

The Herpetological Bulletin

Issue 145, Autumn 2018



Published by the British Herpetological Society

THE HERPETOLOGICAL BULLETIN

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Front Cover: A male Agama mwanzae photographed basking on a kopje in the Serengeti National Park, Tanzania, 2018. See article on page 19. Photograph by Steven Allain.

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Nocturnal variation in population size estimate counts of male palmate and smooth newts (*Lissotriton helveticus* and *L. vulgaris*)

ERIK PATERSON

College of Medical, Veterinary & Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, UK Present address: 205 Telford Road, East Kilbride, South Lanarkshire, G75 0DG, UK Author Email: erikpaterson@virginmedia.com

ABSTRACT - Of the three native species of newt in the United Kingdom, the smooth and palmate newts are the most widespread and abundant. Despite this, there is a dearth of guidelines on sampling for these common species due to the highly protected status of the great crested newt. However, as amphibians can serve as useful bioindicators, recording schemes exist to collect data on the widespread species. This paper reports on an observational study of the peak times for detection of smooth and palmate newts by torchlight at a nature reserve in north Lanarkshire, Scotland. Smooth newts were found to be crepuscular and palmate newts followed a more nocturnal pattern of maximum population size counts. For both species, water temperature seemed to be more relevant for obtaining peak counts than air temperature. I propose that from sunset to approximately 150 minutes after would be the optimal time to sample for both of these species via torchlight.

INTRODUCTION

Mainland United Kingdom has only three native species of newt, the highly protected great crested newt (*Triturus cristatus*), as well as the abundant and more widespread smooth and palmate newts (*Lissotriton vulgaris* and *L. helveticus*; Wilkinson & Arnell, 2011). National surveys such as the Amphibian and Reptile Conservation (ARC) Trust's National Amphibian and Reptile Recording Scheme (NARRS) generate citizen science data on widespread species' distribution and abundance which can serve as a tool for monitoring population status (Wilkinson & Arnell, 2011). To generate more comparable data for small newts between sites, a tailored and standardised effort should be designed and undertaken and one important factor is the optimal time during which to undertake surveys.

As a result of its relative rarity throughout its range, the great crested newt has strict legal safeguards as a European protected species. However, smooth and palmate newts (hereafter small newts) have much more limited protection, safeguarded only from sale and related activity under schedule 5 of the Wildlife & Countryside Act 1981 (as amended). Due to their strict protection status much more documentation and research have been undertaken in maximising detection rates of great crested newts (e.g. Oldham et al., 2000; Sewell et al., 2013; Biggs et al., 2015; Paterson, 2018) than for small newts (Griffiths et al., 1996; Deeming, 2008; Baker, 2013; Sewell et al., 2013). However, given the value of amphibians as indicators of biodiversity and ecosystem health (Wyman, 1990) it is also of value to monitor common species (Wilkinson & Arnell, 2011; Petrovan & Schmidt, 2016).

Several studies exist already which have explored the diel rhythms of smooth newts, these have predominantly shown them to be crepuscular (Himstedt, 1971; Dolmen, 1983a & 1983b; Griffiths 1985; Dolmen 1988). However, little is currently known about the diel rhythms of the

palmate newt although Beebee & Griffiths (2000) state that they are crepuscular. The great crested newt has been shown to have a variable pattern of maximal counts via torchlight relative to sunset, with a statistical peak between 60 and 180 minutes following sunset (Paterson, 2018). This showed that great crested newt populations could be underestimated or undetected if sampled at the wrong time and this may also be true of small newts. This study sought to determine the changes in relative abundance of small newts via torchlight sampling across time relative to sunset. Samples were undertaken throughout the breeding season in order to determine whether smooth or palmate newts showed a similar pattern of differential abundance throughout the survey period as exhibited by great crested newts (Paterson, 2018).

METHODS

The sample site chosen was Gartcosh Nature Reserve in north Lanarkshire (NS 70 68) as it is known that both target species occur there alongside great crested newts (McNeill, 2010; McNeill et al., 2012). Owing to a road development to the north-east of the reserve, access could only be attained to the Bothlin Burn complex of ponds (Fig. 1). This cluster of eight ponds was visited on 17 March 2017 at night and sampled by torchlight to ascertain their suitability for sampling by assessment of accessibility, water clarity, and estimation of small newt numbers. All ponds with the exception of pond B6 which had a thick ring of emergent macrophytes were carried forward for this study.

Ponds were visited on the evenings of 25 March, 8 April, 22 April, 13 May, 26 May, and 17 June 2017 and sampled throughout the night utilising torchlight surveys (as Griffiths et al., 1996). Each pond's accessible perimeter



Figure 1. Gartcosh pond sets showing the six Railway Junction (RJ); seven Garnqueen (GQ); three Stepping Stone (SS); and eight Bothlin Burn (BB) pond cluster locations. Thick dotted line shows the approximate location of a road development which restricted access from the west to the GQ and RJ pond clusters. Inset: Bothlin Burn pond numbers.

was traversed on foot from the same starting point and in the same direction with the water being illuminated by a Clulite Clubman CB3 1,000,000 candlepower LED spotlight. The author undertook the torchlight surveys with an assistant annotating a recording form. This was repeated at hourly, two-hourly, and three-hourly repetition rate treatments in order to control for the effects of torchlight disturbance to the newts (Table 1). Sampling commenced from 30 minutes prior to sunset and continued until sunrise of the following morning. Ponds were visited in the order B1, B2, B3, B4, B5, B8, and B7 including subsets to ensure consistency of timing. Sunset time was determined by the table given on the website timeanddate.com (2017).

Newts (n= 2497) were identified to species and sex where possible utilising secondary sexual characteristics of males (e.g. hind feet webbing, presence and structure of crests, tail filament) to differentiate between species, although a number of records, chiefly of females, which are not distinguishable to species via torchlight, could not be reliably identified (n= 869). However, for statistical purposes, each sample was considered as a discrete unit (n= 174) for both male palmate and smooth newts and for all female small newts. Count data for females and unidentified small newts were excluded from analyses.

At the beginning of each pond sample, water temperature was read in the same approximate location of each pond approximately 10 cm from the shoreline and at approximately 15 cm depth using a TPI digital pocket thermometer. In addition, air temperature was measured approximately 50 cm from the ground at a central location between ponds B1 and B2 (Approx. NS 70575 68400) using a PeakMeter MS6508 digital thermometer. Cloud

Table 1.	Example sampling repetition rate for the Bothlin Burn
complex.	The example shown is the visits undertaken on 16 -
17 June.	

Sample start time (minutes; relative to sunset)	Hourly ponds	Two-hourly ponds	Three-hourly ponds
-30	B1, B2	-	-
+30	B1, B2	B3, B4	B5, B8
+90	B1, B2	B7	-
+150	B1, B2	B3, B4	-
+210	B1, B2	B7	B5, B8
+270	B1, B2	B3, B4	-
+330	B1, B2	B7	-
+390	B1, B2	B3, B4	B5, B8

cover was estimated by the author as the percentage of visible sky obscured by cloud at the beginning of each sample, and the percentage of the visible surface of the moon on that evening was read from the table given by astronomyknowhow.com (2017).

Counts of newts were scaled for comparability whereby the maximum count of each newt species per pond per survey night was considered to be 100% of the potentially detectable population and all other counts from that pond were expressed as a percentage of that count, serving as a measure of population size detection. As sampling of the complete set of ponds lasted up to 50 minutes, visits were staggered and the start time of each can be placed in a half hour window relative to sunset providing the explanatory variable "half hour relative to sunset" as a continuous variable.

Generalised Linear Mixed Models (GLMMs) were built using lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages in R V. 3.4.3 (R Core Team 2017). The dependent variable in all models was the proportional count of newts with separate models built for each of the explanatory variables incorporating half hour relative to sunset, moon percentage, cloud percentage, air temperature, and water temperature. GLMMs were then built incorporating interaction terms test the effects on proportional abundance of newts from the repetition rate treatment, cloud percentage, air temperature, and water temperature. A GLMM was also built to test whether there was any relation between the counts of smooth newt males with palmate newt males. All models included the random effects variables of date and pond number. Likelihood ratio tests were utilised to choose the best fitted models via stepwise backwards deletion of variables.

RESULTS

Both smooth and palmate newts were present in each pond within the Bothlin Burn complex (Table 2). Palmate newts were more abundant in every pond than smooth newts with the exception of B3. A significant, positive relation was found between the numbers of male palmate newts detected, and the numbers of male smooth newts detected (r^2 = 0.41, $F_{(1.160)}$ = 19.7, p= <0.001; Fig. 2).





Figure 2. Counts of male smooth and palmate newts showing a significant positive relation between the counts achieved for both species (r^2 = 0.41, $F_{(1.160)}$ = 19.7, p= <0.001)



Figure 3. Maximal counts of male palmate newts by visit. Peak counts were achieved during the visit on 13th May with a peak count of 27 in pond B1. Plotted curve shows the best fit from a 2nd order polynomial model.



Figure 4. Palmate newt male counts expressed as a percentage of the maximum count achieved per pond per evening shown as boxplots splitting the counts in to quartiles with the thick black horizontal line at each serving as the marker of the median with boxes either side showing the range of the core 50% of data. Dotted lines represent the outer 50% of data and clear dots represent outlying data points. Horizontal lines represent 70, 80, and 90% of the maximum count achieved per evening. Detection of male palmate newts over time showed a more nocturnal pattern of activity with peak abundance at 159 minutes after sunset (vertical line) best fit by a 3rd order polynomial model (curve; r^2 = 0.24, $F_{(3.32)}$ = 9.54, p= <0.001).

Table 2. Maximum counts of small newts of the Bothlin Burn ponds complex. Small newt females are either smooth or palmate newts which could not be identified to species and were excluded from further analyses.

Pond no.	Palmate male	Smooth male	Small newt female
B1	27	9	15
B2	24	15	28
B3	11	16	14
B4	6	3	8
B5	17	8	9
B7	21	7	10
B8	22	7	8

Detection of male palmate newts

Palmate newts were present in broadly similar numbers on every visit with the exception that on 17 June very few were encountered. The peak count was achieved in pond B1 on 13 May (Fig. 3). Palmate newt counts showed a nonlinear relationship with time relative to sunset best fitted by a 3rd order polynomial with a peak of abundance at 159 minutes following sunset ($r^2=0.24$, $F_{(3,32)}=9.54$, p=<0.001; Fig. 4).

Palmate newt males showed peak counts at an air temperature of 6.2 °C ($r^{2}=0.36$, $F_{(2,14)}=11.55$, p=<0.001) and were best fitted by a 2nd order polynomial model (Fig. 5a); the peak counts for three of the sample ponds were achieved below 5 °C, though peak counts per pond were distributed over a range of air temperatures (Fig. 5b). Water temperature also impacted on detection rates of palmate newts with a peak of detection at 12.8 °C ($r^{2}=0.38$, $F_{(4,13)}=9.42$, p=<0.001; Fig. 5c) best fitted by a 4th order polynomial model; peak palmate newt counts per pond were all achieved above 10 °C and clustered around the 12.8 °C (peak (Fig. 5d).

No significant relation was found between the relative abundance of male palmate newts and the percentage visible sky covered by cloud ($r^2 = 0.38$, $F_{(1,22)} = 0.78$, p = 0.39) or by the percentage phase of the moon ($r^2 = 0.39$, $F_{(1,11)} = 0.44$, p = 0.52). No interaction was found between the best-fitted 3rd order polynomial for abundance of palmate males over time with air temperature ($r^2 = 0.26$, $F_{(3,36)} = 0.84$, p = 0.48), water temperature ($r^2 = 0.23$, $F_{(3,151)} = 0.81$, p = 0.49), or repetition rate treatment of the ponds ($r^2 = 0.26$, $F_{(3,147)} = 0.65$, p = 0.59).

Detection of male smooth newts

Smooth newts showed a distinct peak of presence within the pond during April, with the peak count from pond B3 on 8 April. No smooth newts were recorded during the June visit (Fig. 6). Smooth newts showed a distinct crepuscular pattern of maximal counts with a peak abundance at 62 minutes after sunset followed by a low count at 396 minutes after sunset best fit by a 3rd order polynomial (r^2 = 0.37, $F_{(3,113)}$ = 4.42, p= <0.01; Fig. 7).

Smooth newts were most abundant at an air temperature of 3.8 °C ($r^2 = 0.38$, $F_{(2,20)} = 5.28$, p = <0.05) and were best fitted by a 2nd order polynomial (Fig. 8a). Peak counts of



Figure 5. Counts of male palmate newts by temperature. Male palmate newts were statistically most abundant at an air temperature of 6.2 °C (a,b: solid vertical line) and a water temperature of 12.8 °C (c,d: solid vertical line); hashed vertical line shows the 5 °C reported critical minimum for newt activity (Verrell & Halliday, 1985). Plot **(a)** shows all palmate newt counts plotted against air temperature best fitted by a 2nd order polynomial (curve) peaking at 6.2 °C (r^2 = 0.36, $F_{(2,14)}$ = 11.55, p= <0.001); **(b)** shows the peak counts achieved for each pond plotted against the air temperature with the polynomial fit from (a) shown; **(c)** shows all palmate newt counts plotted against water temperature best fitted by a 4th order polynomial (curve) peaking at 12.8 °C (r^2 =0.38, $F_{(4,13)}$ = 9.42, p= <0.001); and **(d)** shows the peak counts of palmate newts achieved at each pond plotted against the water temperature with the curve showing the polynomial fit from (c).







Male Smooth Newt Detection Over Time





Figure 8. Counts of male smooth newts by temperature. Male smooth newts were statistically most abundant at an air temperature of 3.8 °C (a,b: solid vertical line) and a water temperature of 10.7 °C (c,d: solid vertical line), red hashed vertical line shows the 5 °C reported critical minimum for newt activity. Plot (a) shows all smooth newt counts plotted against air temperature best fitted by a 2nd order polynomial (curve) peaking at 3.8 °C (r^2 = 0.38, $F_{(2,20)}$ = 5.28, p= <0.05); (b) shows the peak counts achieved for each pond plotted against the air temperature with the polynomial fit from (a) shown; (c) shows all smooth newt counts plotted against water temperature best fitted by a 3rd order polynomial (curve) peaking at 10.7 °C (r^2 = 0.49, $F_{(3,21)}$ = 4.17, p= <0.05); and (d) shows the peak count of smooth newts achieved at each pond plotted against the water temperature with the plotted curve showing the polynomial fit from (c).

smooth newts were achieved over a range of temperatures, and multiple peak counts for three of the ponds were achieved at contrasting temperatures (Fig. 8b). Smooth newts were most abundant at 10.7 °C water temperature (r^2 = 0.49, $F_{(3,21)}$ = 4.17, p= <0.05) best fitted by a 3rd order polynomial (Fig. 8c) and all peak counts per pond were achieved above 5 °C though they were achieved over a wide range of temperatures (Fig. 8d).

No significant relation was found between proportional smooth newt abundance and percentage cover of cloud (r²= 0.30, $F_{(1,81)}$ =0.13, p=0.73), nor percentage of moon (r²=0.45, $F_{(1,10)}$ =0.08, p=0.78). No interaction was found between the best-fitted detection polynomial with air temperature (r²= 0.36, $F_{(3,96)}$ = 0.51, p= 0.68) or water temperature (r²= 0.36, $F_{(3,107)}$ = 0.81, p= 0.49), and the repetition rate of surveys also had no significant interaction with relative detection period (r²= 0.38, $F_{(3,148)}$ = 0.63, p=0.59).

DISCUSSION

That there was a significant relation between the counts of palmate and smooth newts (Fig. 2) is suggestive that there may be no temporal partitioning of shared resources. However, smooth newts were most abundant during early to late April (Fig. 6) and palmate newts in late April through May (Fig. 3) which may be evidence of a seasonal partitioning of pond use. As other authors have shown high overlap in food resource and habitat use (Griffiths, 1987), there may be a lack of partitioning owing to a "preyunlimited" situation wherein there is enough prey for all the predators to successfully exploit a shared resource (Akani et al., 2008; Griffiths, 1986). However, as no niche modelling was undertaken, this aspect cannot be explored further. Future studies should aim to model niche breadth and occupancy with a view to ascertaining how segregation occurs.

The data presented here on the crepuscular nature of smooth newts accords with observations by other authors (Himstedt, 1971; Dolmen, 1983a & 1983b; Griffiths, 1985; Dolmen, 1988). However, there continued to be high counts achieved throughout the night (Fig. 7) which may be suggestive of behavioural changes. This could be explained if newts were most visible whilst displaying in the open at dusk and dawn, but continued to be active as they fed throughout the night amongst vegetation where they may be obscured (Griffiths, 1985). Palmate newts showed a more nocturnal pattern of abundance, though there was distinct variability in detection proportion throughout the evening. However, this cannot be explained by this experimental protocol and would warrant further exploration wherein recording of behaviour and spatial distribution may provide further explanation. Palmate newts achieved highest counts between sunset and approximately 240 minutes following (Fig. 4) and smooth newts achieved highest counts between sunset and 150 minutes following (Fig. 7). As such, I propose that surveys for small newts take place between sunset and approximately 150 minutes after sunset.

Griffiths (1984) showed that air temperature had a significant relation to finding smooth newts under rocks, with a peak between 9-11 °C. This shows a strong similarity

to the water temperature of peak smooth newt detection in this study (10.7 °C; Fig. 8). However, air temperature of peak detection was 3.8 °C which may suggest that air temperature is less relevant to newt activity when they are in the aquatic phase as when the water temperature dropped towards 5 °C abundance fell (Fig 8). A similar relation was found for palmate newts wherein the peak counts were achieved at a water temperature of 12.8 °C and an air temperature of 6.2 °C, with activity appearing to remain high below 5 °C air temperature, but dropping as the water temperature fell towards 5 °C.

Neither smooth nor palmate newts showed any relation between cloud cover and percentage of visible moon surface unlike that which was shown by Deeming (2008). This may suggest that small newt activity rhythms could be controlled by some other cue than moonlight, for example circadian clocks have been shown to alter with temperature and day length (Majercak et al., 1999).

These data were collected close to the northern fringe of the range for both smooth and palmate newts. It has been found in several other amphibian species at the fringes of their populations that they can show differential developmental and behavioural responses to conditions which differ from core range populations (Brattstrom, 1968; Orizaola & Laurila, 2009; Orizaola et al., 2010, Muir et al., 2014a; Muir et al., 2014b). As a consequence of this, it would be advantageous to repeat this study in southern or central parts of both species ranges. Additionally, great crested newts have been shown to have differing abundance peaks for males and females (Paterson, 2018). However, in this study females were not assessed and so it is possible that female small newts could also show differing peak count periods than males.

Through standardisation of data collection by means of a standardised survey window and by standardised count method, then data collected between ponds at different sites could be comparable and thus provide an overview of the relative importance of breeding pools (Paterson, 2018). The author would propose that surveyors seeking to achieve optimal counts of small newts via torchlight sampling should seek to visit their pond of interest between sunset and c. 150 minutes after sunset during mid April on evenings where water temperatures could be expected to be at least 10 °C.

ACKNOWLEDGEMENTS

I would like to thank my project supervisor Roger Downie for support and constructive review throughout as well as to Debbie McNeill, Nosrat Mirzai, Steven Allain, Victoria Muir, Daniel Haydon, Sofie Spatharis, Graham Sennhauser, Struan Candlish, Hannah Williams, Rosanna Mooney, Bradley Fairclough, and Aisling Gribbin for their assistance with various aspects of this project. Additionally, I'd like to thank two anonymous reviewers for providing useful comment which greatly improved this manuscript.

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Accepted: 2 August 2018

Gerarda prevostiana (Serpentes: Homalopsidae) in Sri Lanka: Distribution and behaviour

SURANJAN KARUNARATHNA¹, THILINA SURASINGHE^{2*}, MADHAVA BOTEJUE³ & MAJINTHA MADAWALA⁴

¹Nature Explorations and Education Team, No: B–1 / G–6, De Soysapura Flats, Moratuwa 10400, Sri Lanka
 ²Department of Biological Sciences, Bridgewater State University. 24 Park Ave, Bridgewater, MA 02325, USA
 ³Biodiversity Conservation Society, No: 150/6, Stanly Thilakaratne Mawatha, Nugegoda 10250, Sri Lanka
 ⁴South Australian Herpetology Group, South Australian Museum. North Terrace, Adelaide, SA 5000, Australia
 *Corresponding Author Email: tsurasinghe@bridgew.edu

ABSTRACT - The homalopsid snake *Gerarda prevostiana* is known from parts of southern Asia and lives in coastal brackish-water habitats. For six years we surveyed 50 such habitats around the coastline of Sri Lanka and recorded the distribution and behaviour of this species. We also observed captive *G. prevostiana* to understand microhabitat use and behaviour. During the fieldwork, we recorded three adult male *G. prevostiana* along the west coast within intertidal mangrove swamps and a tidal river. The low rate of encounters suggests that the snake is difficult to detect due to a very secretive nature and possibly a patchy distribution. The localities where *G. prevostiana* was found in Sri Lanka had an abundance of mud crabs (*Scylla* species), and mud lobsters (*Thalassina* species) that make up the snake's diet as well as crab mounds that are used as refugia. Captive *G. prevostiana* remained inactive in underwater refugia during day-time and only become active at night. The snake's ability to remain underwater for prolonged periods and its prehensile tail are noteworthy. Coastal development, destruction of mangrove swamps, and loss of coastal wetlands may pose a significant threat to *G. prevostiana*. To date both in Sri Lanka and India, *G. prevostiana* has only been recorded from west coasts, where it is suggested that habitats are more favourable than the east coasts. Likewise, it appears that *G. prevostiana* is also restricted to the westerly coasts of countries in the Bay of Bengal and Gulf of Thailand.

INTRODUCTION

erard's Water Snake, Gerarda prevostiana (Eydoux U& Gervais, 1837) is a rear-fanged, mildly venomous snake mostly found in mangrove swamps (De Silva, 1980; Porei, 2001). It is restricted to the Indomalayan biogeographic region (Murphy & Voris, 2014; Whitaker & Captain, 2004; Murphy, 2007; Das et al., 2013). In India it is known from scattered populations along only the west coast, including Gujarat, Maharashtra and Kerala (Vyas et al., 2013). At the start of the current study, G. prevostiana in Sri Lanka was documented from seven museum specimens also from only the west coast (Ferguson, 1877; Haly, 1886; Gyi, 1970; de Silva & de Silva, 1986-87; de Silva, 1990; Christie & White, 1997; Somaweera, 2005; Somaweera et al., 2006; Murphy & Voris, 2014) or from fishing bycatch trawled from shallow open sea 1 km off the northwest coast (Ukuwela et al., 2017). Although listed "Least Concerned" in the Global IUCN Red List (Murphy, 2010), it is considered "Endangered" in the Red List of threatened fauna and flora of Sri Lanka (MoE, 2012). The snake is predominantly nocturnal (Das et al., 2013) and feeds primarily on freshly-moulted crabs by tearing larger body parts into smaller pieces prior to ingestion via "loop and pull" behaviour (Jayne et al., 2002; Chen, 2010). Given its purported rarity and Red List status of G. prevostiana in Sri Lanka, the collection of detailed information on its ecology, natural history, and behaviour is essential for implementing science-based conservation.

In this study of *G. prevostiana* there were three aims, 1) to document the presence of the species in different aquatic habitats on the coastal plains of Sri Lanka, 2) explored



Figure 1. Previous and new records of *G. prevostiana* in Sri Lanka (1, dry zone; 2, wet zone; 3, intermediate zone; 4, arid zone)

Table 1. Morphometric and meristic data of *G. prevostiana* specimens in Sri Lanka; ⁽¹⁾ ZPM/W14A/047, University of Peradeniya; ⁽²⁾NMSL 2013.18.01 and ⁽³⁾ NMSL 2013.19.01, National Museum, Colombo, Sri Lanka

	Source/references of the specimen								
Character/Measurement	Somaweera et al. (2006) ¹	Ukuwela et al. (2017) ²	Ukuwela et al. (2017) ³		Current study				
Locality Name	Kalaoya	Vankale	Vankale	Thirikkapallama	Maduganga	Bolgoda			
Head length (mm)	18.1	15.3	14.7	15.8	16.7	16.2			
Snout vent length (mm)	348	168.6	151.5	187.2	216.3	198.1			
Tail length (mm)	64	27.2	24.7	28.3	28.9	28.6			
Preoculars (L/R)	2	1/1	1/1	1/1	1/1	1/1			
Postoculars (L/R)	2	2/2	2/2	2/2	2/2	2/2			
Supralabials (L/R)	7	8/8	8/8	7/7	8/8	7/7			
Infralabials(L/R)	7	7/7	8/8	7/7	8/8	8/8			
Anterior temporals (L/R)	1	1/1	1/1	1/1	1/1	1/1			
Posterior temporals (L/R)	2	2/2	2/2	2/2	2/2	2/2			
Loreal (L/R)	1/1	1/1	1/1	1/1	1/1	1/1			
Internasal	1	1	1	1	1	1			
Dorsal scale rows (anterior)	17	17	17	17	17	17			
Dorsal scale rows (mid-body)	17	17	17	17	17	17			
Dorsal scale rows (posterior)	15	15	15	15	13	15			
Ventral scales	153	154	159	151	163	156			
Subcaudals (divided)	33	37	33	37	35	37			
Anal	divided	divided	divided	divided	divided	divided			
Pre Anal	entire	entire	entire	entire	entire	entire			

various aspects of its natural history such as habitat associations and behaviour, and 3) investigate threats to provide some insights into better conservation.

MATERIALS AND METHODS

Fieldwork and data collection

We conducted an opportunistic (non-systematic) six-year field survey (2010–2015) targeting G. prevostiana at 52 distinct locations (3000 man-hours in total) in brackish water habitats around the entire coastal plain that encircles Sri Lanka (Fig. 1). We sampled 29 locations on the west coast and 21 elsewhere - north (7), south (8) and east (6). We made repeated visits to 20 west coast locations. Our survey of the coastal plain included the four recognised bioclimatic zones of Sri Lanka (Fig. 1), nearshore aquatic environments and inland tidal rivers. To document the focal species activities, surveys were undertaken in the morning (08.00–11.00h) and after sunset (19.00–22.00h). At each site, we surveyed a total area (both aquatic habitat and the adjacent uplands, including the entire tidal zone) of 2 ha for ~4 hours by visually scanning for surface-active snakes and turning all cover objects for hidden snakes. At each survey location, we measured the average water depth at low tide, average day-time and night-time temperature (using a Digitech QM 1594, 6 in 1 multifunction environment meter, Digitek Instruments Co., Ltd, Hong Kong, China), and recorded the locality and the dominant vegetation types.

We used standard field guides to verify species identification (Whitaker et al., 2004; Somaweera, 2006).

For each *G. prevostiana* captured, we recorded the 18 morphometric and meristic features listed in Table 1, we recorded the same for two preserved specimens from the National Museum of Sri Lanka (NMSL). For any measurements, a digital Vernier calliper or tape measure was used. We sexed captured snakes by everting hemipenes and photographing them.

Mesocosm study

To investigate the behaviour and habitat use of G. prevostiana each of the three male snakes captured during the survey was placed in a small (120 cm long \times 70 cm widex 50 cm high) glass tank. To make the enclosure resemble some aspects of the natural environment of this species, it contained fine sand, silt, leaf litter, small rocks (20–40 mm), two medium-sized logs (48.5 cm in length, 12.5 cm in diameter) as bottom substrates, and brackish water filled up to a depth of 20 cm. After a two-day acclimatisation period, we documented the position of the snake (microhabitat and substrate used) for a total of 8 h per day (03.00–07.00h and 18.00–22.00h) for five continuous days. In each day of the experiment, we offered the snake the following food items: Awaous melanocephalus (largesnout Gobies, average body length: 30 mm); Oreochromis niloticus (Nile tilapia, average body length: 40 mm); freshwater shrimps of genus Machrobrachium (average body length: 30 mm); and, freshwater crabs of the genus Perbrinckia (average body length: 22.5 mm) collected from coastal lagoons. At the end of this test the snakes were released back to the site of capture.

Location and Reference	Date	Coordinates	Closest Town	Distance from shore	Bioclimatic zone	Floral community	Wat Tempera	ter ature °C
							Day	Night
Sri Lanka (Ferguson 1877)	1877	-	_	-	-	-	_	-
Kelani River (Haly 1886)	1886	-	Ja–Ela	-	Wet zone	-	-	-
Sri Lanka Gyi (1970)	1970	-	-	-	-	-	-	-
Kala–oya Somaweera et al. (2006)	1985	-	Eluwankulam	-	Dry zone	Mangrove	-	-
Dandugan–oya Porej (2001)	1997	-	Muthurajawela	-	Wet zone	Marsh	-	-
Doowa Somaweera (2005)	1999	-	Negombo	-	Wet zone	Mangrove	-	-
Vankale (Ukuwela et al. 2017)	2011	08.893550 N 79.928656 E	Mannar	-	Dry zone	Mangrove	-	-
Thirikkapallama (Current study)	12 Dec 2011	08.182692 N 79.823861 E	Eluwankulam	3 km	Dry zone	Mangrove	29.2	27.3
Bolgoda (Current study)	03 Aug 2014	06.699522 N 79.972239 E	Bandaragama	6 km	Wet zone	Mangrove	28.8	26.5
Maduganga (Current study)	24 May 2015	06.273294 N 80.037844 E	Balapitiya	50 m	Wet zone	Mangrove	28.6	27.2

Table 2. Distribution of G. prevostiana in Sri Lanka: previous records and those contributed by the current study



Figure 2. A live specimen of *G. prevostiana* from Maduganga River

RESULTS

During the survey we found only three specimens of G. *prevostiana* (Fig. 2, Fig. 4 a–d) and these were at distant sites along Sri Lanka's western coastal plains (mean elevation 3 m above sea level) (Table 2, Fig. 1). All specimens were male and were captured after sunset (18.00–20.00h) in new moon conditions at low–tide. All three snakes were of similar length (mean 229 mm) and resembled previous records in terms of both morphometric and meristic features (Table 1).

All our new records were made in mangrove swamps with muddy substrates in the coastal intertidal zone (Table 2, Fig. 3a–c). One record was in the dry zone of Sri Lanka while the rest were in the wet zone. In the same habitats, we observed small–sized (15–25 mm) mud crabs (*Scylla* species) and medium–sized (50–85 mm) mud lobsters (*Thalassina* species) as well as mud–lobster mounds. Details of the three new record localities are shown in Table 2. The first record, in Thirikkapallama fishing village (Fig. 3a), was in the north-western coastal arid zone (annual

average precipitation <2,000 mm). The locality was a mangrove swamp (low-tide depth: 0.4 m, muddy-bottom substrate) where Avicenia marina, Bruguieria cylindrica, Rhizophora mucronata, Scyphiphora hydrophyllaceae, and Sonneratia alba were the dominant flora. The second record was at the Bolgoda River (Fig. 3b), 6 km inland from the seashore in the south-western coastal wet zone lowlands. Among the new localities, this was the most inland locality, and located furthest from the shoreline. This locality comprised mostly mangrove vegetation (lowtide water depth: 1.3 m, muddy bottom) dominated by Sonneratia caseolaria, Excoecaria agallocha, Rhizophora apiculata, and Xylocarpus granatum. The third record was from the Maduganga River in the southern coastal wet zone lowlands (Fig. 3c). The locality was a mangrove swamp (average mid-tidal water depth: 0.8 m) dominated by Rhizophora apiculata, Bruguiera gymnorrhiza, Sonneratia caseolaria, Xylocarpus granatum, Lumnitzera littorea, and Excoecaria agallocha.

Observations from the mesocosm study

During the early morning (03.00-07.00h), the snakes remained relatively inactive, and mostly submerged. They retreated under the logs, remained in a coiled position, and resurfaced to breath at various time intervals with individuals resurfacing after only 7.2 mins or after as long as 1.4 h. For breathing, the snakes lifted their nostrils immediately above the water surface, and retreated back after 1-2 secs of inhalation. In contrast, during night-time (18.00-22.00h), the snakes spent much less time underwater (an average of 3.8 mins, range: 1.67-7.42 mins) and emerged from refugia under the logs and remained active by continuously swimming, and even moved onto the log above the water level. All snakes refused the food we provided. We observed tongue-flicking behaviour both above and below water. Above-water tongue-flicking was limited to nighttime where the snakes emerged onto the logs. The snakes also performed tongue-flicking when they were active underwater particularly during the night-time.



Figure 3. Mangrove habitats of G. prevostiana in Sri Lanka: (a) Thirikkapallama, (b) Bolgoda River, (c) Maduganga RAMSAR wetland



Figure 4. Close–ups of *G. prevostiana*: (a) ventral side of the head; (b) lateral side of the head; (c) dorsal side of the head; (d) ventral side of the body; (e) prehensile tail with the knot; (f) prehensile tail; (g) tail locking

Natural history and behaviour

Our observations suggested that the snake's surface activity was exclusively nocturnal. When handled, none of the captured snakes bit nor were aggressive regardless of the time of the day. Their terrestrial locomotion was quite weak, but they were excellent swimmers in both open water and in the heavily-vegetated littoral zone. When disturbed on land, the snakes coiled-up, hid their head within their coils, and remained motionless. We also noted that their tail was robust and prehensile. They can constrict their tail firmly around objects and suspend themselves from their tails (Fig. 4e-g). Moreover, they even dragged objects (12–47 g in weight) using their tails (Fig. 4g). Our observations

indicated that our focal species is sympatric with other native aquatic snakes– *Xenochrophis piscator* (checkered keelback), *Atretium schistosum* (olive keelback), *Cerberus rynchops* (dog–faced water snake), *Hydrophis schistosus* (beaked sea snake), and *Hydrophis viperinus* (viperine sea snake).

DISCUSSION

Gerarda prevostiana is a poorly studied snake in both Sri Lanka and India. In Sri Lanka this species was previously known from seven records (Table 2). Three new records have been added by the current study, two of which are from more southerly sections of the west coast than previously (Bolgoda River and Maduganaga River delta) and suggest that the species may occur along much of the west coast (Fig. 1). Since we documented G. prevostiana across multiple bioclimatic zones, it is unlikely that this snake's distribution is restricted to any of the four recognised bioclimatic zones of Sri Lanka. Instead, prey availability (crustaceans) and mangrove habitats seemed to be the primary determinants of this species. To date all records of G. prevostiana have been from the west coast, despite the presence of significant mangrove habitats along the other coastlines. The more intensive survey effort on the west coast could have biased the result and further surveys are required in other coastal areas where there are mangrove habitats with high densities of crustaceans. However, the fact that in India G. prevostiana is also apparently restricted to the west coast (Murphy, 2010) suggests that our observations do not result from under sampling. The west and east coasts of the two countries are almost contiguous, separated only by the narrow Gulf of Mannar and Palk Strait, giving a distribution of G. prevostiana along the west coast adjacent to the Arabian Sea, from the Gulf of Kutch southwards. In India, the west coast distribution of the species is attributed to more favourable geological, hydrological and climatic conditions. The western coastlines of both Sri Lanka and India are climatically more stable (fewer tornados), receive a greater volume of precipitation year around, and are rich in backwater systems, lagoons, and estuaries, which are ideal physical environments for mangroves (Mag & Sengupta, 1992). Further, the west coast of Sri Lanka has more river deltas, thus sustains a greater freshwater influx, including nutrients and sediments (Survey Department of Sri Lanka, 2007). It may not be a coincidence that the apparent bias for a west coast distribution for G. provostiana is noticeable for the countries bordering the Bay of Bengal and Gulf of Thailand. In the Bay of Bengal, the species has been recorded from the south-east coast of Bangladesh (Rahman et al., 2014), coastal Myanmar (Murphy, 2014) and the west coast of peninsula Malaysia (Tweedie, 1983) to the north coast of Singapore (Karns et al., 2002). Likewise in the Gulf of Thailand, there are no records from the east coast of peninsular Malaysia (Tweedie, 1983) or Thailand until further north in the inner Gulf of Thailand (Chan-ard et al., 2015) and then coastal Cambodia (Murphy, 2014). It may well be that the apparently more favourable conditions of the west coast adjacent to the Arabian Sea are similarly more suitable to G. provostiana in the more westerly coasts adjacent to the Bay of Bengal and Gulf of Thailand.

In general, homalopsids are very secretive and sedentary, which accounts for their low detectability (Karns et al., 2002; Voris & Murphy, 2012). We failed to document *G. prevostiana* in 94% of the locations surveyed and this suggests that the species is rare along the Sri Lankan coastal plains and/or like other homalopsids is difficult to detect. *Gerarda prevostiana* is known to retreat into tidal burrows (Vyas et al., 2013) and although such habitats were searched in our survey, this behaviour would have contributed to low detectability. Nevertheless, this species has been recorded in high abundance at a coastal urban park in Singapore (Karns et al., 2002).

Most previous specimens were documented in coastal habitats with ample mangrove vegetation, such as lagoons, estuaries, and coastal marshes (Voris & Murphy, 2012). These observations are consistent with the natural history of other homalopsids which are associated with muddy substrates and also occupy nearshore coastal wetlands. A specimen documented by Gyi (1970) was the most inland location known for *G. prevostiana* indicating that our focal species can roam inland along aquatic habitats. The specimen we documented in Bolgoda River confirms that *G. prevostiana* could inhabit inland aquatic habitats that are influenced by tidal currents.

During our survey, we failed to make any observations on the foraging ecology of the focal species. However, it is considered to be a crustacean specialists, feeding selectively on recently-moulted crabs (Voris & Murphy, 2002; Rahman et al., 2014). While these snakes swallow small prey whole, with the aid of their prehensile tail, they rip apart larger prey into smaller ingestible pieces by "loop and pull" behaviour, which enables these snakes to ingest prey items that are substantially larger than their gape size (Jayne et al., 2002; Chen, 2010). The prehensile tail could also help them anchor onto coastal vegetation and other substrates to avoid being drifted by tidal currents and coastal waves.

According to Sri Lanka's Red List, G. prevostiana is "endangered" (MoE, 2012). Being a specialist of coastal habitats, G. prevostiana is likely to be negatively impacted by a multitude of anthropogenic disturbances affecting the Sri Lankan coastline and mangrove ecosystems, such as urban development, commercialscale aquaculture, drainage of coastal wetlands, amenitybased development, and recreational activities (Christie & White, 1997; Srinivasan et al., 2012). Sri Lankan brackish-water ecosystems are also heavily impacted by pollution (solid-waste discharge, industrial effluvia, urban runoff, siltation, and nutrient loading) (Dayaratne et al., 1995; Dahanayaka & Wijeyaratne, 2010; Karunarathna et al., 2011). Given the dependency of G. prevostiana on mangrove ecosystems, anthropogenic threats encountered in mangrove ecosystems, and food specialisation, we strongly recommend that the conservation status of G. prevostiana in Sri Lanka be retained as "Endangered". Moreover, Ukuwela et al. (2017) indicated that the Sri Lankan populations of G. prevostiana was genetically distinct from the South-east Asian populations, therefore, we recommend separate assessments of conservation status for those two populations.

ACKNOWLEDGMENTS

We would like to thank N. Karunarathna, D. Gabadage, H. Sanjeeva, C. Soysa, T. Peiris, H. Sanjeewa, A. de Silva, K. Ukuwela, T. Amarasinghe, M de Silva and village fishing communities (A. Kumara, T. Silva, L. Silva, N. Bolgoda) for their support. Also, we would like to give our special thanks to S. Kasthuriarachchi, N. Wickramasinghe, M. Nandasena, L. Somaratne, C. Munasinghe and R. Dasanayake (NMSL) for assistance during examining collections under their care; P. Abeykoon, H. Wickramasinghe, D. Perera, M. Ranaweera and Biodiversity Secretariat of Sri Lanka for partial financial assistance. The Animal Care Protocols used for this research was approved by IACUC of Bridgewater State University (Permit Number 2016–02), MA.

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Accepted: 2 August 2018

Low occurrence of the great crested newt *Triturus cristatus* at the limits of its range: an alarming preliminary study

CELIA GRILLAS^{1*}, ELIOT UGNON-COUSSIOZ¹, PAULINE PRIOL² & JULIEN RENET³

¹Parc naturel régional de Camargue, Mas du Pont de Rousty, 13200 Arles, France ²StatiPOP, 34190 Ganges, France ³Conservatoire d'espaces naturels de Provence-Alpes-Côte d'Azur, Pôle Biodiversité régionale, appartement 5, 96 rue Droite, 04200 Sisteron, France ^{*}Corresponding author Email: c.grillas@parc-camargue.fr

ABSTRACT - The lower Rhône valley constitutes the southern limit of the great crested newt's (*Triturus cristatus*) global distribution range. The initial objectives of this study were to confirm historical presence data, to identify potential new great crested newt populations and to characterise their habitats (with a Habitat Suitability Index) in order to assess their status in the lower Rhône valley. Despite the use of four different survey methods (eDNA, Ortmann traps, collapsible nylon traps, amphibian dip net), great crested newts were not detected except for one known population in Arles. HSI value results highlight the poor quality of wetlands in the studied area. Furthermore, the invasive crayfish *Procambarus clarkii* (detected in 60% of ponds) and the presence of fish (detected by sight in 50% of ponds) are known to have a negative impact on habitat quality and amphibian populations. These limiting factors might be one explanation for the critical conservation state of the *T. cristatus* Rhône populations.

INTRODUCTION

mphibian populations are in decline worldwide (Collins & Storfer, 2003; Stuart et al., 2004). This has led to public concern, particularly because amphibians are indicator species of wetland quality (Welsh & Ollivier, 1998). The great crested newt, Triturus cristatus, is a large urodele species with a wide distribution across central and northern parts of Europe. Despite this wide distribution, southern populations have recently declined significantly in Europe (Gasc et al., 2004; Edgar & Bird, 2006; Denoël, 2012). The middle and lower Rhône valley constitutes the southern limit of its distribution range in France and in the World. Unfortunately during the 20th century, development of hydraulic infrastructure along the river reduced the area of associated functional wetlands. In this southern valley, the species is rare and only a few relict populations have been recorded in Bouches-du-Rhône (Brogard et al., 1996), in Gard (Brogard et al., 1996; Gendre & Rufray, 2005; Gendre et al., 2006; Geniez & Cheylan, 2012), in Vaucluse (Mourgues, unpublished data); in Ardèche (Parrain, 2005; Grossi, 2015) and in Drôme (Parrain, 2005; Parrain, 2010; Grossi, 2015). Among the recorded populations, some have been regularly monitored since the 1990's. For the others, the latest positive data are from the early 2000's or before. The objectives of this study were to confirm those historical presence data, to identify potential new great crested newt populations and to characterise their habitats in order to assess their status in the southernmost part of the Rhône valley.



Figure 1: *Triturus cristatus* populations in the southern Rhône valley and location of studied sites; black dots: prospected historical sites; white dots: prospected non historical sites; diamond: presence of crested newts in 2017 in prospected sites; black stars: known populations outside of the studied area from Geniez & Cheylan (2012) and Grossi (2015). Box at top left: Worldwide distribution range of *T. cristatus* (Esri, USGS, NOAA | Sources: Esri, Garmin, USGS, NPS | IUCN)

Table 1. Results of field survey site by site; H.P.: Historical presence; L.D.: Last data; P.P.: Prospecting pressure (number of survey methods used); HSI score: Habitat Suitability Index score; P.S: Pond suitability (based on HSI score); P.A: Presence (1) – absence (0) of *T. cristatus*

Id	Latitude	Longitude	H.P.	L.D.	P.P.	HSI score	P.S.	P.A.
1	43°32'51.468" N	4°20'47.580" E	No	-	4	0.39	Poor	0
2	43°32'48.660" N	4°20'54.564" E	Yes	2006	4	0.37	Poor	0
3	43°32'47.364" N	4°21'17.892" E	No	-	2	0.44	Poor	0
4	43°34'57.252" N	4°26'58.848" E	No	-	4	0.63	Average	0
5	43°35'52.116" N	4°28'20.280" E	Yes	2002	1	0.54	Below average	0
6	43°37'4.6560" N	4°25'40.548" E	No	-	4	0.4	Poor	0
7	43°37'11.676" N	4°25'36.948" E	No	-	4	0.62	Average	0
8	43°37'30.864" N	4°25'43.608" E	No	-	4	0.49	Poor	0
9	43°41'21.768" N	4°35'40.920" E	Yes	2006	4	0.29	Poor	0
10	43°40'32.952" N	4°36'36.720" E	Yes	2016	2	0.37	Poor	1
11	43°33'34.200" N	4°41'42.360" E	No	-	1	0.44	Poor	0
12	43°33'26.424" N	4°41'42.936" E	No	-	2	0.53	Below average	0
13	43°33'14.148" N	4°41'58.272" E	No	-	3	0.63	Average	0
14	43°43'30.936" N	4°37'6.6360" E	No	-	2	0.33	Poor	0
15	43°44'43.188" N	4°37'9.1920" E	No	-	1	0.25	Poor	0
16	43°45'17.244" N	4°36'59.112" E	No	-	4	0.54	Below average	0
17	43°45'56.628" N	4°37'27.948" E	Yes	2002	4	0.57	Below average	0
18	43°46'7.6080" N	4°37'26.256" E	No	-	4	0.59	Below average	0
19	43°46'42.492" N	4°37'54.336" E	No	-	3	0.57	Below average	0
20	43°46'47.424" N	4°37'48.540" E	Yes	2001	4	0.42	Poor	0
21	43°46'46.992" N	4°37'56.676" E	No	-	3	0.41	Poor	0
22	43°46'59.232" N	4°37'50.664" E	Yes	2006	2	0.54	Below average	0
23	43°48'20.772" N	4°37'40.188" E	Yes	2002	4	0.6	Average	0
24	43°48'22.068" N	4°35'43.620" E	Non	-	2	0.39	Poor	0
25	43°47'20.148" N	4°34'30.360" E	Yes	2004	4	0.23	Poor	0
26	43°47'7.5840" N	4°33'34.452" E	Yes	1995	4	0.66	Average	0
27	43°46'45.120" N	4°32'58.632" E	No	-	3	0.61	Average	0
28	43°46'51.492" N	4°32'22.236" E	No	-	2	0.77	Good	0
29	43°58'53.976" N	4°43'42.636" E	Yes	1956/57	4	0.49	Poor	0
30	43°59'8.1240" N	4°43'53.076" E	Yes	1956/57	4	0.64	Average	0

MATERIALS AND METHODS

Studied sites were geo-referenced historic sites located in the lower floodplain of the Rhône River which have not been confirmed in over 10 years or more except for one pond (n°10), a known presence location, which was used in this study as a control site. Also, other peripheral wetlands potentially suitable for *T. cristatus* detected on orthophotographs (photographs geometrically adjusted for perspective) or on exploratory field work were surveyed.

Field surveys started in March and ended in June 2017. In order to maximize and compare detection probability, four techniques were combined:

• Ortmann funnel traps, which can be applied in aquatic

environments for sampling and monitoring amphibian species both at larval and adult stages (Drechsler et al., 2010).

• Collapsible nylon traps ($45 \times 22 \times 22 \text{ cm}$), also used to detect adult or larval stages of amphibian species, particularly *T. cristatus* (Madden & Jehle, 2013). To provide constant access to air, the traps were placed in order to make sure that part of the net always emerged from the water. Both trapping systems were installed at the end of the day and recovered the following morning.

• Dip netting by day, was used to detect the larval stage during the emergence period (May and June).

• Environmental DNA (eDNA) monitoring, is an emerging detection method which uses nuclear or mitochondrial DNA

released from an organism into the environment (Ficetola et al., 2008). This technique is used to detect mostly rare and discreet aquatic species and is very effective for *T. cristatus* detection even at low density (Rees et al., 2014; Rees et al., 2017). We used the VigiDNA SW1 kit from SPYGEN society. We used one kit (20 samples of 100 ml each) for each water body with a surface area < 1 ha.

In total, 16 sites were sampled with all four methods, four sites with three methods, seven sites with two and three sites with only one of the four methods.

To determine habitat suitability for *T. cristatus* we used the Habitat Suitability Index (HSI) (Oldham et al., 2000). The HSI is a geometric mean of ten suitability variables: location, pond area, pond drying rate, water quality, shade, fowl abundance indicator, fish abundance indicator, number of ponds, terrestrial habitat and macrophyte abundance indicator. HSI scores are between 0 and 1, where 1 indicates habitats that best meet the ecological requirements of the focal species (in our case, T. cristatus). This tool can therefore be used to predict likely presence or absence of T. cristatus (O'Brien et al., 2017). In addition of HSI, two other variables that may impact the presence of T. cristatus were collected: presence of Procambarus clarkii, a common invasive crayfish species in the southern Rhône valley, and water turbidity. The detection of other urodele species was possible with three of the four methods used: we noted these species. We surveyed a total of 30 wetlands (Fig. 1). Among these, 12 were historical presence sites for T. cristatus; the other 18 were peripheral ponds or stagnant branches of the Rhône River.

RESULTS

The crested newt *T. cristatus* was detected in only one pond (n°10) with both Ortmann funnel traps and collapsible nylon traps (eDNA was not monitored in this pond). Palmate newts *Lissotriton helveticus* were found in 16% of the surveyed ponds (n=5). *Procambarus clarkii* were detected in 60% (n =18). The turbidity evaluation revealed that water was clear in 33% of wetlands (n=10), cloudy in 30% (n=9) and opaque in 37% of them (n=11). Fish were detected by sight in 50% of the sites (n = 15).

The mean HSI was 0.49 which represents a "below average" mean habitat suitability according to Oldham's et al., (2000) index interpretation. In the only pond where *T. cristatus* was detected, the HSI was 0.37 ("poor"). Of the searched ponds, 73% had a "below average" or "poor" HSI score (< 0.50) (Table 1).

DISCUSSION

This study failed to confirm the presence of *T. cristatus* in the lower Rhône valley, except in an already known population (pond n°10) that had been monitored for several years (Renet & Olivier, 2012). As only the presence of a species can be unequivocally confirmed, its absence can only be inferred with a degree of probability (Kery, 2002). Therefore, it is necessary to remain cautious about these results, for example survey effort was not equal between

all searched sites. Indeed, it was only possible to use all four detection methods in 54% (n=16) of the ponds due to extremely low water levels. However, a minimum of two detection methods were applied in 90% (n=27) of studied ponds and eDNA was monitored in all of them (except for pond n°10). This study failed to identify the populations recorded around 2000 (Callégari & Gendre, unpublished data) despite using multiple, effective detection methods at most sites. Therefore, it is thought very probable that all of those populations are now extinct.

The population of pond $n^{\circ}10$ is the only known remaining population in the lower Rhône floodplain, and also the most southerly in the worldwide distribution range of *T. cristatus*. Currently, the pond is threatened by an urban project that increases the major conservation issues for this population.

Because only one extant population was detected, it was not possible to:

1 - Statistically compare detection methods

2 -Test the reliability of the HSI in a Mediterranean context.

The HSI results from this study suggest wetlands in the studied area are of poor quality for *T. cristatus*. This bad quality might be one explanation for the critical conservation state of the *T. cristatus* Rhône valley populations.

The present study also recorded fish presence on half of the sites but this could be an underestimate considering that there was no fish detection protocol, and that, in contrast to P. clarkii, fish cannot be detected with the traps used in the study. Generally, amphibians are less abundant, and sometimes absent, when predatory fish are present because of the high predation pressure they exert (Hecnar & M'Closkey, 1997; Denoël et al., 2005; Porej & Hetherington, 2005; Hartel et al., 2007). Procambarus clarkii was also present in the majority of surveyed ponds (63%) following its introduction to the lower Rhône valley in 1976 (Rosecchi & Poizat, 1997; Vigneux, 1997). This invasive crayfish is a polytrophic keystone species that can exert multiple pressures on ecosystems by modifying biotic factors. As an example, it has been demonstrated that the introduction of P. clarkii to ponds can reduce macrophyte biomass, and increase the turbidity (Rodríguez et al., 2003; Gherardi & Acquistapace, 2007; Rodríguez-Pérez et al., 2016; Souty-Grosset et al., 2016). The species is also suspected to directly prey upon amphibian larvae (Cruz et al., 2006; Cruz et al., 2008). Despite the likely disapearance of T. cristatus in the studied ponds, L. helveticus populations persisted in five ponds. Among these, P. clarkii was detected in only one. The deleterious actions of the crayfish are no doubt one of the reasons for this observation.

The low HSI scores of the Rhône wetlands highlight that they probably do not fulfil the ecological requirements of *T. cristatus*. The deterioration of those habitats might be only partly explained by the arrival of *P. clarkii* on the national territory. Indeed, long term environmental modifications induced by land-use policies probably affected the resilience of *T. cristatus* populations by isolating them and diminishing the number of shelter ponds within pond networks. With the arrival of exogenous species like *P. clarkii* the situation has deterioated. Moreover, the geographical location of these *T. cristatus* populations is disadvantageous: range limit populations are particularly sensitive to habitat loss and fragmentation (Sexton et al., 2009; Slatyer et al., 2013).

Nevertheless some *T. cristatus* populations have maintained in the middle and lower Rhône Valley outside of the study area. This might be explained by the isolation of those populations from the hydraulic network that has prevented fish or crayfish invasions and their negative consequences on the habitat. Survey efforts must be maintained in order to confirm the results of this one year study. The effort need to be focused firstly on historical sites and on peripheral ponds isolated from the hydraulic network, less likely to be impacted by *P. clarkii*. Subsequently, efforts should be extended to the surrounding area, especially further north where other small isolated populations are known.

ACKNOWLEDGEMENTS

Permission for this programme was issued by order of the Prefet [2017-s-01], according to French law. This study was supported by the "Compagnie Nationale du Rhône (CNR)" and the French State, we thank Nicolas Rabin and Romain Brusson for their support. The authors would like to thank the "Conservatoire d'espaces naturels du Languedoc-Roussillon" and the "SYMADREM" for providing historical data. The authors would also like to thank numerous persons who helped with the field work, with the protocol development or with data analysis: Olivier Scher, Anthony Olivier, Thomas Gendre, Nicolas Bonton, Cyrille Sabran, Vincent Marty, Pierre-André Crochet, Jean-Paul Lena and Emilien Luquet. We also warmly thank Dr. Nigel Taylor, Philippe Geniez and an anonymous reviewer for providing useful comments.

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Accepted: 7 August 2018

In the shadow of mammals: the use of camera traps to investigate the basking behaviour of *Agama mwanzae* in the Serengeti National Park, Tanzania

STEVEN J. R. ALLAIN

Cambridgeshire & Peterborough Amphibian and Reptile Group Author Email: steveallain@live.co.uk

ABSTRACT - Camera trap images from the Snapshot Serengeti project from 2010 were used to investigate the behaviour of rock agamas (*Agama mwanzae*) on the kopjes of the Serengeti National Park, Tanzania. The species was observed only from four locations in the south-west of Serengeti in September/October. The images were used to analyse the total numbers of basking individuals, the proportions basking morning or afternoon, and to determine what other reptile species used the kopjes as refugia or for basking. Many more females were observed basking in the morning and afternoon did not differ but significantly more females were observed basking in the afternoon. Based on this study *A. mwanzae* appears to be virtually the only species inhabiting kopjes in the south-west of the Serengeti National Park but camera traps may be less likely to detect smaller species.

INTRODUCTION

S ince 2010, the Snapshot Serengeti Project has deployed 225 camera traps across an area of 1,125 km² in the Serengeti National Park, Tanzania to help evaluate both spatial and temporal inter-species dynamics (Swanson et al., 2015a). This data has since been analysed by both the wider scientific community and members from the project to provide insights into the project's original aims, related to mammalian carnivores. With such a large dataset available there are of course many questions that can still be answered and fortunately the data is freely available online (Swanson et al., 2015b). The data was the basis of the current study to answer questions regarding the behaviour and activity of the Mwanza flat-headed rock agama (*Agama mwanzae*) and other reptiles which also inhabit the Serengeti National Park.

Static camera traps are a common method used to observe the behaviour of wildlife without influencing its behaviour; they have become more popular in ecological studies since the introduction of commercially available units in the 1990's (Kucera & Barrett, 2011). The advance of technology has also played a crucial role in their uptake with modern camera traps able to collect photos and videos in high quality formats, no matter the weather or time of day. Technological advances in battery technology have also allowed traps to remain in-situ for longer resulting in more capture days per season (Swann et al., 2011). Cameras are usually fitted with infrared or movement sensors which trigger the camera to take a series of photos or a video. Exotherms such as mammals are more likely to trigger such cameras than smaller ectotherms such as reptiles and amphibians (Ariefiandy et al., 2013).

Agama mwanzae is a relatively large diurnal agamid that lives on boulders and rocky outcrops (Spawls et al., 2006).



Figure 1: A male *A. mwazae* basking on a rock outcrop, captured by one of the camera's traps

The species is fairly common in northern Tanzania and the most dominant agamid in terms of abundance; the visually similar red-headed agamid (Agama agama) is absent from the area of this study (Spawls et al., 2006). Mature males have a bright red/pink upper body and a blue lower body (Fig. 1) while females are a grey-brown colour and are less ornate than mature males (Fig. 2). Their large body size combined with easily distinguishable colouration and known behaviour is ideal for posing retrospective questions related to basking and activity. Snapshot Serengeti placed some of their cameras between rocky outcrops and kopjes in the hope of determining whether or not they were being used by mammals as corridors. From the original dataset, 131 capture events containing reptiles were originally identified (Swanson et al., 2015b), they were used as a starting point for this study with the hope of finding more events that the wider scientific community had overlooked.



Figure 2: A female *A. mwanzae* photographed basking on a rock outcrop at the Serengeti National Park, Tanzania

MATERIALS AND METHODS

The dataset came from the 2010 season (June-November) of the Snapshot Serengeti Project, when DLC Covert II camera traps (5 MP CMOS camera with 40° FOV) were being used at 50 cm above ground level. During 2010, only 200 camera traps were used with each of the cameras placed towards the centre of a 5 km² grid cell to allow a systematic coverage (Swanson et al., 2015a). Trapping events are defined by a single series of three photos taken one second apart when the camera traps are triggered. Using the dataset provided by the project online, camera trapping events were investigated in batches of 36 (108 images) in order to look for the presence of reptiles, including the target species A. mwanzae. The events originally identified by the wider scientific community were used to first establish which areas were suitable for use by A. mwanzae and then later to further establish times of the day when it was most likely that the trapping event was triggered by a reptile and not passing grazing mammals. Once all of the appropriate capture events had been identified and the files had been downloaded, the photos from each were then analysed. This involved identifying the species in frame, the maximum number of individuals photographed during the event and their sex (Fig. 1). Abiotic factors such as the time of day and date were also recorded in order to allow later analysis. Besides A. mwanzae, other reptile species using the kopjes were noted. Individuals of A. mwanzae were classified by both their size and colouration into males, females and juveniles. Sub-adults whose gender could not be seen clearly and all juveniles which can't be sexed from photos were excluded from the analysis. Despite what has previously been described by Yarnell & Jones (2001), immature males were seen to have the beginnings of mature colouration, which was used to identify them.

RESULTS

The trapping events with A. mwanzae occurred in only four different locations, all towards the south-west corner of the

Snapshot Serengeti study area. The traps at the locations where the agamas were observed were only active during September and October in the 2010 season, this is when the camera traps were placed in the field. The cameras at the four sites detected *A. mwanzae* with different frequencies, varying as follows: J13 (n=23), O10 (n=353), T13 (n=407) and U13 (n=2) giving a total of 785 trapping events. Of these, 778 events included individuals of *A. mwanzae* basking, with very little other behaviour observed.

The number of agamas photographed during each capture event varied with only one individual per event being the most numerous (n=621), followed by two individuals (n=141), three individuals (n=20) and finally four (n=3). Of the total trapping events containing *A. mwanzae*; 318 were in the morning (before noon) and 467 occurred in the afternoon. When the numbers of observations of *A. mwanzae* in the morning and afternoon were compared (Table 1) there were significantly more in the afternoon (One-way Chi Square, χ^2 = 27.9, df= 1, p= <0.0001).

When looking at the basking behaviour of *A. mwanzae*, significantly more females were observed basking than males, including 4 immature males (One-way Chi Square, $\chi^2 = 5.09$, df=1, p= 0.024). The time of day (Table 1) apparently had no effect on the number of males basking (One-way Chi Square, $\chi^2 = 0.14$, df=1, p= 0.7083) but more females were basking in the afternoon than morning (One-way Chi Square, $\chi^2 = 52.34$, df=1, p= <0.0001). Besides *A. mwanzae*, only one other reptile species, the striped skink (*Trachylepsis striata*), was observed using the kopjes. This species was only seen in two trapping events.

Table 1. The frequency of adult male and female Agama mwazae detected by camera traps during the morning or afternoon (based on peak counts from each camera trapping event)

Time of day	Male	Female	Total
Morning	57	295	352
Afternoon	62	500	562
Total	119	795	914

DISCUSSION

This study showed that camera traps can be an effective way to monitor the behaviour of reptiles such as lizards, despite the fact that they are not commonly used for this purpose. The main result from this investigation is that more female *A. mwanzae* were observed to be basking than males, which is expected given the known social structure of the species (Yarnell & Jones, 2001). More females were seen basking in the afternoon which is a likely consequence of the kopjes having warmed up after the heat of the midday sun, meaning that lizards could gain warmth from both direct sunlight and radiation from the kopjes.

The lack of reptile diversity on the kopjes is startling given the potential benefits that they provide to species wishing to sun themselves. More species may have been using the kopjes than reported as the camera traps may not have been sensitive enough to have been triggered by their smaller size. In experiments in Australia, Welbourne et al. (2015) found that reptile species with a larger critical mass were more likely to trigger camera traps than smaller ones.

ACKNOWLEDGEMENTS

I'd like to thank the following people for their involvement with helping to sort the camera trap data so that the analysis could be possible: Adam Newport & Giulia Iscru. I'd also like to thank Alexandra Swanson and Margaret Kosmala for their help in getting this project off of the ground, helping with access to the data and finding a solution to download and manually analyse it all. And finally, I'd like to thank the reviewers of The Herpetological Bulletin for extensively helping me to prepare this manuscript for publication.

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Accepted: 23 August 2018

Spring basking by *Vipera aspis*: Observations from Italy and France on the displacement distances of basking vipers from their hibernacula

LORENZO RUGIERO¹, LEONARDO VIGNOLI², LUCA LUISELLI^{14,5} & ROGER MEEK^{3*}

¹IDECC–Institute for Development, Ecology, Conservation and Cooperation, via G. Tomasi di Lampedusa 33, 00144 Rome, Italy
 ²Department of Science, University Roma Tre, Rome, Italy
 ³7 rue George Clemenceau, Chasnais, 85400, France
 ⁴Department of Applied and Environmental Biology, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria
 ⁵Département de Zoologie, Faculté Des Sciences, Université de Lomé, BP 6057 Lomé, Togo
 ^{*}Corresponding author Email: Rogermeek85@aol.com

ABSTRACT - Patterns of movement are an important component in animal ecology. In temperate zone reptiles this includes movement soon after emergence from hibernation, often a crucial period for courtship and mating. Due to the effects of climate or habitat, intra-specific differences in movement in different areas might be expected. We examined this possibility using long-term data on the displacements of aspic vipers (*Vipera aspis*) from their hibernation dens in Italy and France; the two sites differed in latitude, altitude and vegetation cover. We found no statistically significant differences between Italy and France in displacement distances from the dens. However, in both countries displacements were significantly greater in the afternoon in both males and females. This was thought to be due to differences in cover between the localities. The likely explanation is that the movement of the sun across the sky may leave morning basking positions in the shade and require the snakes to move further from their dens to continue basking.

INTRODUCTION

K nowledge of movement patterns and frequencies for movement are integral to understanding animal ecology (e.g., Turchin, 1957; Larsen, 1987; Hansson & Åkesson, 2014). For snakes and other ectothermic animals, movement will be limited both spatially and temporarily by the structure and thermal qualities of their environment. Consequently, the movement patterns among different populations of a species in different environments could differ. Although the movement of snakes has been studied in detail in some species (e.g., Gregory et al., 1987; Macartney et al., 1988; Webb & Shine, 1997; Blouin-Demers & Weatherhead, 2002; Himes et al., 2006), the data remain scarce even for many abundant snakes and especially for reptiles after emergence from winter dormancy in temperate zones (Zuffi et al., 1999; Reed et al., 2012). This is often the period for reproductive activity and may be energetically costly for both female and male snakes (e.g., Shine, 1980; Luiselli et al., 1996; Olsson et al., 1997; Bonnet et al., 1999). For example, in the aspic viper Vipera aspis, males were reported to lose around 350 mg per day due to post hibernation sexual activity (Saint Girons, 1996).

The aspic viper (Fig. 1) is widespread in Europe occupying a series of different habitat types (Arnold, 2002; Speybroeck et al., 2017) and in most of its range undergoes winter hibernation, frequently occupying the same winter dens across several years (Meek, 2016; Luiselli et al.,



Figure 1. Male of V. aspis from Oriolo Romano (Italy)

2018). A small species (maximum total length at around 85 cm; Corti et al., 2010) it is a capital breeder (Lorioux et al., 2013) that reaches maturity after around 3 to 4 years (Bonnet et al., 1999). Currently much of what is known of temperate viper movements in Europe involves reactions to thermal constraints, including outside of the spring mating season. For example distance movements of up to 164 and 361 m were observed during summer in *V. ursinii* in Hungary, and were associated with avoiding high temperatures (Ujvari & Korsos, 1997). In the Swiss Alps *V. berus* males and females moved similar distances after



Figure 2. Google Earth map of the study area at Oriolo Romano (Italy), showing the location of the four hibernacula (A, B, C, D)

the mating period (Neumeyer, 1987). Seasonal movements have also been observed in non-viperids, for example, male grass snakes *Natrix natrix* were more active during the spring than summer although females travelled greater distances during July following oviposition (Madsen, 1984).

In this paper we report on the movements of *V. aspis* soon after their emergence from communal hibernacula in two areas of Europe, Italy and France, using data from long-term studies (Rugiero et al., 2012, 2013; Meek, 2013, 2014; Luiselli et al, 2018). This time of year is usually the mating period for *V. aspis* and we were interested in comparing their movements in habitats that differed in latitude, altitude and vegetation cover and establish whether or not these differences impacted on their movement patterns. The data come from observations on four hibernacula in a single area of Italy and in western France from a single den situated in a fragmented landscape (Luiselli et al., 2018).

METHODS

Italy: Observations were made at four hibernation dens (A, B, C, D in Fig. 2) in an area in the Tolfa Mountains north of Rome, Italy (coordinates: 42°19°N, 12°12°E, altitude 387 m; Fig. 2). The four hibernacula were situated in a mixed area of bushy pastures (*Citysus scoparius* and *Rubus ulmifolius* bushes) and oak forest (*Quercus cerris*), thus in a relatively wooded zone although not far from ecotones created by paths and tracks (Fig. 3). Surveying started from February 1987 and continued throughout the active year until November 2017 (30 years of records). Sampling intensity varied substantially across years and seasons: in some years there were 2-3 visits a week 20 February to 05 March but in other years only once a week. Overall, the



Figure 3. Landscape surrounding hibernaculum A at Oriolo Romano (Italy)

number of annual days in the field was similar across years (for more details see Rugiero et al., 2013 and Luiselli et al., 2018) and thus the accumulated long-term observations represent a large data set.

Linear distances of snake locations from the hibernation dens were determined with a tape measure, during the period 10 October-20 March (Rugiero et al., 2013) although only spring time observations are reported here. In most instances, sightings occurred during sunny and warm days. We restricted the daily observations in spring to avoid impacting on the natural behaviour of the snakes since our primary aim was to measure daily movement (Macartney et al., 1988). In order to minimise disturbance we measured distance travelled from dens only once every ninth site visit. Surveying was between 08.30h and 12.00h and again between 15.00h and 18.00h Central



Figure 4. Google Earth map of the study area at Chasnais, western France. Arrow shows location from where the photograph in Fig. 5 was taken and also the direction.



Figure 5. Chasnais hibernaculum with white arrow showing approximate location of the den entrance. The view also illustrates the narrow linear structure of the hedgerow system. Only one viper, a female, was located in the hedgerow to the right in the autumn of 2016 giving birth (Meek, 2016).

European Time (CET) and usually completed within 90 minutes. Detection was made by visual encounter and time of sighting (CET) recorded. Snakes were individually marked by ventral scale-clipping and dorsally painted with a white number allowing the surveyors in the short-term to identify specimens that were already captured without any need for further recaptures.

France: Observations were made at a hibernaculum in Vendée, western France (46°27`N;1°53`W; altitude 25 m; Fig. 4). The area surrounding the hibernaculum was mainly composed of agricultural land and patches of woodland,

usually connected by hedgerows (Fig. 5). The den area was situated at the northern end of a hedgerow system and formed of a series of discontinuous drainage pipe remnants of approximately 1 m diameter. European ash (*Fraxinus excelsior*) with a dense understory of bramble growth (*Rubus fruticosus*) covered the den area. A combination of autumn leaf fall, drifting soil from agricultural land resulted in debris entering the drainage pipes, leaving openings of around 15 cm at the top of the pipes for entry.

Surveying was carried out between 22 March - 28 May (2013), 12 March - 22 April (2014), 04 April - 28 April (2015), 21 April - 27 April (2016). Most visits were carried out twice daily between 09.20h and 10.50h and 15.30h and 18.40h (CET) and usually completed within one hour. Time of each sighting (CET) was recorded. As in Italy, detection was by visual encounter by walking along both sides of the hedgerow surrounding the den at a distance of 4 - 6 m. This included hedgerows to the north/north-east, west and south (Fig. 4). Farmland stretching from approximately 5-10 m east and west of the hedgerows and the grassy areas next to the road were also searched for snakes. These areas had little vegetation except during the summer months of 2015. Each hedgerow was surveyed once during a visit. All snake locations were recorded and plotted on a map (Google Earth) to determine their approximate distances from the den entrance along with dates of observation.

Statistical Analysis: In Italy there were 505 observations from 59 snakes around their dens (mean = 8.6 sightings per individual) and in France 76 observations on 6 snakes (mean = 12.7 sightings per individual). To test for differences in displacement from hibernacula before and



Figure 6. Boxplots of displacement data (in metres) in *V. aspis* from the hibernation dens in Italy and France. Horizontal boxes represent the interquartile ranges, vertical lines within the boxes medians and horizontal lines the ranges. Triangles in the Italy pm data are statistical outliers. Samples sizes are number of *means* of *means* of individual snakes.

after midday and avoiding pseudo-replication, analysis has been applied to the means of the displacements of individual snakes. Statistically, this method employs the properties of the Central Limit Theorem; that is the *mean* of the *means* is equal to the population mean. This produced sets of 'morning' means and 'afternoon' means for comparison. To test that the mean values followed a normal distribution the data were subject to Anderson-Darling normality tests. The results gave morning means $a^2 = 0.28$, p = 0.51 and afternoon means $a^2 = 0.37$, p = 0.29 for France but in Italy for morning means $a^2 = 0.96$, p = 0.01 and afternoon means $a^2 = 1.19$, p = 0.004. We therefore used a non-parametric Mann-Whitney U-test to make comparisons of median displacement distances with α at 5%. Levens tests were used to test for homogeneity of variances in displacement, which is more robust for smaller samples (i.e. viper data in France) and less sensitive to departures from normality (Box & Jenkins, 1976). The test considers the distances of the observations from their sample medians and assumes a null hypothesis of H₀: $\sigma_1^2 / \sigma_2^2 = 1$. All calculations were performed on Minitab.

RESULTS

Comparisons between Italy and France: Figure 6 shows a box plot of the data from Italy and France. Although Italian snakes were on average found further from den entrances in the morning than those in France, the difference was not significant (Mann Whitney, W = 127, p = 0.13) neither was the greater average afternoon

Table 1. Mean post hibernation displacement distances (m) for male and female *V. aspis* in the morning and afternoon. Note that to avoid pseudo-replication data are shown as *means* of *means* of individual snakes where adequate amounts of observations for individual snakes were made. See text for details.

Italy					Fra	ance		
	Males Females			Males		Females		
	Mean±sd	n	Mean±sd	n	Mean±sd	n	Mean±sd	n
AM	14.7±7.3	21	10.9±7.0	36	5.5±4.6	4	11.3±6.5	2
PM	27.5±23.6	13	21.3±15.0	25	30.8±20.2	4	61.5±6.6	2

displacement by snakes in France (W = 185, p = 0.09). There was no significant difference between morning or afternoon variance in displacement between Italy and France either during morning (Leven's test, L = 0.86, p = 0.36) or afternoon (L = 0.075, p = 0.78).

Italy: During the morning (before 12.00h) mean linear distance from the den for 57 post hibernation vipers was 12.32m±7.28, with a range 7-27m. The movements were in general greater in the afternoon: *mean* distance = 23.38m±18.29, range 2 – 88m with differences between morning and afternoon significant (Mann-Whitney, W = 2272, p = 0.0004). This pattern applied to both sexes and was significant in females (Mann-Whitney W = 909.5, p = 0.002 but just outside α at W = 316, p = 0.07 for males. Variation in displacement was also greater in the afternoon (*mean* = 25.1±27.0m) in comparison to morning (*mean* = 4.4±4.6m) with the differences were also significant

in both sexes; males L= 4.7, p = 0.03, females L = 9.7, p = 0.003.

France: During spring, post hibernation *V. aspis* sightings of a maximum 6 snakes (however numbers declined with time see Luiselli et al., 2018) during the morning surveys (< 12.00h) gave indicated movements from the den area from 1.23–17.8 m (*mean* 7.43m±6.21, n = 6). Snakes moved further in the afternoon with *mean* distances of individuals ranging from 20.1–68.8 m (*mean* = 38.2±19.4, n = 6) with differences between morning and afternoon statistically significant (Mann Whitney, W = 21, p = 0.005). Variation in movement was greater during the afternoon with the difference significant (Leven's test *L* = 12.3, p = 0.001). The employment of means of means for statistical analysis negated comparison between sexes for the French snakes but as can be seen in Table 1 there was greater afternoon displacement in both sexes.

DISCUSSION

Although the study sites in Italy and France differed in latitude and altitude, displacement distances in V. aspis were in statistical agreement. In both countries, V. aspis generally travelled further from their hibernacula in the afternoon than in the morning. Shorter morning movement is expected in temperate latitudes where it is well known that snake hibernacula are often located in positions where they will catch the early sunshine. Consequently, snakes can begin the day by basking close to their hibernacula. These basking areas will very often be in shade later in the day so that snakes will necessarily have to move further from their hibernacula to remain in the sun. Viitanen (1967) mentions westward movement of V. berus in the afternoon and this is comparable to the results reported here, he also quotes Duguy (1958) on movements in V. aspis suggesting that V. aspis and V. berus are similar in this respect.

The tendency for longer displacements in the afternoon in France, compared with Italy, may in part have been due to the immediate habitat in France mostly consisting of a long narrow hedgerow (Figs. 4 & 5). To avoid breaking cover the snakes may have undertaken fewer lateral movements than the Italian vipers, which had more comprehensive cover around them. This is possibly reflected in Table 1 which shows that at the French den females travelled further than males and further in the afternoon (but see above for problems with statistical testing). One of the females regularly travelled to the same basking location approximately 90 m along the hedgerow from the den entrance each afternoon. This is high-risk movement unless it can be achieved under cover (Meek, 2013). However, after mating females must thermoregulate precisely (Lorioux et al., 2013) and to do so are constrained to move to track the sun during the course of the day so that basking is optimised. The benefits for female V. aspis of investing in movement to achieve high and stable body temperatures include larger, fitter offspring and fewer stillborn (Lourdais, et al., 2004; Lorioux et al., 2013). However, risk of predation may be increased if snakes repeatedly use the same basking areas as predators may regularly search such localities (Meek, 2013).

We are deeply indebted to Dr Massimo Capula, who coworked with us in the field during the earlier years of monitoring at the Italian site. Capture of vipers in Italy was authorised on an annual basis by the Environmental Ministry of Italy. Professor Rick Hodges made many helpful comments and suggestions and also acted as sole deciding editor for the manuscript.

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Accepted: 17 September 2018

Patterns of amphibian diversity in the Western Palearctic

CHRISTOPHE DUFRESNES

Department of Animal and Plant Sciences, University of Sheffield, Alfred Denny Building, Western Bank, Sheffield S10 2TN, UK Author Email: Christophe.Dufresnes@hotmail.fr

ABSTRACT - The discovery of cryptic species by molecular tools is leading us to revisit our perception of amphibian diversity and distribution. Here an analysis is presented of amphibian diversity across the Western Palearctic based on an up-to-date taxonomic list accounting for recently discovered taxa. While glacial refugia locally host many species, the most diverse regions are found at intermediate latitudes, where amphibians colonising from different refugia coexist. At least eight major diversity hotspots are identified. This data will be useful for future biogeographic and conservation assessments.

INTRODUCTION

Inderstanding patterns of amphibian diversity is instrumental to prioritise conservation efforts towards hotspots of endemism and species richness. An up-todate spatial diversity framework is also fundamental to implement ecological, biogeographic and evolutionary research. In the Western Palearctic, i.e. Europe, North-Africa and the Middle East, the current distribution ranges of species, especially ectotherms, were mainly shaped by the Quaternary ice ages (Schmitt, 2007). In particular, the mild conditions, ecological stability and complex topography of southern regions have promoted the persistence and diversification of many radiations (Schmitt, 2007). Two decades of phylogeographic work have lifted the veil on this cryptic diversity, ultimately leading to the description of new species. However, this diversity is rarely taken into account in spatial assessments, because the legitimacy of cryptic species is debated, assessing their distribution requires molecular tools, and their discovery and/or specific assessment is often very recent (e.g. Lissotriton, Pabijan et al., 2017; Pelodytes, Díaz-Rodrígues et al., 2017; Ommatotriton, van Riemsdijk et al., 2017; Hyla, Dufresnes et al., 2018b). As a result, only 114 native Western Palearctic amphibians appear on the IUCN red list database (IUCN 2018), while about 20% more (137) could now be considered combining recent research (Dufresnes, 2019). Similarly, the 2014 European atlas for amphibians includes 73 native species (Sillero et al., 2014), instead of the 91 now known from Europe.

Here I have compiled distribution data from Western Palearctic species, including newly discovered cryptic species, to get an up-to-date picture of the global patterns of amphibian diversity.

MATERIALS AND METHODS

All analyses were conducted in QGIS 2.18.4 (QGIS

Development Team 2018). Distribution data was gathered from the IUCN red list (IUCN 2018) and updated according to recent phylogeographic work. This included species splits for Bufo spinosus, Bufo eichwaldi (Recuero et al., 2012), Bufotes cf. turanensis (G. Mazepa, unpublished data), Hyla cf. intermedia (Dufresnes et al., 2018b), Hyla molleri, Hyla orientalis (Stöck et al., 2012), Hyla felixarabica (Gvoždík et al., 2010), Pelobates vespertinus, Pelobates balcanicus (CD unpublished data), Pelodytes atlanticus, Pelodytes hespericus (Díaz-Rodrígues et al., 2017), Pelophylax cypriensis (Plötner et al., 2012), Lissotriton graecus, Lissotriton kosswigi, Lissotriton lantzi, Lissotriton schmidtleri (Pabijan et al., 2017), Triturus macedonicus, Triturus ivanbureschi, Triturus anatolicus (Wielstra et al., 2013), Ommatotriton nesterovi (van Riemsdijk et al., 2017), Salamandrina perspicillata (Canestrelli et al., 2006), Lyciasalamandra billae (Veith et al., 2016); merging between Bufotes viridis and Bufotes variabilis (Dufresnes et al., 2018a); and fine-tuning of distributions, prepared for a new field-guide of Western-Palearctic amphibians soon to be published (Dufresnes, 2019). In total, individual distribution layers were produced for 137 native species (full list in Table S1).

A grid of 20×20 km was generated the region of interest using the MMQGIS plugin (http://michaelminn.com), drawn along the Arctic ice cap in the north, the Atlantic Ocean in the west, the Saharan and Middle Eastern deserts in the south and south-east, and the Ural Mountains in the north-east. Distribution shapefiles were then matched against the grid to count the number of species present in each quadrat using the Join Attributes By Location feature.

RESULTS AND DISCUSSION

Amphibian diversity in the Western Palearctic is maximal at intermediate latitudes across western and central Europe (Fig. 1), where different species originating from separate glacial refugia (Iberia, Balkans, Black Sea area) meet



Figure 1. Diversity of Western Palearctic amphibians, based on 20×20 km grids, and accounting for the 137 native species present in the region

in so called "melting pots" of diversity. This pattern is paradigmatic of European biogeography (Hewitt, 2011), and matches the results of previous comparative studies of European amphibians and reptiles, although not accounting for recent species split (Meliadou & Troumbis, 1997; Sillero et al., 2014). In addition to this key area, I identified seven localised hotspots of diversity (i.e. with more than ten cooccurring taxa), mostly driven by regional endemism and long-term persistence of species due to ecological stability throughout the Quaternary: (1) the north-western African coast; (2) the central and northern Iberian Peninsula; (3) the Apennine belt in Italy; (4) the Balkan Peninsula, especially the foothills of the Carpathians; (5) the Anatolian and Caucasian Black Sea coasts; (6) The Levant region (eastern Mediterranean coast); (7) the Hyrcanian region (southern shores of the Caspian Sea). In contrast, islands and deserts expectedly bear low species richness (Fig. 1).

These patterns summarised well two decades of amphibian phylogeography. The diversity of the Palearctic seems mainly shaped by diversifications between and within regional hotspots, acting both as shelter and active promoters of diversity. A similar picture holds at the intra-specific level (e.g. Dufresnes et al., 2016). It is quite fascinating to notice that the richest areas in terms of specific richness (Western and Central Europe) are also the poorest in terms of intraspecific diversity. Indeed, most of the many amphibians found in France, German, Belgium, Switzerland and adjacent countries have little genetic variation, due to postglacial expansions (as shown in *Hyla arborea*, Dufresnes et al., 2013). This lack of adaptive potential likely contributes to their generally poor conservation situation in these areas (Dufresnes & Perrin, 2015). Hence, the high yet vulnerable diversity of amphibians in Western and Central Europe is particularly of concern in these heavily impacted regions. This data will be useful in future research and conservation efforts.

ACKNOWLEDGEMENTS

I am grateful to the IUCN red list for sharing their data, as well as the Swiss National Science Foundation for funding (fellowship P2LAP3_171818). I also thank V. Pospisilova for support. The diversity layers are archived on ResearchGate (https://www.researchgate.net/ publication/327792262_GIS_shapefiles_of_amphibian_ diversity); the species distribution shapefiles are available upon demand.

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Accepted: 29 August 2018

Please note that the Supplementary Material for this article is available online via the Herpetological Bulletin website: https://thebhs.org/publications/the-herpetological-bulletin/issue-number-145-autumn-2018

Comparison of the effectiveness of four materials used in constructing coverboards for sampling amphibians during their terrestrial phases in a Cambridge allotment

KATHRYN LEWIS

Pulborough, West Sussex Author Email: kate.n.l.lewis@gmail.com

Most of the effort devoted to monitoring amphibians in temperate climates has focused on the relatively short aquatic phases of their life cycle. As most amphibians in these areas spend a substantial fraction of their lives on land, methods for sampling populations during these phases will be of some importance. Such methods are not as welldeveloped as those for the aquatic phases, and there are not, as yet, generally-accepted procedures for sampling amphibians on land (see Beebee, 2013, for example).

The work reported here formed part of a larger study of amphibians during their terrestrial phases in a number of allotments in Cambridge (UK). Four species were found in this habitat: common frog (*Rana temporaria*), common toad (*Bufo bufo*), palmate newt (*Lissotriton helveticus*) and great crested newt (*Triturus cristatus*). Great crested newts are rare in this habitat and are not considered further in this note. All amphibians found were assigned to the categories sub-adult (metamorphs or juveniles) or adult, based mainly on size and colour.

Amphibians in all six categories (see Table 1) were sampled using coverboards (also sometimes called artificial cover objects or mats) at an allotment south-east of central Cambridge. Each coverboard measured 0.5 x 0.5 m and was constructed of non-nylon backed wool fibre carpet (carpet), untreated 9 mm thick plywood (wood), roofing felt (felt) or 1000-guage black polyethylene (plastic). The coverboards were distributed as widely and evenly as possible on a variety of substrates, including grass, concrete, scrub, dirt and ivy. Grouping depended on available space, and was either lengthwise (as in Fig. 1) or two-by-two to form a large square. The coverboards were put in position on 4th February 2017, they were then examined on 12 occasions between 25 February and 30 July.

Overall, 116 amphibians were found beneath the coverboards. Table 1 shows the total numbers of common frogs, common toads and smooth newts, both juveniles and adults, utilising the four materials, expressed as percentages of the total for each category. Because of small sample sizes, very few of the differences in numbers of amphibians between materials were significant at P<0.05 (univariate analysis) of raw data utilising the bootstrap option in SPSS, Field, 2012). The overall pattern, however, is clear: carpet was the most utilised material in all six cases, being responsible for 55% of the amphibians found. Plastic was the least utilised material in three out of six cases.



Figure 1. The coverboards, from left to right: plastic, carpet, roofing felt and wood

Table 1. Total numbers and percentages of amphibians in six categories found beneath coverboards constructed from four different materials. Newts refers to *L. helveticus*. Significant differences are denoted by asterisks.

	Total number found	Material				
		Carpet	Wood	Felt	Plastic	
Juvenile frogs	34	68*	21	0	12	
Adult frogs	5	60	40	0	0	
Juvenile toads	24	29	13	29	29	
Adult toads	7	57	0	14	29	
Juvenile newts	18	61	17	17	6	
Adult newts	28	57*	14	21	7	

It has been well-known for many years that the materials from which they are constructed may affect the ability of cover boards to attract reptiles and amphibians (e.g. Hampton, 2007); the reasons may be complex (e.g. Grant et al., 1992). Why was carpet more successful than the other three materials at attracting amphibians at this allotment site? It may be significant that 2017 was a relatively dry year in Cambridge. Carpet retains moisture well, and therefore even in the drier months provided a moist microclimate. The other materials prevent rainfall from reaching the ground and so produce a drier microclimate This may unless rainfall has been particularly high. explain why the results reported here contradict those of Scheffers et al., (2009), which showed that wood attracted more amphibians than carpet at a site in Missouri.

The study reported here is very much a preliminary one and is based on small sample sizes. Nevertheless, in view of the fact that there are as yet no generally accepted, standardised methods for sampling amphibians using coverboards during their terrestrial phases (partly because the detailed requirements seem to vary between habitats), the data may well prove useful to other workers when devising methods that are relevant to the particular circumstances of the habitats that they are sampling.

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Accepted: 23 July 2018

An observation of scavenging by a Spectacled cobra *Naja naja* on a road killed Russell's viper *Daboia russelii*

VIJAY PATEL¹, SHIVA CHETTIAR², SAGAR KUMBHANI³, & KRUNAL TRIVEDI^{4*}

¹C-202, Nidhi Complex, Nana Varachha, Surat-395006. Gujarat, India
²96/748, GHB, Haatkeshwar, Ahmedabad-380001. Gujarat, India
³B-904, Jainam Residency, Pal, Surat-395009. Gujarat, India
⁴26, Kasturba Mahila Society, Palanpur Patiya, Surat-395009. Gujarat, India
*Corresponding author Email: krunal.trivedi.7567@gmail.com

Roads have direct and indirect negative effects on wildlife and pose a significant threat to vertebrates at the global level (Forman et al., 2003) due to the numbers of animals killed on roads. Road kill carcasses are also a potential food source and frequently scavenged by other animals. This is the case in India but it is a behaviour often overlooked in the literature. For instance snakes are known to scavenge on birds, frogs, lizards, mammals and other snakes on road kills (Romulus Whitaker Per. Comm.). In this note we report on an observation of road scavenging by a spectacled cobra *Naja naja* on a road killed Russell's viper *Daboia russelii*.

On the evening of 13th November 2014, while travelling from Navsari to Surat (21° 3'19.65"N, 72°54'44.00"E) we observed a *N. naja* consuming a road killed Russell's viper *D. russelii* (Fig. 1). Due no doubt to being repeatedly run over, the carcass of *D. russelii* was firmly adhered to the road. The approximate length of the cobra was 120 cm and *D. russelii* 80 cm and it was apparent that it was experiencing some difficulty removing the carcass in attempt to swallow it (Fig. 2). After consumption the *N. naja* disappeared into roadside bushes. Our observation of the feeding event was over 8 minutes but one-third of the viper was already consumed when we arrived and hence the total feeding period was longer. The present observation highlights the increased risk of mortality from traffic from snakes feeding on road-killed animals due to



Figure 1. Spectacled cobra in the early stages of consumption of road-killed viper



Figure 2. The feeding event almost complete. See text for details.

the increased time they must spend on the road surface. To our knowledge, there has been no previous report of road scavenging in *N. naja*, so our observation is the first record of this novel behaviour in *N. naja*. The scavenging event was filmed and can be viewed on https://youtu.be/ QprTB4UYBIo.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the anonymous reviewers, Romulus Whitaker and Bartosz Nadolski, who provided valuable input and comments on the previous versions of the manuscript. KT would also like to thank the Nature Club Surat for support and constant motivation.

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Received: 21 June 2018

Attempted predation of *Varanus bengalensis* (Squamata: Varanidae) by *Varanus flavescens* (Squamata: Varanidae)

ANIRBAN CHAUDHURI¹ & SUBHADEEP CHOWDHURY^{2*}

¹6/7, Nature Mates, Bijoygarh, Jadavpur, Kolkata 700032, West Bengal, India ²Krishnachak, Dhurkhali, Howrah 711410, West Bengal, India ^{*}Corresponding author Email: isuvodeep@gmail.com

The yellow monitor, *Varanus flavescens* (Hardwicke & Gray, 1827), is a diurnal carnivorous lizard that feeds on a range of animal prey (Auffenberg et. al. 1989). It is distributed in Bangladesh, Bhutan, India, Nepal, and Pakistan (Wangyal, 2011). The Bengal monitor *V. bengalensis* (Daudin, 1802), is also diurnal and a generalist feeder found in India, with a range extending from Afghanistan to Mayanmar including Pakistan, Sri Lanka, Bangladesh and Nepal (Das, 2002).

On 31 July 2007 at c.a 15:18 h, a foraging adult V. flavescens (about 1 m in length) was observed trying to predate a juvenile *V. bengalensis* (approx. 12 inch in length) at Bongaon, 24 Parganas (north) district, West Bengal, India (23°04'40" N, 88°82'77" E, WGS 84, elev. 15 m) (Fig. 1). The V. flavescens held on to the cornered juvenile *V. bengalensis* by its mid-body while in response the latter bit and held onto one of the digits of the front left leg of the predator. This situation continued for approximately 7 min. The V. flavescens was observed thrashing its head sideways at intervals in an attempt to release its foot which it was eventually able to do but then was seen to loosen its grip probably in attempt to secure a better hold on its prey. At this moment the juvenile V. bengalensis freed itself and escaped into to a small crevice in the mud wall of the village house where the incident was observed. The V. flavescens followed and tried to scratch the crevice with its forelegs to get access to the prey but after a several attempts gave up and left the area. The authors waited for another half an hour to see if the V. bengalensis would re-appear out of the crevice but it did not resurface during this time.



Figure 1. Attemped predation of yellow monitor lizard on Bengal monitor lizard

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Received: 11 August 2018

First report of leucism in Anguis fragilis

DANIEL JABLONSKI & ADRIÁN PURKART

Department of Zoology, Comenius University in Bratislava, Ilkovičova 6, Mlynská dolina, 842 15 Bratislava, Slovakia *Corresponding author Email: daniel.jablonski@balcanica.cz

The common European slow-worm (*Anguis fragilis*) is a member of family Anguidae distributed from the central Balkans to western Europe including the British Islands (Jablonski et al., 2016). Although it is a common reptiles species and quite diverse in coloration, colour aberrations (e.g. albinism, melanism or ventral blue coloration) are generally very rare in this genus (Robert et al., 1965; Knight, 1966; Jablonski & Meduna, 2010; Gleed-Owen et al., 2012; Hails, 2017). According to the best of our knowledge, leucism has never been reported for this species and genus.

During a field trip on 19th September 2017 six juvenile specimens of A. fragilis were observed during cloudy weather under an artificial refuge (large piece of metal) that had been placed in the grassy area. Three were leucistic and three with normal juvenile coloration for the species given their size (Fig. 1). These individuals were around 9-10 cm of total length with an intact tail. The coloration of leucistic juveniles was pinkish with dark eyes. The vertebral strip and lateral coloration were visible and lightly brown. The locality is at 51.4141°N, -1.7195°E near Marlborough, Wiltshire, United Kingdom. Together with slow worms there were three Vipera berus (Linnaeus, 1758) (two adults, one juvenile) under the shelter. At the same locality Natrix helvetica (Lacepède, 1789) were very abundant. After photographs were taken, all individuals were released.

Leucism with diminished number of iridophores and very low number of melanophores and xanthopores is known and reported in reptiles, predominantly in snakes (Bechtel, 1995; Krecsák, 2008). Therefore, is it not surprising that this colour aberration is found in *A. fragilis* but there is also a possibility that the reports of previous authors did not distinguish between leucism and albinos. Individuals with this colour defect have probably lower levels of survival since they would be conspicuous to predators. This is probably the reason why such aberrated adult individuals are rarely observed in nature (see Gezova et al., 2018). Due to the rarity of similar observation in the genus *Anguis*, any other such information will be much appreciated.

ACKNOWLEDGMENTS

We would like to thank Stephen Todman who informed us about this observation and provided photographs. This work



Figure 1. The leucistic and normal coloured individuals of *A. fragilis* from Marlborough, United Kingdom

was supported by the Slovak Research and Development agency under the contract no. APVV-15-0147.

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Received: 5 September 2018

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Chair	Ms. K. Le Breuilly	9 Anvil Close, Streatham, London SW16 6YA. Tel: +44 (0)208 6969733; Mob: +44 (0)7732 949344. kim.lebreuilly@gmail.com
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Tel: +44 (0)1935 432557. rom@froggy-bottom.co.uk (3rd year) steveallain@live.co.uk (1st year)

Mr. R. Muryn

Mr. Steven Allain

Mr. Jim Labisko

Ms. Helena Turner

jl693@kent.ac.uk (1st year)

Tel: +44 (0)7936 140587, hturnerjsy@hotmail.com (1st year)

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THE HERPETOLOGICAL BULLETIN

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