specifically, at the southern of the municipality of Armenia (4°28'47.6" N, 75°45'4.3" W) and the municipality of Quimbaya (4°36'27.5" N, 75°49'16.7" W). In addition, in the collection of Amphibians and Reptiles of the University of Quindío, there are two individuals (ARUQ-374, ARUQ-231) collected in the municipality of Calarcá (4° 30' 44.712" N, 75° 39' 6.641" W). Altogether, those turtles represent the first published record of the species in the whole western versant of the Central Andes of Colombia (Fig. 1). The presence of this species in the department of Quindío may possibly because the intentional release of unwanted pets (Rueda-Almonacid et al., 2007); however, observations in March 2017 of a female nesting and laying six eggs (Fig. 2B), suggest the possibility of the establishment of viable populations of *T. v. callirostris* in the region.

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# Orange-bellied racer (*Alsophis rufiventris*); diet and arboreality

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The orange bellied racer, *Alsophis rufiventris* (Duméril, Bibron & Duméril, 1854), is one of four *Alsophis* species endemic to the Caribbean Lesser Antilles. Historically, it was found on St. Eustatius, Saba, and St. Kitts and Nevis but was extirpated from St. Kitts and Nevis during the 1900s, so that it is now confined to the Dutch Caribbean (Sajdak & Henderson, 1991; Savit et al., 2005). Thus, only two populations exist with a combined range of just 34 km<sup>2</sup>, which is 10.9% of the known historical range (Sajdak & Henderson, 1991; Savit et al., 2005; Daltry & Powell, 2016). Despite being the most geographically widespread colubrid genus in the West Indies, little is known about the ecology of most *Alsophis* species (Savit et al., 2005; Daltry & Powell, 2016). Here, we provide new dietary and natural history information that was collected during a field study of *A. rufiventris* on St. Eustatius from September 2016 to February 2017, as well as other ad hoc observations.

From observational work the diet of *A. rufiventris* is known to include *Anolis schwartzi* (Savit et al., 2005; Heinz et al., 2005) and hatchling *Iguana delicatissima* (Debrot et al., 2013). Daltry et al. (1997) performed a preliminary faeces study on samples collected from Saba and St. Eustatius and identified *A. sabanus* and *A. schwartzi*, including one *A. bimaculatus* and one *Ameiva erythrocephala*. Although some samples likely contained remains of the only frog species that occurs on these islands, *Eleutherodactylus johnstonei*, these were not conclusive (Daltry et al., 1997). Here, we report on observational predation events involving three different prey species and their life stages, of which two are previously unpublished. Two observations were opportunistically recorded on Gilboa Hill and the Quill: a subadult *Ameiva erythrocephala* (2008; Fig. 1) and an adult *A. schwartzi* (2010), respectively. Additionally, on 8 December 2016 while conducting a transect-based study involving the capture of individuals; one snake was caught with a clutch of 15-30 eggs, from the frog *E. johnstonei*, in its mouth. The eggs were similar to those of *E. coqui* as shown in Elinson & Del Pino (2012, Fig 3 therein); in both species there is no free tadpole stage and the froglets complete development within the egg. Predation on *E. johnstonei* eggs could explain field observations reported by Heinz et al. (2005) and White et al. (2008), who documented snakes “rooting and digging in loose soil and probing small holes” (White et al., 2008), especially given *E. johnstonei* lays nests inside leaf and soil layers (Bourne, 1997).

Although commonly referred to as ground-dwelling or



Figure 1. *A. rufiventris* eating a subadult *A. erythrocephala*

terrestrial species, aquatic and arboreal behaviour has also been observed in some species of *Alsophis* (Henderson & Sajdak, 1986; Pérez-Rivera & Laboy-Rivera, 1996; Powell et al., 2015), even above 18 m (Thomas & Kessler, 1996), however, to our knowledge this behaviour is unreported for *A. rufiventris*. On many occasions on St. Eustatius we observed *A. rufiventris* in trees as high as 3 m from ground level (Fig. 2). These observations were made on the outer western and eastern slopes and inside the crater of the Quill within floral vegetation types: Myrcia-Quararibea Mountains, Coccoloba-Chionanthus Mountains and Chionanthus-Nectandra Mountains (both high and low variant) (de Freitas et al., 2014). Behaviour observed by White et al. (2008) for *A. sibonius* on Dominica, in which individuals were observed to stalk anoles resting on tree trunks at heights of ~80cm, suggests that our observations were of arboreal foraging individuals that identified prey items at greater heights. Similarly, arboreal foraging has also been described for other species of presumably ground-dwelling or terrestrial snakes (Brown et al., 2018).

Our observations suggest that *A. rufiventris* is likely to predate on many herpetofauna species, of different life stages, present on St. Eustatius (Table 1), including - given its potential for arboreal foraging - the large bodied *A. bimaculatus* that perches at heights greater than *A. schwartzi* (Roughgarden, 1995; see Daltry et al., 1997). Given the size of subadult and adult *I. delicatissima*, as

**Table 1.** Overview of confirmed and probable herpetofauna species in the diet of *A. rufiventris* on the islands of St. Eustatius and Saba. . C= Confirmed prey species, P= Probable prey species, X= Too large to be prey. <sup>1</sup>Debrot et al. (2013); <sup>2</sup>Heinz et al. (2005); <sup>3</sup>Savit et al. (2005); <sup>4</sup>Daltry et al. (1997); <sup>5</sup>This paper. Data from Daltry et al. (1997) did not include life stage information.

Prey species	Island	Eggs	Juvenile	Sub-Adult	Adult
<b>Lizards</b>					
<i>Anolis schwartzi</i>	St. Eustatius	P <sup>2</sup>	P	P	C <sup>2</sup>
<i>Anolis bimaculatus</i> <sup>4</sup>	St. Eustatius	P	P	P	P
<i>Anolis sabanus</i> <sup>4</sup>	Saba	P	P	P	P
<i>Ameiva erythrocephala</i>	St. Eustatius		P	C <sup>5</sup>	X
<i>Iguana iguana</i>	Saba		P	X	X
<i>Iguana delicatissima</i>	St. Eustatius		C <sup>1</sup>	X	X
<i>Sphaerodactylus sabanus</i>	Both		P <sup>3</sup>	P <sup>3</sup>	P <sup>3</sup>
<i>Sphaerodactylus sputator</i>	St. Eustatius		P <sup>3</sup>	P <sup>3</sup>	P <sup>3</sup>
<b>Frog</b>					
<i>Eleutherodactylus johnstonei</i> <sup>4</sup>	Both	C <sup>5</sup>	P	P	P



**Figure 2.** Arboreality of an adult *A. rufiventris* at 3 m above ground in a *Vachellia macracantha*.

well as adult *A. erythrocephala*, we expect these to be physically too large to be consumed by *A. rufiventris*. In addition, the venom of *A. rufiventris* is believed to be weak and only capable of subduing smaller prey (Powell et al., 2015). Furthermore, with only one observation of *A. rufiventris* eating a hatchling/juvenile *I. delicatissima* and low recruitment within the local iguana population (van den Burg et al., 2018), we expect these predation events are rare.

*Alsophis rufiventris* is listed as vulnerable on the IUCN Red List of Threatened Species. This classification is based on its limited range and the likely threat of mongoose introduction, which has led to its extirpation elsewhere (Daltry & Powell, 2016). This risk is increasing due to enhanced traffic routes between these islands and larger nearby islands on which the mongoose is established. Feral and free-roaming cats pose a lesser but nevertheless potential threat as human populations expand on both islands. This is strengthened by recent observations of iguana mortality caused by feral cats, which included both hatchling and adult iguanas (van den Burg et al., in press). Before Hurricanes Irma and Maria impacted Saba and St. Eustatius in 2017 the snake was thought to be abundant

with an apparently stable population (Daltry & Powell, 2016). Using baseline data (Savit et al., 2005; Zobel, 2017), a post-hurricane population assessment in the Quill and parts of Boven National Parks on St. Eustatius is being undertaken to determine the impacts of these events on the population.

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## Behaviour of a common cobra (*Naja naja*) stranded in a well

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The common cobra (*Naja naja*) is widely distributed in India and a frequent cause of snakebite mortality. The species is active both at night and during daylight hours and is mostly terrestrial in habit. It has been reported to be a good swimmer although to date there is no record of how long the species may remain underwater.

During a visual survey undertaken with an Irula snake catcher in a rice paddy field near Mahabalipuram, Tamil Nadu, India (12°42'18" N, 80°8'27.6" E), at around 11.10h on 27 March 2011, a girl in the village drew our attention to an adult cobra that had fallen into a well (Fig. 1). The well was at ground level, with no parapet, and had an estimated depth of 9 m. The water surface was 1.5 m below ground level.

According to the villagers, the snake had been confined in the well for five days and had failed to escape despite several desperate attempts. Throughout this period the snake was either fully or partially submerged. When we saw the snake floating on water, the ambient air temperature was 42°C and relative humidity 40%. While the snake catcher made a rescue effort, the snake submerged and remained underwater for 45 minutes. The depths to which it submerged ranged from 1 cm to a depth where it was no longer visible. The snake was found to position itself more vertically than horizontally, in the water column (Fig. 2). During this period, it surfaced only twice and breathed for a maximum of two minutes after remaining submerged for approximately 20 minutes on each occasion. Subsequently, the snake resurfaced and began looking for possible resting places, in particular ridges along the wall. At this point, it was rescued in response to repeated requests from the villagers who needed access to the well. The snake appeared to be in sound health when rescued and was an adult measuring 137 cm; on average adult cobras grow to 150 cm (Whitaker & Captain, 2004).

Various snakes that are either aquatic or semiaquatic show specific morphological and physiological adaptations to aquatic environments (Seymour, 1982; Lillywhite & Ellis, 1994; Aubret, 2004; Brischoux & Shine, 2011) that make them potentially better divers than terrestrial species (Brischoux et al., 2011; Lillywhite et al., 2012). However, even within species there may be distinctly different capabilities. Individuals of two disjunct populations of Australian tiger snake (*Notechis scutatus occidentalis*), one that seeks prey in water the other not, have very different capacities for holding their breath before resurfacing from



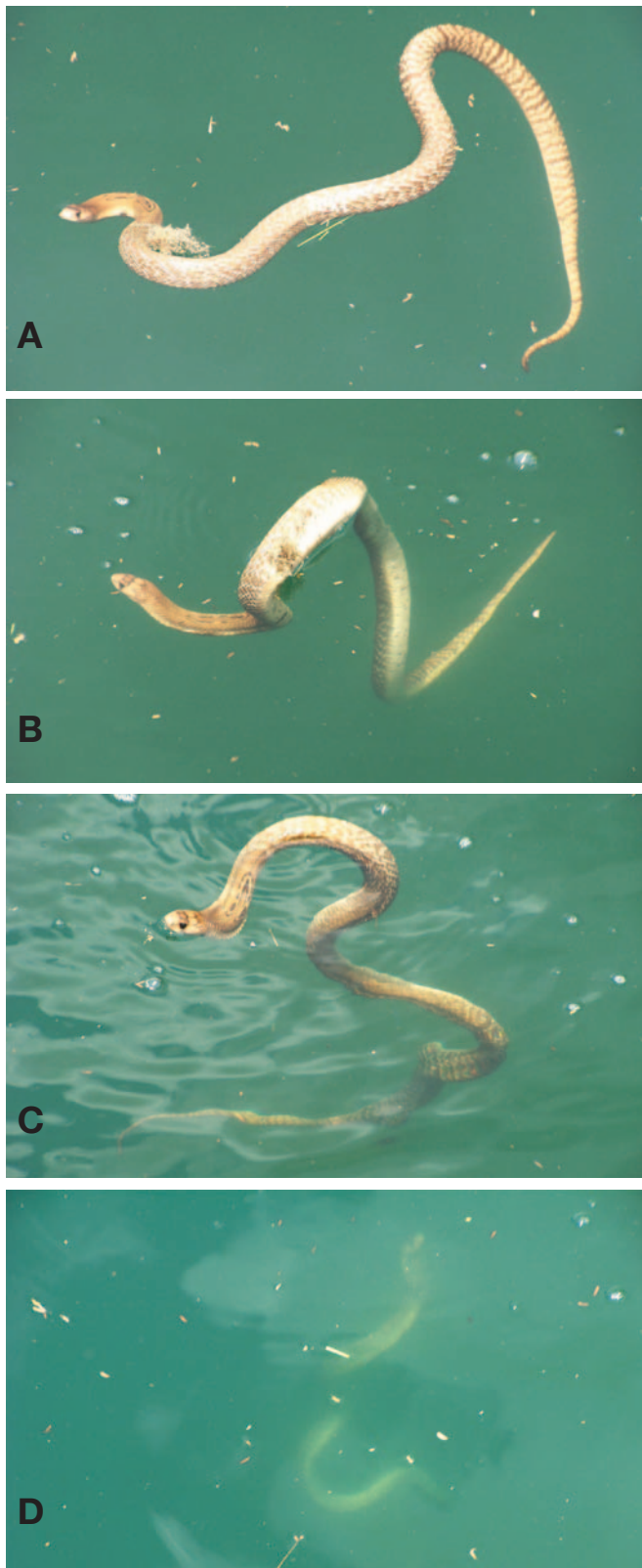
**Figure 1.** *N. naja* floating inside a well in a farmland. The large diameter well made at ground level is typical for the study area in Tamil Nadu.

a dive, i.e. they differ in their apnoea score (Aubret et al., 2007). Viperid snakes, even though largely ground-dwellers, show cardiopulmonary characters similar to those of aquatic snakes giving them the potential to perform well in an aquatic environment (Lillywhite & Smits, 1992); this is supported by two reported instances of underwater breath-holding behaviour in *Trimeresurus malabaricus* from India (Bhaisare & Pelling, 2015). The current observation makes it clear that the common cobra has considerable ability to dive and even at relatively high temperatures can remain without breathing for lengthy periods.

### ACKNOWLEDGEMENTS

I thank Mr. T Velan who accompanied me during the fieldwork. I am thankful to the Marine Biology Regional Centre, Zoological Survey of India, Chennai for their support for research while the observation was made. I am grateful to the Irula Snake Catcher's Industrial Cooperative





**Figure 1.** Sequences of submergence (A-D) in *N. naja*. The body was aligned rather vertically during gradual movement under water.

Society, Tamil Nadu who provided immense help during the course. I acknowledge the Tamil Nadu Forest Department who provided necessary permit for the study. My sincere thanks to Dr. P A Azeez for his comments on the draft. I am

thankful to the reviewer for suggesting necessary changes in the manuscript.

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## Winter activity of the smooth newt *Lissotriton vulgaris* in Central Europe

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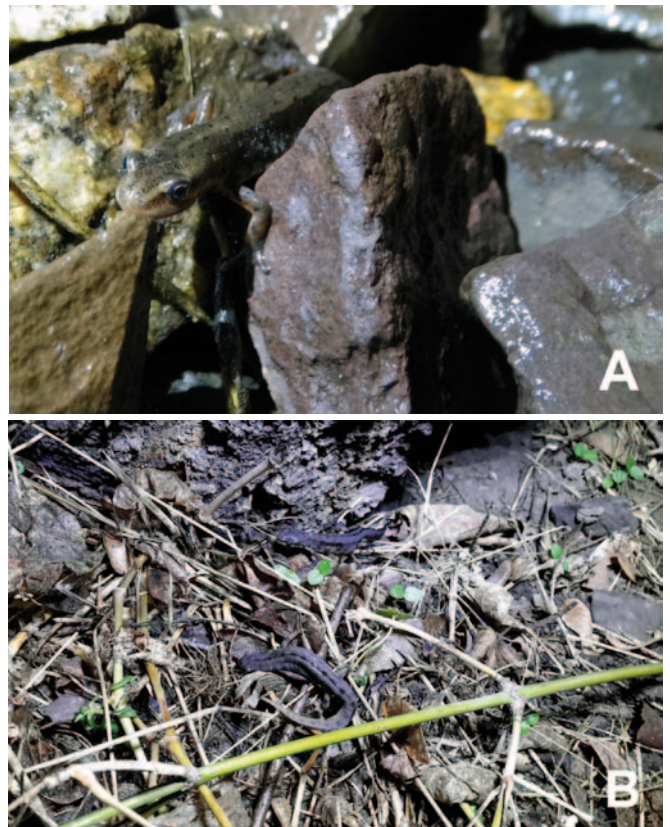
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Climate change deeply affects animal phenology. This is especially true for poikilothermic animals such as amphibians (Beebee et al., 2002; Walther et al., 2002; Root et al., 2003; Corn, 2005). In the global context, both anurans and urodeles now tend to begin their spring migration earlier than in the past (Gibbs et al., 2001; Chadwick et al., 2006; Ge et al., 2015; Todd et al., 2011). In Europe, such changes in phenology are relatively easy to detect among explosively-breeding species such as the common frog *Rana temporaria* or the common toad *Bufo bufo* (Tryjanowski et al., 2003; Carroll et al., 2009; Neveu, 2009; but see Beebee et al., 2002). In contrast, much less is known about changes in the phenology of small and secretive species, such as the smooth newt *Lissotriton vulgaris* (but see Chadwick et al., 2006; Jablonski, 2013). In the temperate oceanic climate of western Europe, the species is known to begin breeding migration as early as January, with a long-term trend towards earlier onset of migration (Chadwick et al., 2006). In some cases, animals may remain active throughout the year (Bell, 1977). In contrast, in the continental climate of central and eastern Europe, *L. vulgaris* usually hibernates on land between November and March (Kowalewski, 1974; Juszczak, 1987; Baruš & Oliva, 1992). Moreover, no long-term data on phenological changes are available for the species. However, as amphibians at similar latitudes adjust their phenology to temperature in analogous ways (Sparks et al., 2007), records of winter activity of newts are expected to occur more often not only in Western Europe (Chadwick et al., 2006), but also in the eastern part of the continent (Jablonski, 2013). Here we report unusual winter activity of *L. vulgaris* in a lowland population in western Poland.

In 2014, we initiated monitoring of a *L. vulgaris* population located in an urban park, 'Traszki Ratajskie' (16°58'29" N; 52°23'32" E) in the centre of the city of Poznań (ca 540,000 inhabitants). Individuals from this population breed in two small ponds located in the park and hibernate in an embankment adjoining tram tracks bordering the park (for more details on the population studied, see Kaczmarski & Kaczmarek, 2016). From 2014 to 2018, between December and January, we made several opportunistic observations on winter activity of newts. Observations were performed after dusk, exclusively on days characterised by high humidity and temperatures over 5°C (in the studied area, the mean daytime temperature in December and January is 0.2°C and -0.8°C, respectively;



**Figure 1. (A)** A male smooth newt *L. vulgaris* active on a stone embankment near tram tracks on 29 January 2018 **(B)** Two *L. vulgaris* males active on land on 19 December 2018, Poznań municipality, western Poland

data for 1981–2010). The transect we used was located along a tram track, which, for the studied population, serves as a terrestrial habitat as well as a hibernation site (Kaczmarski & Kaczmarek, 2016). Air temperature and humidity were measured at the beginning of each observation using a CEM TH321-S thermohygrometer. Animal handling was performed according to guidelines from local conservation authorities (permit no. WPN-II.6401.190.2016.AC.2)

In the course of two midwinter observations, we found active individuals of *L. vulgaris*. On 19 December 2014, we found 6 active animals (3 females and 3 males; air temperature 11°C, humidity 93%). On 29 January 2018, we observed 5 newts active on the ground (3 females and 2 males; air temperature 9°C, humidity 100%). Newts



were observed on open ground (i.e. without vegetation) of stone aggregate (Fig. 1A), between stones, and on soil under trees planted on the slope of the embankment. No individuals were found on the park trails between the embankment and the breeding ponds, where substantial mortality occurs during the spring and autumn migration (Kaczmarski & Kaczmarek, 2013). Two males in 2014 and one in 2018 exhibited traces of mating colouration and crest development (Fig. 1B).

The results of our observations show that, in central Europe, *L. vulgaris* is able to engage in winter activity during mild weather. December migration into ponds by some individuals is known from western Europe (Bell, 1977); however, to date, such activity has been seldom reported from central and eastern Europe, probably due to this region's more severe climate (Jablonski, 2013). Published observations of winter activity of *L. vulgaris* in Slovakia concerned only males and were described as very early spring migration attempts, as males move to breeding ponds earlier than females (Jablonski, 2013). In our population, we found both adult males and females during our winter observations, and only three males showed any signs of breeding colouration. Additionally, all of the individuals observed were found in a terrestrial habitat where newts were abundant during autumn surveys (Kaczmarski & Kaczmarek, 2016).

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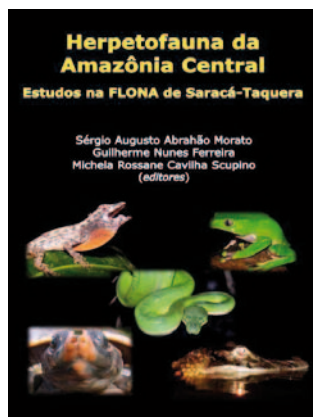
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# Herpetofauna da Amazônia Central-Estudos na FLONA de Saracá-Taquera (2018)

Sérgio Augusto Abrahão Morato, Guilherme Nunes Ferreira, Michela Rossane Cavilha

Publisher: Scupino (Eds.). STCP Engenharia de Projetos Ltda./MRN S.A. 210 pp. (In Portuguese)  
ISBN 978-85-68814-03-1



This book on the herpetofauna of the Saracá-Taquera National Forest (FLONA Floresta Nacional: protected areas subject to sustainable exploitation regimes) is based on a series of studies by 37 researchers from several Brazilian universities. The reserve is located in the central Amazon basin of north-east Brazil, occupies a surface area of 4413

km<sup>2</sup>, and has a humid tropical climate. Within its limits there are records of 52 amphibian species (48 Anura and 4 Gymnophiona) and 126 reptiles (4 Crocodilia, 13 Testudines and 109 Squamata).

The book has 7 chapters dealing with 1. Distribution and habitat of reptiles; 2. Distribution and habitat of amphibians; 3. & 4. Assessment of the impact of forestry and mining activities on the herpetofauna; 5. Study of the genetic diversity of *Podocnemis expansa* (Giant South American turtle); 6. Impact of bauxite mining on the richness and abundance of freshwater turtles; and, 7. Diversity and abundance of crocodilians in Lake Sapucaá. Each chapter provides an initial summary giving the environmental background and main conservation problems of the region. The texts are in Portuguese, but all the chapters start with a brief abstract in English and the graphics and tables are easily interpretable, even by those who do not speak Portuguese.

The first two chapters provide an exhaustive list of the amphibians and reptiles occurring in the reserve (based on bibliographical references and the authors' surveys), and describe how this diversity is structured depending on several landscape and geomorphological features. However, the enormous richness of species prevents the authors from making a detailed description of their habitat use, although additional details are provided for freshwater turtles and crocodilians in the final two chapters. The chapters are accompanied by some high-quality photos, but in order to find pictures of most species the reader should consult Morato et al. 2014. The chapters also contain maps and graphs, but lack images of the main landscape types or maps of these showing species richness by taxonomic order. Even so, the text provides novel and relevant information on the ecology of reptiles and amphibians in the reserve, with emphasis on the type of ecosystems where exploitation must be minimised in order to maintain biological diversity.

The third and fourth chapters go deeper into the subject, and discuss the effect of the destruction of primary forests on the herpetofauna, supported by abundant graphic material. The fifth chapter assesses the possible impact of disturbance by boats on the genetic diversity of *P. expansa*. It also provides new and interesting data on the metapopulation structure of this chelonian in the affluents of the Amazon river and on the factors that influence the gene-flow among subpopulations. The sixth chapter assesses the richness and local abundance of freshwater turtles. The authors found 11 species in the reserve (65% of the known species for the Amazon basin), which confirms the exceptional value of this protected area. The seventh chapter evaluates the habitat use and the status of the populations of the 4 crocodilian species in Lake Sapucaá and confirms that the Saracá-Taquera reserve maintains important populations of these species.

In summary, 'Herpetofauna da Amazônia Central-Estudos na Flona from Saracá-Taquera' is a specialised text for those interested in Amazonian herpetofauna, but also for those interested in a broad sense in the ecology of crocodilians and in the effect of anthropic activities in hyper-diverse tropical biotic communities.

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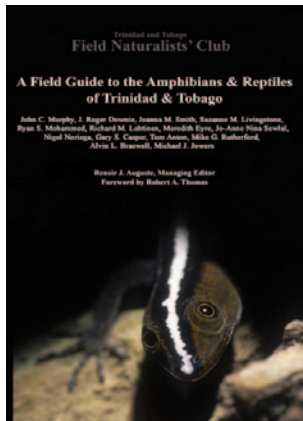
Received: 8 May 2018



## A Field Guide to the Amphibians & Reptiles of Trinidad and Tobago

John C. Murphy, J. Roger Downie, Joanna M. Smith, Suzanne M. Livingstone, Ryan S. Mohammed, Richard M. Lehtinen, Meredith Eyre, Jo-Anne Nina Sewlal, Nigel Noriega, Gary S. Casper, Tom Anton, Mike G. Rutherford, Alvin L. Braswell, Michael J. Jowers

Trinidad and Tobago Naturalist's Club, pp. 336  
ISBN: 978-976-8255-47-1



This field guide is a user-friendly, detailed, step-by-step guide to the herpetofauna of Trinidad and Tobago. The foreword gives a warm welcome with tales from Robert Thomas's own experiences starting with, "I bounded into the dark woods towards a chorus that included Milky Treefrogs (*Trachycephalus typhonius*)". Thomas draws the reader in with, "Such encounters

with tropical amphibians imprint nature lovers with the wonder of herpetofaunal biodiversity that is brought to life in the excellent book that follows". This leads into a guide which is primarily aimed at field naturalists and researchers and is fully documented by a collaboration of numerous specialists including three who are members of the British Herpetological Society. Content includes waifs and questionable species in Trinidad and Tobago. Expect to find approximately thirty-five frog and toad species, nine turtles, two tortoises, two caimans, eight geckos, eight anoles, sixteen lizards, fifty-two snakes and a plethora of photographs to compliment them.

There are detailed descriptions of each animal, including morphometrics, habitat descriptions, ranges and climatic information. The layout of the book is clear and easy to understand. It breaks down into multiple sections with an easy to use index at the back for readers who want to just look at a few species. There are six appendices giving information regarding amphibian life stages, handling, chytridiomycosis, and museum specimens. Also there is an appendix with useful information including multiple guides and tips such as contact details for research stations and lodgings. The photos show the interior and exterior of these lodgings. Details are provided such as facilities to expect including how many individuals can be housed, location details, travel and, for example, the best location to see turtles. This makes contact for field trips easier and potentially less time consuming. Hopefully, reducing the need to locate lots of places and explore which are the best for the purpose of your trip. Appendix five gives details regarding museum specimens, locations, what was collected and when. It even provides information about how the specimen has been stored which is useful if you are looking to examine or process stored specimens for research purposes. There is another appendix covering

health and safety, both for humans and animals. This covers snakebite and amphibian handling to reduce the risk of spreading disease, all essential information for field workers. The writing in these appendices is simple, straight to the point, explaining advice in layman's terms.

Extensive referencing and a glossary are included to give further understanding and explanation if not gained in the main text. The book is not a mini pocket guide but due to it including all herpetofauna for both Trinidad and Tobago, the size makes sense considering the illustrations and photographs. Anyone interested in amphibians and reptiles could pick up this book and enjoy the pictures and information. Herpetoculturists would find it of interest but it is obviously aimed at a more specialised reader with regards to field research or scientific exploration of the animals identified.

There are already varied sources for the herpetofauna of Trinidad and Tobago, amphibians and reptiles (Murphy, 1997) or just snakes (Boos, 2001), but this book collates it all. Detailed animal drawings/sketches have been used from Murphy (1997) but many have been redrawn, assuming to update or give more detail. Similar maps have also been used for distribution of species but are much more compact compared to the previous book.

The photographs are generally excellent, match up to the text and in several cases there are multiple pictures of one species, with different angles and colourations. A few of the photographs could have been made smaller to make more space and lack some sharpness but are better than no photographs at all. Both adults and juveniles are illustrated which is very helpful when there are significant colour differences between them. An example of useful comparative photographs is seen with the *Typhlonectes* and the synbranchid eel on p.49 showing that they could be easily mistaken for each other. Additionally, different life stages such as tadpoles and eggs are shown which again are useful in aiding identification.

All photos and illustrations are labelled clearly. Photographs of habitats are given which are a useful addition as they illustrate where these animals reside and where they can be found, e.g. Tobago glass frogs eggs on the underside of leaves over streams.

The sketches show excellent detail of scale patterns, locations and body parts e.g. Plate 20 & 21. These are labelled well with correct terminology and morphometric information for data collection. Some of these illustrations are colour coded to illustrate different scale patterns seen in different species e.g. Windward skinks.

Distribution maps are given for most species identified

and the terrain is colour coded giving a simple, easy to read, distribution visual. The maps are small, leaving more room for written information.

Having carried out field research myself, this guide would be a wonderful aid. The information included would save time and make anyone's trip a lot easier. This is a huge positive regarding further research in these areas and having read this, I can only say it is a book that encourages more research to be done. The book does have areas where it readily admits information is lacking for example *Mannophryne olmonae*, where "eggs are presumably laid in forest leaf litter". But, this again identifies that although there is a massive amount of knowledge here, there is still more research to be done. Although this guide is not flawless in presentation, there are minor issues with bold writing and font size inaccuracies, altogether it is a wonderful read with up to date information and a great aid for anyone researching herpetofauna on these islands.

This guide is only currently available to buy from The Trinidad & Tobago Field Naturalists' Club website and has been priced for \$TT290 (= £32). At this price it is excellent value for money.

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## Corrigendum

The following shows changes to the original text since publishing:

**The Herpetological Bulletin 141: 16-22**

**Daniel F. Hughes, Katrina Johnston & Walter E. Meshaka Jr (2017)**

**Nesting characteristics of three turtle species along a wetland matrix in western Pennsylvania, USA**

- In Tables 2, 3, and 4, the “Overall” for “Air” temperature should be: **15.4 C° (-2.8–27.8 C°)** instead of 12.9 C° (-13.9–27.8 C°).
- In Table 2, the “Air” temperature for “October” is missing and should now show this corrected figure, **9.8 C° (-2.8–26.1 C°)**.

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