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Front Cover: A northern viper *Vipera berus* photographed by Clifford Seabrook in Kent (England). There is an article about this species on p. 20.

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RESEARCH ARTICLE

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Body-bending behaviour in three snake species in the Russian Far East

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ABSTRACT – - Body-bending behaviour or kinking is a cryptic form of immobility, believed to be used by snakes to avoid predation. Originally, this defensive strategy was thought to be used only by arboreal species in the Neotropics but recent reports suggest that it may be more widespread than previously thought. For the first time, data is presented on this type of behaviour for three snake species of east Asia - *Elaphe dione, Elaphe schrenckii* and *Gloydius ussuriensis*.

INTRODUCTION

Effective antipredator strategies are vital to the survival of all living beings, including reptiles. Snakes, in particular, demonstrate a wide variety of antipredator tactics ranging from immobility such as death-feigning to fleeing or to biting, and the choice of a particular antipredator response is determined by a combination of both intrinsic and extrinsic factors (Passek & Gillingham, 1997; Durso & Mullin, 2014; Fuentes et al., 2021). Immobility is a common defensive pattern, which can manifest itself not only in death-feigning but also in a more cryptic form known as body-bending behaviour or kinking.

Body-bending behaviour (BBB) is a poorly understood defensive mechanism in which a snake contorts its body into small curves resembling a zigzag. Due to such shape, a kinking snake resembles a liana or a stick, which makes it harder for a potential predator to see. This cryptic behaviour was originally described exclusively for arboreal tropical snakes (Beebe, 1946; Abuys, 1986), and to date there are reports for less than thirty snake species using this defensive strategy. Recent publications have described first cases of BBB from Asia and Europe (Kathe & Deshmukh, 2020; Hauser et al., 2022), while the bulk of the existing records still refer to the Neotropics.

To the best of our knowledge, there have been no records of such behaviour from Russia. Therefore, we report here the first BBB cases for several snake species inhabiting the Russian Far East, namely the Amur rat snake *Elaphe schrenckii* (Strauch, 1873), the steppe rat snake *Elaphe dione* (Pallas, 1773) and the Ussuri pit viper *Gloydius ussuriensis* (Emelianov, 1929) (Uetz et al., 2024).

All our records were made during field studies in the period 2015–2024 in the southern and western parts of Primorsky Krai, Russia. The cases presented in our study refer to Khankaysky, Pogranichny, Nadezhdinsky and Partizansk Districts. We took pictures of all the snakes mentioned in our study and usually made brief notes on the surroundings and weather conditions. We have observed a total of twelve cases of BBB for three snake species; these cases are separated into groups by species and given in chronological order.

OBSERVATIONS

Elaphe dione

Case 1
On 30 June 2020 at 14:29 h, an adult *E. dione* was found by N.E. Pokhilyuk in the middle of an unpaved road. The observation occurred on the road to Dvoryanka (Khankaysky District) approximately 12 km north-east of the village. It was a warm cloudy day (20 ˚C) with a light wind. Since it had rained the previous night, the road was still moist. The snake lay stretched out in the middle of the road displaying a distinct BBB (Fig. 1A). It did not try to escape or curl up in a defensive posture when the author approached and touched it. When handled, the specimen stopped expressing BBB and returned to its normal body posture.

Case 2
On 18 September 2021 at 13:46 h, a snake was found by N.E. Pokhilyuk on an unpaved road in the Studenaya River valley ca. 3–7 km west of the village of Dukhovskoe in Pogranichny District. It was a warm day (25 ˚C) with few clouds and almost no wind. The snake was an adult steppe rat snake *E. dione* (ca. 70 cm in total length) vividly expressing BBB (Fig. 1B). Even when picked up and handled, the snake did not abandon this behaviour completely, it remained tense with a couple of small bends and a nearly square loop at the anterior part of its body. After taking several photographs, the rat snake was put on the roadside, where it quickly escaped into dense vegetation.

Case 3
On 24 April 2022 at 12:54 h, an adult *E. dione* was found by N.E. Pokhilyuk on an unpaved found road in the vicinity

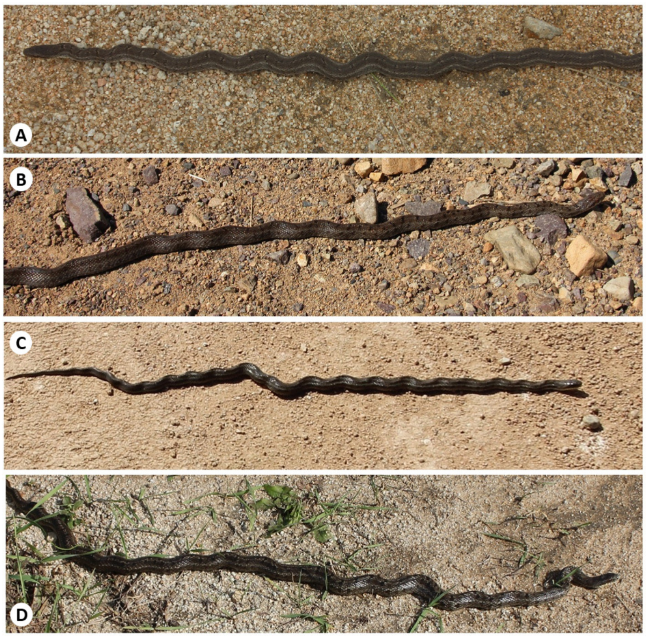

Figure 1. *Elaphe dione* displaying BBB

of Dvoryanka, Khankaysky District. It was a cool cloudy day (16 ˚C) with a light wind. The snake was crossing the road. When approached, the specimen stopped moving and started displaying BBB (Fig. 1C). The snake lost all its bends immediately after it was picked up. The snake moved rather slowly and did not try to provide any aggressive, defensive response.

Case 4

On the afternoon of 11 September 2022, the authors found an adult *E. dione* on an unpaved road between the villages of Zharikovo and Komissarovo (the Ilyinka-Komissarovo route) in the vicinity of Ilyinka, Khankaysky District. It was a warm cloudy day (25 ˚C) with a light wind. The snake expressed BBB and curled the anterior part of its body into a typical defensive S-posture. When picked up and handled, the snake lost its bends and actively tried to escape.

Case 5

On a warm and sunny afternoon on 25 September 2022 at 14:37 h, a steppe rat snake was observed by N.E. Pokhilyuk on the roadside approximately 3.5 km north-east of Dvoryanka, Khankaysky District. The snake was lying stretched out on the road with its tail still in the grass with multiple small bends along its whole body (Fig. 1D). It was an adult rat snake of at least 70 cm in total length. It did not try to escape when the author approached it. When picked up, the snake lost its bends and demonstrated no tendency to bite or take up a defensive posture. After being photographed, it was released into the wild.

Elaphe schrenckii

Case 6

On 13 September 2015, I.V. Maslova found a subadult *E. schrenckii* in the lower course of the Litovka River, Partizansk District. The specimen was seen on an unpaved road located in the hills covered with oak forests. It was a relatively warm afternoon (19 ˚C) with some clouds. The snake lay stretched out with clearly visible small bends along its whole body (Fig. 2A).

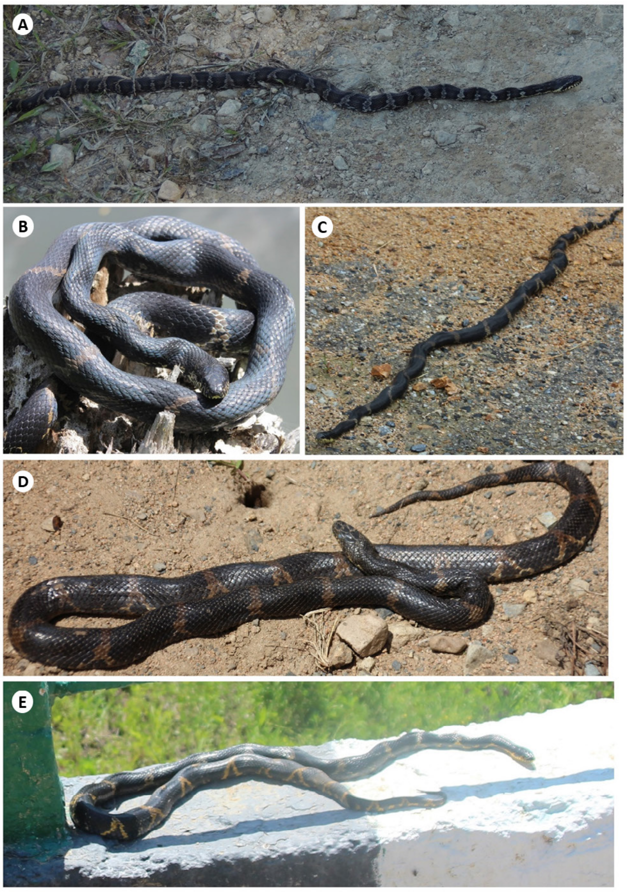

Figure 2. *Elaphe schrenckii* displaying BBB

Case 7

On 16 June 2019 at 16:10 h, N.E. Pokhilyuk collected two adult steppe rat snakes *E. dione* and an adult Amur rat snake *E. schrenckii* at the bank of the Komissarovka River in the vicinity of Dvoryanka, a remote village in Khankaysky District, Primorsky Krai. After taking pictures of the animals, all the snakes were released into the grass. The Amur rat snake slowly made its way through the grass and then started climbing up a dead tree stump (ca. 1 m tall) by the river. Next, it curled up on the top of the stump demonstrating BBB (Fig. 2B). The snake did not react to the author approaching it. When the author stepped back by ca. 8–10 m, it remained in the same position and continued to display BBB. In this case, it is difficult to tell whether such behaviour was solely a defence mechanism or the snake used it for effective basking. It should be noted that the weather on that day was relatively cool (17 ˚C) and moderately cloudy, and the tree stump was exposed to direct sunlight.

Case 8

On the afternoon of 14 July 2019, N.E. Pokhilyuk observed an adult *E. schrenckii* (over 130 cm in total length) on a small bridge on the Zharikovo-Komissarovo route at the border between Khankaysky and Pogranichny Districts. It was a warm day (23 ˚C) with storm clouds and rain showers; therefore, both the unpaved road and the asphalt surface of the bridge were damp. The Amur rat snake lay motionless, distinctly displaying BBB (Fig. 2C), but when we approached it, the snake attempted to flee into vegetation along the roadside. When picked it up, the snake was very agitated and tried to escape. A few minutes later, it became significantly calmer and was released into the grass.

Case 9

On 22nd June 2021, N.E. Pokhilyuk saw a snake curled up on the roadside about 2 km south-west of Barabash-Levada, a small village in Pogranichny District. It was an adult *E. schrenckii*, most likely a gravid female over 130 cm in total length. The day was warm (22 ˚C) and cloudy. The snake lay about a metre from the grass edge, in a location between the foot of a mountain and the Komissarovka River. It was preparing to shed its skin which could be told by the blue colour of its eyes (Fig. 2D). The snake demonstrated BBB, however it's hard to tell whether it was a part of the basking process on a warm day or a defensive reaction to our car passing by.

Case 10

On 12 June 2022 at 13:52 h, while examining a bridge over a small creek for snakes, 3.5 km south-west of Barabash-Levada (Pogranichny District), N.E. Pokhilyuk recorded one *E. schrenckii* displaying BBB. It was a hot sunny afternoon (27 ˚C), and the snake was lying on the edge of the bridge basking (Fig. 2E). It was an adult of at least 140 cm total length. More than a half of snake's body lay on a black-painted section of the bridge, which seemed unusual, given the heat. The snake's head was slightly lifted indicating that it was awake and obviously had seen both our car and the author approaching it. When handled, the snake expressed neither BBB nor any aggressive defence mechanisms. It did not make any attempts to bite but actively tried to escape.

Gloydius ussuriensis

Case 11

On the evening of 17 September 2016, a subadult Ussuri pit viper *G. ussuriensis* displaying BBB was found by I.V. Maslova in the lower course of the Vtoraya Rechka River. It was lying on a track across a flood meadow in the vicinity of Senokosnaya, an abandoned village in Nadezhdinsky District. The snake was stretched out with the anterior part of its body having several slight bends (Fig. 3A). When approached, it initially tried to flee but when followed the pit viper soon resorted to aggressive defence coiling up and striking at the potential threat.

Case 12

On 9 September 2021, at 19:34 h, N.E. Pokhilyuk encountered an adult *G. ussuriensis* crossing the road approximately 3 km north-east of Dvoryanka, a small remote village in Khankaysky District, Primorsky Krai. It was a sunny evening, shortly before sunset. The snake was lying on the roadside less than 0.5 m from the grass. It remained motionless with its head slightly turned towards the first author who approached it and started taking pictures (Fig. 3B). The body of the snake displayed several small bends that seemed an unusual posture for a pit viper. The snake did not react to the camera but when our automobile was passing by it, it turned its snout towards the car and jerked the head back further in a defensive S-shaped posture.

Figure 3. *Gloydius ussuriensis* displaying BBB

DISCUSSION

Given the number of cases presented in this study, we can suggest that BBB is common among at least some of the snake species of the Russian Far East. The previously known accounts of such behaviour would refer almost exclusively to tropical and subtropical species. All previous records referenced the Neotropics, south and south-east Asia, Spain and the USA (Marques et al., 2006; Doherty-Bone, 2009; Duarte, 2012; Torres et al., 2015; França et al., 2020; Kathe & Deshmukh, 2020; Hauser et al., 2022). The latter account refers to the grey rat snake *Pantherophis alleghaniensis* (Holbrook, 1836) (Doherty-Bone, 2009) which is, to the best of our knowledge, the only account of kinking in the temperate climate zone. Thus, the cases we have provided are the first published records of BBB in north-east Asia.

It was suggested by Marques et al. (2006) that BBB is most common among arboreal snakes dwelling in forest habitats which is consistent with the fact that all early publications referenced either arboreal or semiarboreal species. Subsequently, kinking was described for a number of non-arboreal snakes, e.g. *Coniophanes fissidens* or *Psomophis joberti* (Maddock et al., 2011; Miranda et al., 2012). Maddock et al. (2011) conjectured that this kind of defence mechanism is typical among not only arboreal but also terrestrial species which dwell on the forest floor littered with lianas and different branches. This suggestion seems reasonable, and most of our cases refer to *E. schrenckii* and *E. dione*, which are known for their tendency to climb (Table 1; Dunaev & Orlova, 2014). Nevertheless, the study by Hauser et al. (2022) mentions two cases of BBB in *Fowlea piscator* (Schneider, 1799), which is a semiaquatic species. This leads us to suggest that kinking may be a convergent defensive mechanism typical of most land snakes, their lifestyle notwithstanding.

Table 1. Natural history traits of the three snake species displaying body-bending behaviour

Traits / Species	***E. dione***	***E. schrenckii***	***G. ussuriensis***
Family	Colubridae	Colubridae	Viperidae
Adult body length	100–120 cm	160–180 cm	60–65 cm
Venom	Non-venomous	Non-venomous	Venomous
Reproductive mode	Oviparous	Oviparous	Ovoviviparous
Diet	Rodents, birds, amphibians	Rodents, birds	Frogs, fish, small mammals
Foraging mode	Active forager	Active forager	Ambush forager
Lifestyle	Semiarboreal	Semiarboreal	Terrestrial
Habitats	Eurytopic species	River valleys, montane forests	Eurytopic species (prefers wet habitats)
Activity pattern	Diurnal	Diurnal	Diurnal, crepuscular, nocturnal

It should be noted, we have also observed this behavioural trait in the Ussuri pit viper *G. ussuriensis* (Cases 11 & 12), which, as far as we know, is the first record of BBB in the family Viperidae. All previously documented records refer to different genera within the family Colubridae.

Hauser et al. (2022) noted that BBB is closely connected with diurnal species and open spaces. Our observations partially correspond to these criteria as nearly all snakes described in this study are diurnal (Table 1) and have been found in open spaces. The only exception is *G. ussuriensis* whose foraging behaviour is after sunset. It should be noted that we also cannot correlate BBB displays with a foraging mode as, unlike rat snakes, *G. ussuriensis* are ambush predators that do not actively search for potential prey.

All three species share similar types of habitats with *E. schrenckii* preferring montane areas while *E. dione* and *G. ussuriensis* can reside in both forested and open habitats. In all of our cases, snakes were found on flat open surfaces, usually a road. Such position might make a snake feel exposed to potential predators, but, at the same time, it provides better visibility of the surroundings with a chance of spotting a threat much earlier. Since the snakes we recorded had had a better opportunity to see us approaching from a distance, we have failed to observe the initial moment when they began exhibiting BBB.

Based on our observations and those already published, BBB cannot be correlated with the size of a snake. We observed this behaviour in *G. ussuriensis*, which did not exceed 60 cm in total length, and in *E. schrenckii* of more than 140 cm. Although most of our cases refer to adult snakes, we have recorded BBB in one subadult *E. schrenckii*, while other researchers have reported juvenile snakes with this defensive display (França et al., 2020; Hauser et al., 2022). Thus, this type of behaviour does not appear to be age-dependent. According to Hauser et al. (2022), this type of behaviour may also be connected with thermoregulation. Snakes are known to turn to more aggressive defensive strategies at high temperatures (Keogh & DeSerto, 1994). Therefore, BBB should have been a common passive antipredator display in snakes whose body temperature is suboptimal. Hauser et al. (2022) suppose that BBB serves as a warming up for further aggressive defence or fleeing. Nevertheless, we are more inclined to agree with França et al. (2020) who mention that some individuals were ready to attack a possible threat or flee rapidly, thus nothing hindered their mobility. We have observed similar traits in some *E. dione* and *E. schrenckii* that were actively trying to escape when handled. Those individuals exhibited no signs of suboptimal temperature; their movements were rapid, and the snakes immediately escaped into the vegetation when given a chance.

Apparently, BBB has rarely been observed in captive snakes, although BBB was displayed by *Elaphe taeniura* while both basking and just lying in its shelter (M.V. Akulenko, pers. comm.).

We presume that body-bending behaviour is a common defensive mechanism for many species, regardless of the climate zone they inhabit. Unfortunately, there have still been relatively few reports on this behaviour in the wild. We hope that our observations will encourage further studies that will contribute to understanding this phenomenon.

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RESEARCH ARTICLE

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Diet of the Dattatreya night frog *Nyctibatrachus dattatreyaensis* from the central Western Ghats, India

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ABSTRACT – The Dattatreya night frog *Nyctibatrachus dattatreyaensis*, found in the Chandra Drona Parvatha massif, is a stream-dwelling, evolutionarily distinct and globally Endangered species threatened by increasing habitat loss and alteration. We examined the stomach contents of 104 individuals, from ten different streams, of which 42 had prey in their stomachs. The prey items were in 12 orders across 4 classes, mainly dipterans, hymenopterans and lepidopterans. The frog exhibits a passive foraging mode, has a moderate trophic niche breadth ($B_{st} = 0.43$), and may have a preference for agile prey. Apart from this, there were plant materials, sand grains and plastic debris found in the stomach contents, with 0.82 mm³ of plastic debris found in eight individuals across three streams. The presence of plastic debris indicates the impact of anthropogenic activities leading to a form of habitat degradation. The data presented indicates the need for immediate and efficient conservation strategies to be put in place for this understudied species.

INTRODUCTION

Feeding ecology plays a pivotal role in the overall biology of a species, impacting its survival, reproductive strategies, population dynamics, habitat preferences and social patterns (Hohmann et al., 2006). Anurans may be active or passive foragers (Duellman & Lizana, 1994) but these foraging strategies are not mutually exclusive and even within a species may vary depending on the individual and the habitat (Crnobrnja-Isailović et al., 2012). Additionally, anurans are classified as specialist, intermediate or generalist predators, according to their prey preferences and the diversity of available prey in their habitats (Toft, 1981). Understanding the feeding ecology of a species provides insights into its foraging mode and associated physiological processes (Taigen & Pough, 1983; Navas et al., 2008), as well as interactions with sympatric species, and the impact of invasive species (Sabagh et al., 2010; Mohanty & Measey, 2018). Furthermore, the outcomes of dietary studies can aid in designing ex-situ conservation initiatives for threatened species (Michaels et al., 2014; Jayson et al., 2018). They are also essential for assessing ecosystem health and understanding the impacts of environmental factors like habitat fragmentation and alteration due to human activities (Anderson et al., 1999; Hocking & Babbitt, 2014). Nevertheless, there is limited information available on the feeding ecology of post-metamorphic anurans from the Indian subcontinent, with just a few studies on species endemic to the Western Ghats, such as *Rhacophorus pseudomalabaricus* (Kanagavel et al., 2017) and *Indirana leithii* (Modak et al., 2018).

The genus *Nyctibatrachus* Boulenger, 1882 represents a fascinating and diverse assemblage of nocturnal amphibian species, paleoendemic to the Western Ghats, a biodiversity hotspot (Abraham et al., 2022). It currently consists of 34 recognised extant species known for their cryptic nature and unique modes of reproduction (Kumar et al., 2022). *Nyctibatrachus*, with 84% of its species threatened, is the fourth most threatened genus in the Indomalayan biogeographic realm (Re:wild, Synchronicity Earth & IUCN SSC ASG, 2023). However, little is known of their ecology, including diet and feeding preferences.

Dattatreya's night frog *Nyctibatrachus dattatreyaensis* is an evolutionarily distinct and Globally Endangered (EDGE) species with a global priority for conservation (Gumbs et al., 2023). Discovered by Dinesh et al. (2008), this species is endemic to the central Western Ghats of India. It is a stream-dwelling, range-restricted species, currently known only from the Chandra Drona Parvatha massif, in the Chikmagalur district of Karnataka, at elevations between 865 and 1,515 m a.s.l.. Unfortunately, it is severely threatened by habitat destruction and fragmentation resulting from residential and commercial development and agriculture. Climate change and associated changes in the seasonal rain patterns will likely have a negative impact on their population and distribution (IUCN SSC, 2023). Despite this, the species remains significantly understudied to the extent that the only available literature pertains solely to its taxonomy. Given this premise, we report here attributes related to the feeding ecology of *N. dattatreyaensis*, which include its diet composition, foraging mode and niche breadth.

MATERIALS & METHODS

Study site

Streams of Chandra Drona Parvatha massif were randomly sampled to assess the presence and abundance of *N. dattatreyaensis*. From these, ten streams with well-established breeding populations were selected for this study (Figs. 1 & 2). The altitudes of the sampling sites ranged between 1,113

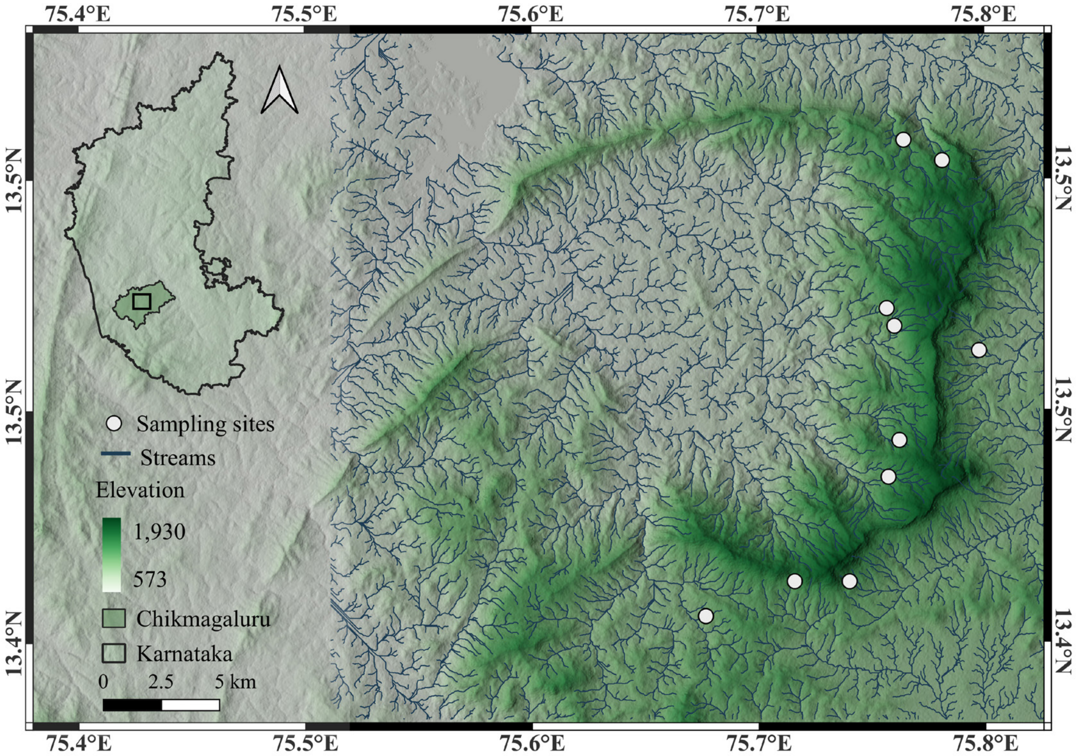

Figure 1. Map depicting Chandra Drona Parvatha massif and the sampling sites

and 1,420 m a.s.l.. The higher-elevation region of the massif has shola forests, where most of the streams originate. The massif also contains evergreen, semi-evergreen and moist deciduous forest types, and is fragmented with coffee estates, homestays and human settlements. Due to the cooler weather, picturesque mountains and waterfalls, the massif attracts numerous tourists throughout the year. The Bhadra Wildlife sanctuary borders and occupies a small portion of the massif.

Sampling

The frog population was sampled during the post-monsoon and winter seasons, between November 2022 and March 2023. The study area receives heavy rains during the monsoon season, which decreases the probability of detecting the species. A 100 m line transect was laid at each of the ten sampling site and surveyed from 20:00 to 23:00 h. Adult individuals were located using visual and acoustic encounter methods (Heyer et al., 1994). Individual frogs were captured gently using scoop nets and were handled using disposable vinyl nitrile gloves. Each stream section was sampled only once to prevent the recapture of individuals. Sex was determined by checking for the presence of femoral glands and nuptial pads which are secondary sexual characteristics of male *Nytcibatrachus* (Biju et al., 2011). In the case of females, pale, yellowish-coloured eggs were visible through the lateral side of the abdomen. We assigned adult individuals that lacked these characters to unknown sex. Snout-vent length (SVL) of all the individuals was measured using a digital vernier calliper with an accuracy of 0.1 mm.

Figure 2. Female *Nyctibatrachus dattatreyaensis* in its natural habitat

All individuals were weighed, and then their stomach contents were collected by flushing their stomachs. We followed the protocols of Solé et al. (2005) for stomach flushing and this conforms to the BHS Ethics Policy. To ensure minimal animal stress, the entire procedure on a single individual was completed within 10 minutes on-site. The frogs were then promptly returned to their original capture locations and monitored to ascertain if there were any adverse effects after stomach flushing. A sterilised, 14 Fr (4.6 mm diameter) silicone catheter was carefully inserted into the frog's stomach, and 10 ml of stream water was flushed through the catheter a maximum of three times using a syringe. Any stomach contents expelled from frogs during this procedure were collected in clean containers. The stomach contents were subsequently sieved and preserved in separate vials containing 70% ethanol. All the intact and partially digested prey items were assorted and identified under a stereomicroscope. The length and width of only the undamaged prey items were measured to the nearest millimetre using a graph sheet (Seshadri et al., 2021). The preserved prey items were identified to their lowest taxonomic level, usually to order, using literature (Tripplehorn & Johnson, 2005; Ramani et al., 2019).

Data analyses

To quantify the dietary intake of *N. dattatreyaensis*, we calculated the following parameters, Numeric percentage

$$N\% = \frac{N}{p} \times 100$$

where N is the number of prey items of a specific order/class and p is the total number of prey items. Frequency of occurrence percentage

$$F\% = \frac{F}{n} \times 100$$

where F is the frequency of occurrence and n is the number of individuals it was extracted from. The volume of each prey item was calculated using the ellipsoid volumetric formula,

$$V\% = \frac{4}{3}\Pi \left(\frac{length}{2}\right)\left(\frac{width}{2}\right)^2$$

(Griffiths & Mylotte, 1987). For each prey category, we calculated the index of relative importance following Pinkas (1971), *IRI = (V% + N%) F%*, and then converted it to IRI%. Higher IRI% signifies higher relative importance of that prey category.

Statistical analyses

Shapiro-Wilk normality test indicated that the data do not follow a normal distribution, therefore non-parametric tests were selected for all the analyses. We used the Mann-Whitney test U to analyse the difference in size and weight between the male and female individuals. To assess the heterogeneity and evenness in diet, we used reciprocal Simpson's heterogeneity index 1/D

$$D = -\sum[ni(ni-1)]/([N(N-1)])$$

where N is the total number of prey items and *ni* is the number of prey items in the prey category *i* and Simpson's evenness index

$$E_{1/D} = \frac{\frac{1}{D}}{S}$$

where S is the number of species (Krebs, 1999). We calculated trophic niche breadth using Levin's measure of niche breadth (B) (Levins, 1968) and standardised trophic niche breadth (B_{st}) (Hurlbert, 1978; Krebs, 1999). The index ranges from 0 to 1; values closer to 0 indicate usage of narrow niche breadth (specialist diet), and values closer to 1 indicate usage of broader niche breadth (generalist diet). All the analyses were performed using R Statistical Software (R Core Team 2022).

RESULTS

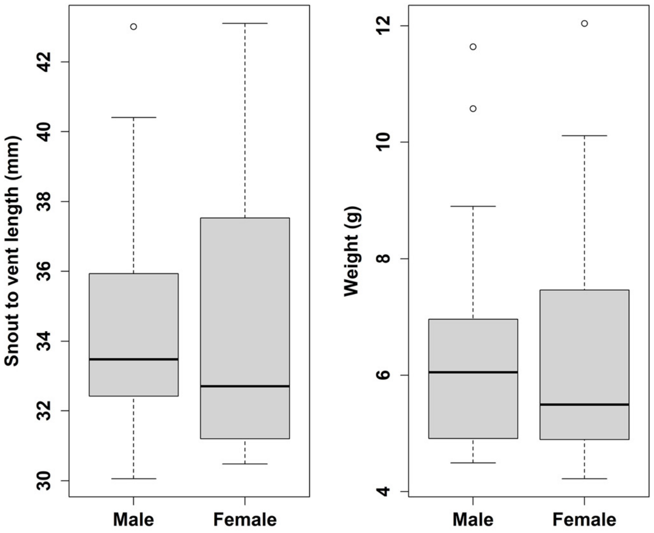

Figure 3. Comparison between female and male *Nyctibatrachus dattatreyaensis* in (left) SVL and (right) body weight

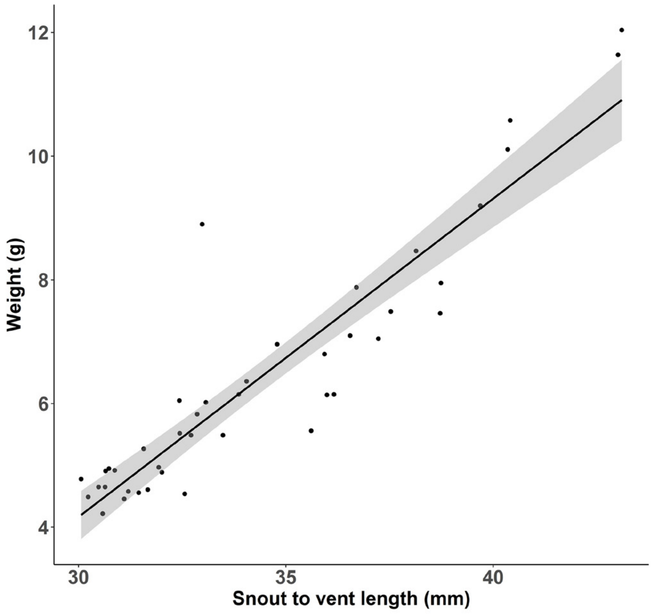

Figure 4. Relationship between body weight and SVL of adult *Nyctibatrachus dattatreyaensis*

We collected a total of 104 individuals of *N. dattatreyaensis* from across the sampling sites: 59 females, 37 males and 8 individuals whose sex could not be determined. Males had a mean SVL of 34.20 ± 3.35 mm and a weight of 6.49 ± 1.99 g. Females had a mean SVL of 34.57 ± 3.94 mm and a weight of 6.36 ± 2.10 g. There was no significant difference between males and females in either SVL (W = 225, p-value = 0.9207) and body weight (W = 233.5, p-value = 0.7532) (Fig. 3). At the same time, there was a strong positive correlation between the SVL and body weight (Fig. 4) with a correlation coefficient of 0.92 (Spearman's Rank Correlation).

After stomach flushing, we obtained prey items from 42 individuals (29 females and 13 males). We identified 67 prey items belonging to four classes: Oligochaeta, Insecta, Arachnida, and Crustacea. The prey items were identified to their respective orders, ten altogether (Table 1) except for two of the prey items, which remain unnamed. Apart from these, we also found plant materials and plastic debris in the

Table 1. Diet composition of *Nyctibatrachus dattatreyaensis* represented in numeric percentage (N%), percentage of frequency of occurrence (F%), volumetric percentage (V%), Index of relative importance (IRI) and its percentage representation (IRI%).

Class	Order	N%	F%	V%	IRI	IRI%
Oligochaeta	Unidentified	4.48	4.76	-	-	-
Insecta	Orthoptera	1.49	2.38	12.05	32.24	0.78
	Dermaptera	1.49	2.38	7.82	22.17	0.54
	Hymenoptera	17.91	16.67	6.44	405.78	9.82
	Coleoptera	10.45	7.14	1.51	85.38	2.07
	Lepidoptera	8.96	11.90	6.02	178.27	4.31
	Trichoptera	7.46	7.14	4.52	85.56	2.07
	Hemiptera	1.49	2.38	0.23	4.11	0.10
	Diptera	37.31	33.33	60.94	3274.98	79.25
Crustacea	Isopoda	1.49	2.38	0.17	3.97	0.10
Arachnida	Araneae	4.48	7.14	0.08	32.57	0.79
	Unknown	2.99	2.38	0.23	7.66	0.19

stomach contents. Plastic debris was found in eight individuals from three streams: two bisected by roads and one within a coffee estate. The total volume of the plastic debris was 0.82 mm³, with a mean volume of 0.07 ± 0.05 mm³. This excludes plastic debris with a width smaller than 0.1 mm such as lint microfibres and other microplastics. Dipterans (mosquitoes, crane flies) and hymenopterans were the most consumed among the prey items, with IRI 79.25% and 9.82%, respectively, followed by lepidopterans and coleopterans. The index of relative importance is calculated for all the prey categories, and the same can be found in Table 1.

The diet of *N. dattatreyaensis* is diverse with distinct prey items (Reciprocal Simpson's Heterogeneity Index (1/D): 4.960221), and the prey items preferred are moderately even (Simpson's Evenness Index E1/D: 0.41335). Based on the percentage utilisation of 12 food sources, the trophic niche breadth of the species is 5.76 according to Levin's measure of niche breadth (B) and 0.43 after standardisation (B_{st}). This indicates that the species has a moderate niche breadth and may favour certain types of prey over others.

DISCUSSION

Some anurans are specialist foragers, while others feed on diverse groups of prey items and exhibit preferences for certain specific taxa (López et al., 2007; Blanco-Torres et al., 2021). However, most anurans exhibit generalist feeding behaviour (Santos et al., 2004; Solé & Rödder, 2010). *Nyctibatrachus dattatreyaensis* is a generalist but would appear to have some preferences. We found 12 prey orders from four classes in their stomach contents, including undetermined prey items. Among them, dipterans were predominant in volume and frequency of occurrence (V = 550.85 mm³, F = 14). Volumetrically, the largest single prey item they consumed belonged to dipterans (crane fly, V = 301.6 mm³) and the smallest single prey items were hymenopterans (ant, V = 0.03 mm³). Although they consume prey from 12 different orders with varying sizes, dipterans had the highest relative importance (IRI% = 79.25) followed by hymenopterans (IRI% = 9.82) and lepidopterans (IRI% = 4.31). The trophic niche breadth value of B_{st} = 0.43 indicates a relative predominance of some prey items (dipterans) over others in the composition of their diet. Whether there is any actual preference for specific prey can only be confirmed with a study on prey availability in their habitat (Woodhead et al., 2007).

Studies on other anurans have shown a correlation between the foraging mode, prey preference and metabolic characteristics of a species. The anurans that prefer sedentary prey tend to have high aerobic capacity and resting metabolism, apt for active foraging. In contrast, agile prey is selected by the anurans that have low aerobic capacity and resting metabolism that correlates with the opportunistic mode of foraging (Taigen & Pough, 1983; Twardochleb et al., 2020). The predominant prey type in the diet of *N. dattatreyaensis* in this study was agile dipterans. They also frequently consumed other highly mobile prey items, such as Lepidoptera, Coleoptera and Trichoptera, exhibiting a passive foraging mode (sit-and-wait foraging strategy) similar to *Fejervarya moodiei* (Mohanty & Anujan, 2022). Frogs that are active foragers are often known to have toxins to deter their predators while those that are passive foragers rely on camouflage to attack and consume their prey (Toft, 1981; Wells, 2010). We only observed *N. dattatreyaensis* sitting inactive, well camouflaged in the slower sections of the stream near vegetation, ambushing prey that moved in front of them; they are not known to have any toxins. These observations are consistent with the species being a passive forager.

Some anurans consume plant materials as a major part of their diet, intentionally and regularly, for example, *Boana albomarginata* (Tupy et al., 2021). But in most frogs, it is thought to be consumed accidentally (Rodrigues et al., 2023). Ingestion of sand grains may aid the mechanical digestion of insects such as beetles or could have been passively ingested from their microhabitat (Evans & Lampo, 1996). The plastic debris in the stomach content might have been ingested while consuming insects. Previous studies have documented the presence of microplastics or plastic debris in the stomach content of tadpoles (Kolenda et al., 2020) and toads (Döring et al., 2017) highlighting the impact of anthropogenic activities surrounding the freshwater streams inhabited by *N. dattatreyaensis*. Microplastics have been detected even in remote, high-altitude freshwater bodies, similar to the study area for *N. dattatreyaensis* (Iannella et al., 2019). When ingested and accumulated in the organs of tadpoles, microplastics lead to alterations in their external morphology, genetic mutations and cell damage (Araújo et al., 2020). They can be fatal, induce stress, and increased levels of microplastics in the water could impair the tadpole's ability to feed (Balestrieri et al., 2022). When coupled with chytridiomycosis, the bioaccumulation of microplastics amplified vulnerability among tadpoles (Bosch et al., 2021). Chytrid infection has been previously reported in species of *Nyctibatrachus* (Dahanukar et al., 2013). These findings highlight the importance of conservation efforts to protect freshwater ecosystems and the need to develop strategies tailored to conserve stream-dwelling anurans such as *N.*

dattatreyaensis. This study is the first to provide ecological information crucial for understanding the ecological role of this species and the health of its habitat. However, further research is needed to address how prey availability, seasonality, and ontogenic changes affect prey selection in this species.

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RESEARCH ARTICLE

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Initial field data for the Critically Endangered Alchichica salamander *Ambystoma taylori* from Lago Alchichica, Mexico

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ABSTRACT – The Lake Alchichica salamander *Ambystoma taylori* is a Critically Endangered, neotenic inhabitant of a single, hyposaline lake in Puebla, Mexico. Between 2015 and 2018, initial field data were collected for this understudied species, to inform future conservation action. Using unbaited submerged passive traps at depths of 0–<10, 10–<20 and 20–30 m, we captured 240 salamanders (137 males, 82 females, 18 juveniles) over 32 visits, for which length and mass were collected, and which were individually identified. Generalised linear models showed that males were captured at greater rates than females, and capture rates were lower in the dry-warm and wet-cold seasons than the wet-warm and dry-cold seasons. Salamanders were more likely to be captured at depths of 20 and 30 m than 10 m, but this effect was not detected when using only presence/absence data, suggesting clustering of animals in the lake. No evidence was found that depth of capture was predicted by size, sex or season. No eggs and only a small number of juveniles were detected, which were found in all seasons and without a clear trend in size across the year, meaning that a clear breeding season could not be identified. It is noteworthy that hatchling larvae would be able to escape the traps due to mesh size. Adult mass had a significant, positive cubic relationship with SVL, but females were heavier for their lengths, especially at larger sizes. Conversely, males were slightly but significantly longer than females for their weight (despite shorter absolute SVLs than females). These trends are typical in salamanders and likely associated with investment in eggs. Approximately 12% of animals carried an unidentified ectoparasitic burden and endoparasites *Hedruris siredonis* were detected in opportunistically encountered dead animals that were necropsied. Data reported here provide an initial insight into the biology and phenology of this species, as well as offering viable survey methods in a challenging environment.

INTRODUCTION

The Lake Alchichica salamander *Ambystoma taylori* is an ambystomatid salamander endemic to a single, hyposaline crater lake (Lago Alchichica) on the border of Puebla and Veracruz states, Mexico. This obligate neotene is assessed as Critically Endangered on the IUCN Red List (IUCN SSC ASG, 2015), is an Evolutionarily Distinct and Globally Endangered (EDGE) species (Safi et al., 2013) and is threatened by pollution, increasing salination and water extraction from its native lake, as well as regional anthropogenic water stress, and habitat damage from tourism (IUCN SSC ASG, 2015). The stromatolite-rich nature of the lake bed, which limits access to and use of nets in the lake, the extreme depth of the lake (64 m), and hyposaline conditions protect the population from overharvesting and introduction of non-native fish, two important threats for ecosystems in neighbouring freshwater lakes (CJM & JAHD, pers. obs.). The lake is a complex environment, with thermally driven, seasonal cycles of water circulation and stagnation, and associated oxygenated and anoxic periods, and phyto- and zoo-plankton blooms (Lugo et al., 1999). Besides the salamander, the lake hosts a unique ecosystem, with widespread endemism of organisms including algae, zooplankton, invertebrates and silverside fish (Alcocer et al., 2007; 2015; Chiappa-Carrara, 2022; Macek et al., 2008; Vilaclara et al., 2022).

Beyond its neoteny, and limited work investigating phylogeny (Percino-Daniel et al., 2016), parasitology (Michaels et al., 2016) and more broadly concerning its ecosystem (Kaźmierczak et al., 2011), little is known about the salamander. A Conservation Needs Assessment (CNA) for the species recommended, among other actions, that in-situ research is conducted in order to better understand the biology of the species (Marcec, 2019). Between 2015 and 2018, surveys sponsored by Africam Safari and ZSL EDGE of Existence Programme were conducted for *A. taylori* at Lago Alchichica to estimate salamander abundance in the lake and investigate species biology. Results of these surveys are summarised herein.

MATERIALS & METHODS

Ethics and legality

All methods complied with the British Herpetological Society Ethics Policy and with local laws and regulations. The following permits were in place for all fieldwork: SGPA/DGVS/07294/15 and SGPA/DGVS/09323/16; all work was ethically approved by the Zoological Society of London.

Field methods

Thirty-two surveys were conducted from an inflatable boat, manoeuvred by paddle. Torpedo Crawfish Traps from Heavy-Duty Steel Mesh (USA) (Fig. 1) were used to trap salamanders; bait was not required to trap animals. On each survey visit, 21 traps were distributed around the lake and lowered to the substrate at three different ranges of depth; 0–10 m, 11–20

Figure 1. (Top left) *Ambystoma taylori* adult in habitat, Lago Alchichica, Mexico; (top right) stromatolithic habitat both above and below the water line harbours salamanders readily snags traps and precludes drag netting; (bottom) salamander in minnow trap, having been slowly drawn up from the bottom of the lake

m and 21–30 m depth, measured by marking strings attached to the traps signifying depth. Trap location was previously determined by using a bathymetric map of the lake to randomly distribute traps within appropriate depth zones (Kaźmierczak et al., 2011). Each trap was equipped with a plastic bottle as a buoy at the end of the string, and small pieces of polystyrene were attached along the string every 3 metres to avoid it sinking and tangling around stromatolites. Traps were deployed in the morning and retrieved after 24 hours. Visits were scheduled in each of four climatic seasons (cold-dry: November to February; warm-dry: March to May; warm-wet: June to August; cold-wet: September to October, all inclusive).

During the first surveys, traps were retrieved slowly (~6 metres/minute) to avoid potential deleterious effects of depressurisation on salamanders. During some surveys, however, the strong wind made it impossible to retrieve the traps at a slow rate, so they were retrieved quickly without any apparent deleterious effects on salamanders. Trapped salamanders were handled with nitrile gloves and were processed at the lake shore. Animals were sexed (female, male or juvenile) through inspection of secondary sex characteristics including cloaca shape and size, then measured (total length [TTL]; snout-vent length [SVL]) and weighed. To aid in individual identification, animals were photographed dorsally in a standardised manner, and marked with Visible Implant Elastomer (VIE; Moon et al., 2022); animals were also compared to historical records held by JAHD of opportunistically photographed animals to identify any recaptures. Salamanders were checked for external parasites and deformities, recording the number of affected animals. Individuals were then released into the lake, in the closest possible spot to the trap where they were caught, close to aquatic shelters in which they could hide.

Statistical methods

Recorded data were subject to the analyses presented in Table 1, with an alpha of 0.05 used throughout. These models comprised: 1) a Poisson generalised linear model (GLM) investigating the effect of sex, season and depth on counts of captured salamanders, allowing for variation in trapping effort between seasons (visits were not evenly distributed by season – see Table 2); 2) a logistic regression investigating the influence of season and depth on presence/absence (i.e. empty nor non-empty traps) of salamanders; 3) a multinomial regression investigating the influence of sex, season and weight of salamanders on the depth at which they were captured; 4) a linear regression investigating the effect of SVL and sex on mass; 5) a linear regression investigating the effect of mass and sex on SVL. Analyses were conducted in R 4.2.3. using the stats package (R Core Team, 2023) for all analyses other than the fitting of multinomial models, for which the nnet package was employed (Venables & Ripley, 2002). Residuals for all models were checked using the DHARMa package (Hartig, 2022) to ensure that model assumptions were met. A backwards elimination approach to hypothesis testing was used, with initial models fitted with interactions and non-significant terms removed; non-significant main effects were retained

Table 1. Research questions posed and analyses performed to answer them using the collected data

Analysis ID	Research question	Response variable	Explanatory variables	Type of model used
1	Do encounter counts differ between sexes, seasons and depths?	Count of captures per visit	Main: Sex (male; female); season; depth (10; 20; 30 m) Offset: number of traps by season	Poisson generalised linear model
2	Is presence/absence related to season or depth?	Empty or non-empty trap	Season; depth (10; 20; 30 m)	Logistic regression
3	Is depth predicted by season, sex or size of salamander?	Depth category (10; 20; 30 m)	Sex (male; female), season or weight	Multinomial regression
4	How is mass related to length, and is this different between sexes?	Mass	Sex (male; female), SVL (cubic)	Linear regression
5	How is length related to mass, and is this different between sexes?	SVL	Sex (male; female), Mass (cubic)	Linear regression

Table 2. Survey, capture and morphometric summary of *Ambystoma taylori* from Lago Alchichica, Mexico; lengths and weights are given as mean(standard deviation)[a]

			Count			SVL (cm)		TTL (cm)		Weight (g)		Number with ectoparasites	
Season	**Number of visits**	**Depth (m)**	**Males**	**Females**	**Juveniles**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Cold-dry	11	10	11	5	3	8.54 (1.47)	7.66 (1.38)	16.35 (3.07)	14.25 (2.67)	28.32 (14.23)	18.5 (7.7)	3	1
		20	34	10	0	7.95 (1.37)	7.68 (1.34)	14.85 (3.24)	13.94 (2.91)	20.66 (11.17)	22.76 (11.51)	3	3
		30	21	12	3	8.49 (1.65)	8 (0.58)	15.12 (3.42)	14.9 (1.51)	23.38 (14.01)	22.15 (10.36)	0	3
Warm-dry	7	10	2	4	2	10.57 (3.11)	7.94 (0.65)	20.39 (6.7)	15.37 (1.8)	57 (49.5)	25.25 (7.27)	0	1
		20	6	3	4	8.2 (1.69)	8.6 (2.48)	15.77 (3.89)	17.03 (6.08)	28.33 (17.52)	34.67 (29.14)	0	0
		30	3	4	0	8.53 (0.88)	8.7 (1.09)	15.98 (1.7)	16.61 (1.59)	20.67 (6.51)	31.75 (11.21)	0	0
Cold-wet	5	10	1	0	0	-	-	-	-	-	-	0	0
		20	2	1	0	8.22 (1.43)	-	15.86 (3.08)	-	15 (1.41)	-	0	0
		30	3	5	0	8.86 (0.28)	7.84 (0.62)	16.63 (0.45)	15.12 (1.61)	25.67 (5.03)	26 (8.94)	0	0
Warm-wet	9	10	14	8	2	7.74 (0.69)	8.53 (1.54)	14.87 (1.81)	16.39 (3.23)	18.5 (6.75)	47.4 (24.64)	2	3
		20	13	18	3	7.43 (1.1)	7.35 (1.23)	14.22 (2.49)	14.41 (1.93)	20.77 (7.91)	22.69 (10.9)	2	2
		30	27	12	1	7.62 (0.78)	8.29 (1.48)	16.01 (5.82)	15.85 (3.12)	21.05 (8.13)	29.4 (16.66)	2	0

[a] single animal for which sex was not recorded was captured in the warm wet season, with and SVL of 6.994cm, and a TTL of 13.531cm; weight was not measured.

in models. Only final models are presented. Visits were necessarily treated as independent, which is an assumption of the analysis, but a reasonable one given that no recaptures were detected (see Results). Random effects models treating year as a random effect were explored as potential means of controlling for any year-level effects that may influence repeated measures, but could not be fitted due to insufficient observations across seasons for some years.

Juveniles were removed from the analysis due to small sample size and the impossibility of sexing animals. For model 1, the use of an offset was validated by checking that the correlation coefficient between the number of traps and counts was close to 1. There were too few observations of ectoparasites for a robust formal analysis.

RESULTS

In the research period, 32 survey visits were made with a mean(SD) duration between visits of 38.6(91.5) days. A total of 240 live salamanders were encountered in traps, from which useable data were collected for 237 and for which full data (collection date, TTL, SVL, weight and depth) were available for 207. These are summarised in Table 2. No recaptures were documented across any surveys and population size estimates could therefore not be made, although it might be inferred to be large (see Discussion). Moderate prevalence of ectoparasites was detected, with about 12% (29/242) of captured animals having a visible ectoparasitic burden, with no clear seasonal pattern (formal analysis was not feasible). Separate to trapped individuals, two animals were found dead near the lake shore, without apparent cause. These animals were subject to a necropsy, and parasites of the digestive tract were found: the gastro-intestinal nematode *Hedruris siredonis*. No emaciated animals as described in the context of nematodiasis (Michaels et al., 2016) were captured. Several other dead animals were found floating on the lake surface, primarily during the spring; no cause of death or other pathology could be ascertained.

Statistical results of analyses are presented in Table 3. Analysis 1 estimated a relatively low rate of capture in the reference state (dry-cold, female salamander at 10 m depth), at 0.0953 captures per trap (exponentiated -2.35). The model found that males were captured at a significantly higher rate than females (rate ratio of 1.65 – nearly twice as frequently), and salamanders were captured at significantly higher rates at depths of 20 and 30 metres than 10 metres (rate ratios of 1.89 for both depths); parameters for 20 m and 30 m were almost identical. Capture rates were significantly lower in the dry-warm and wet-cold seasons than in the dry-cold season (rate ratios of 0.37 and 0.31, respectively – approximately a third as frequently), with no significant difference observed in the

Table 3. Statistical outcomes of models. For each model, the intercept estimate represents the outcome when all variables are at their reference level, and the estimates for other terms indicate changes to the intercept based on other categories (categorical variables) or a 1-unit change in continuous variables. Log odds and rate ratios may be converted to natural scales by exponentiation. Significant p-values are in **bold**. Note that the p values given for models 4 and 5 are derived from an overall likelihood ratio test for the major terms as it was not necessary to dissect the cubic term into each constituent polynomial; for other models, p values are Wald Chi-squared tests of regression coefficients.

Analysis ID	Response	Term	Reference	Estimate scale[a]	Estimate (SE)	P-value
1	Count of captured salamanders	Intercept	N/A	Log rate ratio	-2.35 (0.19)	**<0.0001**
		Season: dry-warm	Dry-cold		-0.99 (0.24)	**<0.0001**
		Season: wet-cold	Dry-cold		-1.18 (0.31)	**<0.0001**
		Season: wet-warm	Dry-cold		0.19 (0.15)	**<0.0001**
		Sex: Male	Female		0.5 (0.14)	**<0.0001**
		Depth: 20m	10m		0.64 (0.18)	**<0.0001**
		Depth: 30m	10m		0.64 (0.18)	**<0.0001**
2	Likelihood of non-empty trap	Intercept	N/A	LogOR	-1.52 (0.24)	**<0.0001**
		Season: dry-warm	Dry-cold		-0.81 (0.32)	**0.01**
		Season: wet-cold	Dry-cold		-1.63 (0.49)	**<0.001**
		Season: wet-warm	Dry-cold		-0.34 (0.26)	0.19
		Depth: 20m	10m		0.30 (0.28)	0.28
		Depth: 30m	10m		0.16 (0.28)	0.57
3	Likelihood of capture at given depth vs 10 m	Intercept	N/A	LogOR	20m: 1.12 (0.43) 30m: 0.92 (0.43)	**20m: 0.009** **30m: 0.034**
		Season: dry-warm	Dry-cold		20m:-0.44 (0.63) 30m:-0.49 (0.66)	20m: 0.48 30m: 0.46
		Season: wet-cold	Dry-cold		20m: 0.07 (1.20) 30m: 1.31 (1.11)	20m: 0.95 30m: 0.24
		Season: wet-warm	Dry-cold		20m:-0.48 (0.43) 30m:-0.18 (0.44)	20m: 0.27 30m: 0.69
		Sex: Male	Female		20m:-0.17 (0.42) 30m:-0.28 (0.42)	20m: 0.68 30m: 0.51
		Weight	Mean Weight		20m:-0.02 (0.01) 30m:-0.01 (0.01)	20m: 0.08 30m: 0.274
4	Mass	Intercept	N/A	Response scale	26.856 (0.80)	**<0.0001**
		SVL	Mean SVL		191.48 (11.38)	**<0.0001**
		SVL^2			37.49 (13.12)	
		SVL^3			-22.17 (11.76)	
		Sex: Male	Female		-4.25 (0.96)	**<0.0001**
		SVL^{1*} sex : Male	N/A		-53.85 (13.83)	**<0.001**
		SVL^{2*} sex : Male			11.28 (15.98)	
		SVL^{3*} sex : Male			19.81 (15.20)	
5	SVL	Intercept	N/A	Response scale	7.82 (0.09)	**<0.0001**
		Mass	Mean Mass		15.63 (0.71)	**<0.0001**
		Mass^2			-2.54 (0.70)	
		Mass^3			0.05 (0.70)	
		Sex: Male	Female		0.38 (0.11)	**<0.001**

[a] for intercepts, this is equivalent to a log rate for model 1, log odds for analyses 2 and 3, and mean measurement for analyses 4 and 5

wet-warm season (Fig. 2; Table 3). There were no significant interactions between variables, so the effect of sex, season and depth did not depend on one another. No overdispersion was detected for the Poisson model. Analysis 2, which was designed to control for any clustering effect by reducing the count response variable to a binary response (whether any salamanders were caught or not), produced consistent results for season. The likelihood of capturing at least one salamander was significantly lower in the dry-warm and wet-cold seasons than in other seasons (odds ratios of 0.44 and 0.20, respectively; i.e. approximately 56% and 80% less likely). However, for depth, although the same trends were identified as in analysis 1, differences in the likelihood of captures at each depth were not significant (Table 3). The Poisson GLM suggested approximately 30 traps (95% confidence interval (CI): 22 to 47, calculating CIs from standard error of estimates)

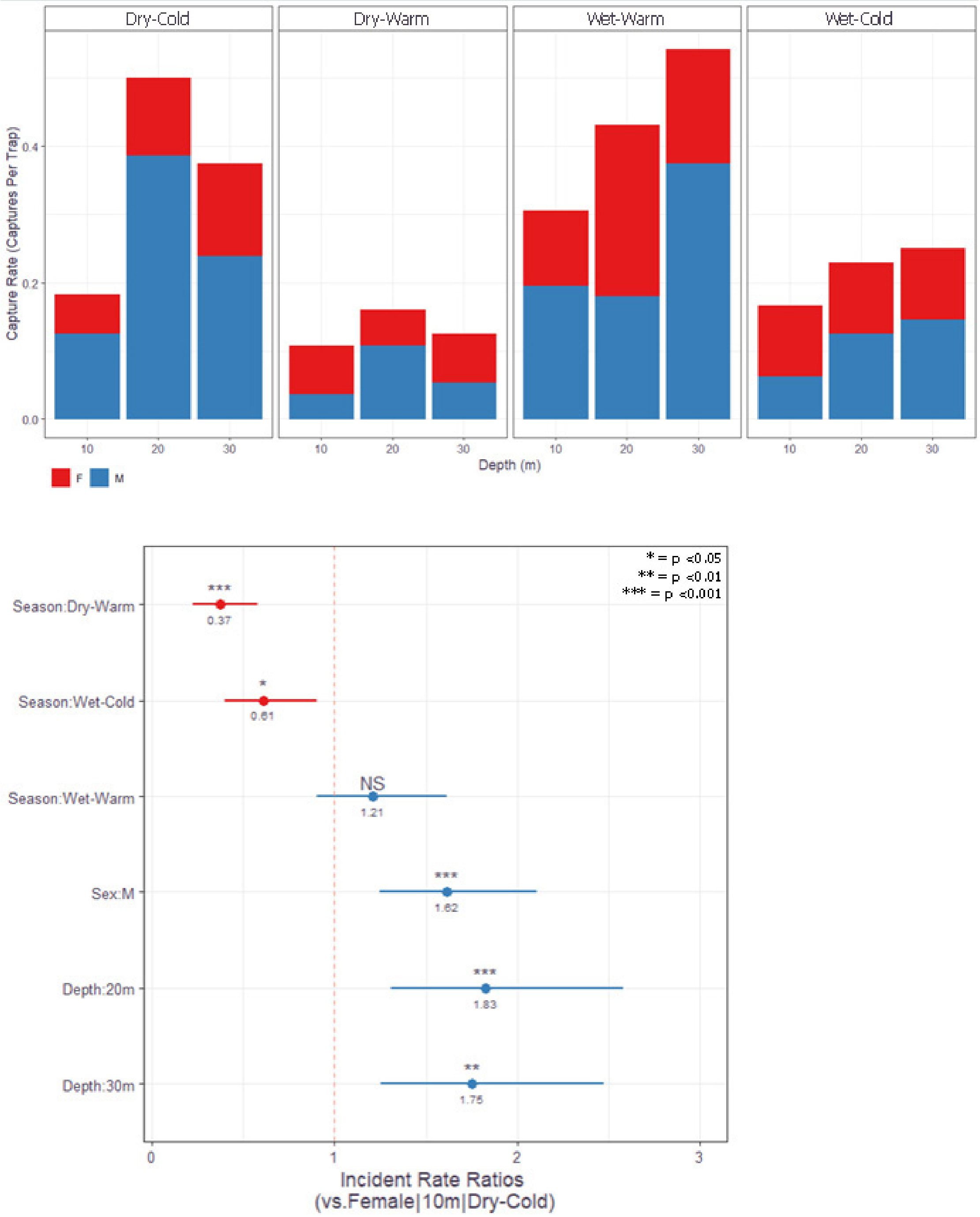

Figure 2. Capture rate ratios (captures per trap) of male and female *Ambystoma taylori* from Lago Alchichica, Mexico, (top) across depths and seasons; (bottom) incident rate ratios estimated from analysis 1, associated with seasons, sex and depth relative to captures of female salamanders at 10 m during the dry-cold season

are needed to ensure a 95% probability of capturing at least one female salamander in the dry season at 10 m depth (i.e. at reference), while the logistic regression model suggested that approximately 15 traps (CI: 10 to 28) are needed under the same conditions. The overlapping confidence intervals indicate that the baseline estimates of likelihood of capture from both models are not statistically different. Analysis 3 found no evidence that the likelihood of capturing salamanders at each depth (10, 20 or 30 m) was significantly predicted by size, sex or season (Table 3). Analysis 4 showed that mass had a significant cubic relationship with SVL, but that males had a significantly shallower curve than females, indicating that females were heavier for their length than males (by around 4 g on average), especially at longer SVLs (Fig. 3; Table 3). Analysis 5 showed that, adjusting for the effect of mass, males were slightly but significantly longer on average (by about 0.4 cm in animals of average weight) than females and that there was a significant cubic relationship between SVL and mass; no significant interaction between mass and sex was detected (Table 3). Importantly, accounting for the effect of mass provided a more accurate insight into differences in SVL between sexes (showing that males are slightly longer for their mass) than a simple comparison of means (which appeared to show that males were smaller).

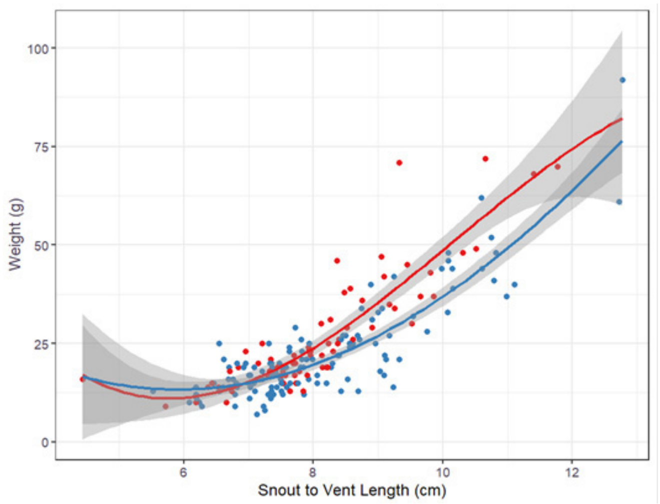

Figure 3. Masses of male (blue) and female (red) *Ambystoma taylori* from Lago Alchichica, Mexico plotted against their snout-vent length. Curves represent smoothed estimates of the relationship between variables based on model estimates, with their standard errors plotted in grey. The trends demonstrate the interaction between snout-vent length and sex in their effects on weight, such that females are heavier than males at larger sizes, but more similar at smaller sizes. Lines diverge at ~7 cm SVL, which may indicate the point at which sexual dimorphism becomes apparent; this was not formally investigated.

DISCUSSION

We present some initial field data for *A. taylori*, a species about which little is known of its biology. Our data suggest fluctuations in capture rates, but not in depth selection by salamanders, with season as well as potential aggregation of animals, rather than an even distribution of individual animals. Males were captured at a higher rate than females. Females were heavier than males for their length, especially at larger sizes, while males were conversely longer for their weight. Despite the capture of a large number of individuals, no recaptures were made. These results are discussed and contextualised below.

The field methods developed for surveys may be useful for conducting further work on the species. Animals were collected successfully in traps without bait. This suggests that trapping relied on passive interception of animals and therefore indicates relatively high density of animals, given the routine success of traps and their random placement. It is unclear whether use of bait might improve trap efficiency, but preliminary informal experience of trialling dead fish as bait did not result in any apparent change in numbers of captured salamanders. One methodological point of note is the ease with which traps´ string snag irretrievably on the rough stromatolites; polystyrene pieces attached along the string every three metres helped us solve this problem to avoid damage to or loss of equipment. Although slow retrieval of traps to avoid damage to salamanders was generally employed out of caution, where this was not possible, no deleterious effects were encountered, suggesting that rapid retrieval may be safe. However, caution is encouraged, and slow retrieval should be employed wherever possible to avoid potential problems that may not be immediately noticeable. Due to the rough, snagging stromatolites and the depth of the lake, other means of survey (e.g. drag netting or visual encounter surveys) are not feasible in the lake (netting) or would only detect animals in the very shallow margins (visual encounters).

Our data suggest that males were captured at higher rates in traps than females, that trapping was more successful for both sexes at depths of 10–20 and 20–30 m than 0–10 m and was less successful in the dry-warm and wet-cold seasons than the wet-warm and dry-cold seasons (all allowing for trapping effort variation between seasons). It is unclear whether the difference between sexes in trapping success is indicative of a male-skewed population, or a higher trapping rate for males. If it were the case, the latter could be caused by higher activity in males, which, although not yet investigated in *A. taylori*, is often reported in male salamanders across the caudate phylogeny, including the Ambystomatidae (Finkler et al., 2003; Verell & Palton, 1996; Helfer et al., 2012), though not ubiquitously (Schulte et al., 2007). Given that the population is restricted to the single lake, and animals live for more than year, seasonal differences in trapping success must reflect differences in capture rate rather than in the presence of animals. This is perhaps caused by seasonal variation in activity of salamanders and/or of weather conditions affecting trap drift, and consequently in the rate at which animals encountered traps. Movement of animals into depth zones outside of those sampled in this study (i.e. deeper than 30 m) should also be considered, given that the anoxic zone of the lake is strongly influenced in size and depth by seasonal changes in temperature that determine circulation cycles in the lake (Lugo et al., 1999). The anoxic zone, which would exclude salamanders, is largest during the period of the year (Lugo et al., 1999) when highest salamander trapping rates were recorded in the present study, which may indicate that salamanders are driven into higher concentrations in shallower water and, consequently, are more readily trapped. Trapping at greater depths may help to elucidate this and survey work of this sort is encouraged. Notably, when presence/absence rather than count data were analysed, the effect of depth was no longer significant. This may indicate that while seasonal and sex differences are linked directly to changes in animal activity and occupation, the effect of depth may be influenced by clustering of animals, with depths >10 m supporting higher concentrations of salamanders, thereby exaggerating the observed differences in capture rates between depths. Further survey work may disentangle these hypotheses and determine whether or not the apparent aggregation may be the result of an attractant effect of already trapped animals.

The 18 juveniles collected ranged from ~6–15 cm TTL and were found in all seasons, with no clear pattern in size across months. Eggs were never encountered, despite the fact that in other species (e.g. *Ambystoma lermaense*), eggs are often attached to traps by trapped females (CJM, pers. obs.). Further fieldwork is recommended to better understand the reproductive cycle of this species and whether it forms a distinct pattern, or if reproduction occurs throughout the year in this relatively stable aquatic system. Observations from captive populations may augment insights from fieldwork, as well as data for other species. Other lake-dwelling neotenic species from Mexico have been documented to breed in the warm-wet season and so, in the absence of other information, this period may be the most likely candidate for the breeding period of *A. taylori* (Brandon, 1973; Zambrano & Reynoso, 1993; Sanchez-Herrara, 1980).

We detected a typical cubic relationship between length and mass (Peig & Green, 2010), and evidence for size-dependent sexual dimorphism in length and mass. While smaller (and therefore likely younger) females shared a similar relationship between length and mass with males, larger (and therefore likely older) females were heavier for their length than males. Additionally, male salamanders were longer for their mass than females; this trend was statistically disentangled as the higher mass of females generated a higher raw mean SVL for this sex. Salamander females in general are typically more heavily bodied due to carrying eggs and the fat stores necessary to produce them, while males are typically more elongate for their mass (e.g. Bruce, 2000; Church, 2008; Howard, 1983; Jakob & Marx, 1992; Scott & Fore, 1995; Semlitsch, 1985) . The point at which trend lines for mass separate may therefore indicate the point at which sexual maturity occurs in females, as this may correspond with production and storage of eggs. This divergence typically occurred at around 7 cm SVL (see Figure 3, where trend lines for males and females diverge), but was not subject to formal analysis in the scope of this work. Data are not available to indicate the age at which animals reach this size and may be different for males and females, given the existence of sex-specific growth patterns in the genus (Blackwell et al., 2003). Body condition is a variable of particular interest in captive populations of amphibians (Ferrie et al., 2014), which exist for this species (Marcec, 2019). However, monitoring body condition is constrained by access to body condition data for wild populations (e.g. Jayson et al., 2018). Consequently, the data presented in the present work may provide a robust dataset of population norms against which comparisons could be made.

Ecto- and endo-parasitism was detected in the salamanders; a reasonable estimate for prevalence of ectoparasitism of about 12% could be made, but endoparasites were detected by chance in a small number of dead individuals and so a robust endoparasitosis prevalence estimate cannot be calculated. It was not possible to identify ectoparasites as these were left intact on animals to avoid injuries associated with their removal, given the absence of appropriate veterinary resources, and could not be properly visualised in identification photographs. Endoparasites were identified as *H. siredonis*, which has been previously reported from the salamander in emaciated individuals (Michaels et al., 2016), and extends detection to animals of apparently normal body condition, albeit still found dead near the shore with no apparent cause. Further work (either in the field or using museum specimens) to understand the prevalence of this parasite and its relationship with salamander health is recommended. Characterisation of the ectoparasites of this species is of particular interest and should be the subject of future work in order to inform understanding of their potential impacts on salamander biology and interactions in the ecology of the lake.

There were no recorded recaptures despite overall capture of 240 adults between 2015 and 2018. The use of a combination of photographic and VIE methods suggests that this is not a case of undetected recaptures. The apparent absence of recaptures may result from one of a number of reasons including a very large population of salamanders, and effect of capture on recapture (e.g. animals becoming wary of traps), or high population turnover (this latter is highly unlikely given the strictly closed population and long lifespan of *Ambystoma* salamanders (e.g. Trenham et al., 2000)). It is considered unlikely that field methods resulted in the death of all captured animals given that none of methods used were novel for amphibians and no signs of distress or injury were noted in any animals on release. Further fieldwork is encouraged to better understand population size, but given all available data, a large population of this range-restricted species seems likely, highlighting the pivotal role of point endemism in driving conservation status.

Overall, our data provide an initial insight into the biology, distribution and phenology of *A. taylori* and provide suggested methods for successful surveying of this species in a challenging environment. Further surveys are required to fully elucidate species biology, which may be importantly supplemented by observations from animals in existing populations in captivity.

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RESEARCH ARTICLE

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Comparison of scale anomalies in populations of northern viper *Vipera berus* from habitats differing in size and degree of fragmentation

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ABSTRACT – Northern vipers *Vipera berus* are declining and increasingly confined to fragmented and isolated populations in England. Scale anomalies that occur during development can potentially be related to environmental stressors. Using photographs of the dorsal view of viper heads, we compared levels of head scale fragmentation and asymmetry between four populations to identify the extent of anomalies. Although all populations had vipers that displayed scale fragmentation and asymmetry, the incidence of such anomalies was lowest at Fackenden Down which supports the largest population and is probably the least isolated. Two populations that displayed particularly high levels of scale anomalies – Talbot Heath and Turbary Common – are isolated and surrounded by urban and suburban development. Supraocular and intercanthal scales showed a higher incidence of fragmentation and asymmetry than did apical scales, and there was a general tendency for the vipers with scale fragmentation to also display asymmetry. We speculate that the relatively high incidence of scale anomalies in small, isolated populations may be a result of environmental or genetic pressures and that head scale fragmentation and asymmetry may provide an early indication of such stressors.

INTRODUCTION

How and why living organisms deviate from bilateral symmetry has long fascinated biologists. Such deviations may frequently be a result of injury, such as the loss of digits or limbs. However, there has been a longstanding debate over whether genetic and environmental stressors can lead to asymmetry (e.g. Parsons, 1992; Lens et al., 2002; Beasley et al., 2013). Within a population, deviations from symmetry may be non-directional and occur randomly on traits on both sides of the body (fluctuating asymmetry) or may occur more on one side than the other (directional asymmetry). Either way, reptiles are good models for such studies as their scale patterns are easily quantified and compared between both sides of the body. Moreover, several studies have indicated links between genetic and environmental stressors and asymmetry (see review by Laia et al., 2015). Equally, it has been shown that suboptimal incubation conditions can cause developmental instability and increase the incidence of scale anomalies in hatchling reptiles (e.g. Brown et al., 2017). Consequently, measuring scale anomalies in reptiles can potentially be used as an index of stressors that might be impinging on a population (Shamiminoori & Bull, 2015).

In Britain, concerns over declines of *Vipera berus* have led to its classification as Vulnerable in England and Near Threatened in Scotland, Wales and Great Britain as a whole (Foster et al., 2021). Small populations are particularly vulnerable to anthropogenic threats including habitat loss and fragmentation as well as seasonal temperature fluctuations due to climate change (Gardner et al., 2019). Moreover, nearly 40% of viper habitat had been lost by 2011, and the species now only occurs in fragments of its historic range in Great Britain (Gardner et al., 2019). However, both the drivers of these declines and the interventions needed to mitigate them may be complex (Julian & Hodges, 2019). Such recent trends have precipitated a number of long-term studies across the country with the aim of determining drivers of population dynamics and potential threats (e.g. Hills, 2018; Hodges et al., 2023; Struthers, 2023). As individual northern vipers are relatively easy to identify through their distinctive head scalation and colour patterns, these studies are providing valuable datasets from which head scale anomalies can be analysed.

In this study, *V. berus* individuals from four populations differing in size, geographic location and proximity to human encroachment in southern England were analysed after being photographed; and head scalation was examined with specific emphasis on degrees of fragmentation and asymmetry. Scale fragmentation and asymmetry were visually scored with the specific aims of 1) identifying and comparing the extent of scale fragmentation and asymmetry in the four populations of vipers; and 2) discussing the potential drivers of scale anomalies in relation to population histories and environmental factors.

MATERIALS & METHODS

Study sites

Observations of *V. berus* were compiled from four sites in southern England studied from 2005 to 2021; two sites in Dorset: Talbot Heath and Turbary Common, and two sites in Kent: Fackenden Down and Kings Wood. Talbot Heath and Turbary Common are approximately 37 ha and 40 ha in area respectively, each site supporting a population of less than 100 vipers which may also be in decline (Dobbs, pers. obs). Both sites are isolated and surrounded by urban and suburban development. The Kings Wood study sites comprise three small clearings that total 3 ha, separated by distances of between 0.5 km and 3 km within a wider mixed woodland of 588 ha. The population of vipers in these clearings has declined over the past 20 years and currently comprises less than 50 individuals (Hills, 2018). Fackenden Down is a 28 ha chalk downland site that has supported a largely stable population of over 500 vipers between 2007 and 2019, with about 50 individuals recognised per year (Myall, 2021; Struthers, 2023).

A total of 1,287 images were processed from the four populations, ultimately identifying 391 individuals using visual identification of scale patterns and natural markings. Photographs were taken of each individual with a clear view of the head; individuals in Dorset and Fackenden were photographed in-situ, while those in Kings Wood were captured and photographed on a piece of light-coloured foam (ethical approval for the procedure was provided by the University of Kent). Images were organised into six datasets by location, with Kings Wood divided into three smaller sites, designated main site, earthworks and Soakham Down, with associated dates of sighting and the individual snake identification code or name. Images were visually compared to examine scale patterns between populations. The analysis omitted vipers whose images were too out of focus to accurately delineate scales, and the small number of juvenile individuals that were photographed.

Criteria for assessing asymmetry and fragmentation

The methodologies used to assess scale fragmentation and asymmetry were adapted from those used in previous studies of snakes (Brown et al., 2017; Bauwens et al., 2018). Three groups of scales were used to score fragmentation and asymmetry in each individual; the apical scales, the intercanthals and the supraoculars (Fig. 1).

The symmetric configuration that occurred most often (Fig. 1) consisted of two apical scales, a group of five intercanthals and six small supraoculars stacked in groups of three on either side of the centre frontal scale. Fragmentation and asymmetry in these three types of scales were defined as follows:

1. Fragmentation: Clear fracture lines splitting one of the scales. Such fragmentation did not always result in asymmetry. An individual may have had additional scales due to fragmentation, but if those scales occurred equally on the left and right sides of the head, they did not contribute to the asymmetry score (Brown et al., 2017). Any scales that had suffered trauma (e.g. through a predator attack) usually showed evidence of scarring and healing and were excluded from the analysis. Individual snakes were then scored as 1 = any fragmentation present, 0 = fragmentation absent.
2. Asymmetry: A difference in the number of scales of the same type on either side of the head. Thus, if the total count of scales of the same type was an even number those scales were scored as symmetric, while if the total count was an uneven number the scales were scored as asymmetric. Individual snakes were then scored as 1 = any asymmetry present, 0 = asymmetry absent.

Figure 1. Dorsal view of the head of a male northern viper showing the most common symmetric scale pattern, (in descending order from the apex) two unfused apical scales (red), five intercanthals (blue) and six (three on each side) supraoculars (yellow)

Statistical analysis

Preliminary analyses showed no difference in fragmentation or asymmetry between vipers at the three sites in Kings Wood and were therefore pooled for further analysis. Binomial Generalised Linear Models (GLMs) were then used to compare fragmentation and asymmetry between the four sites using R version 4.1.0 (Field et al., 2012). As Fackenden Down historically supported the largest population of vipers, this was used as a baseline against which other populations were compared.

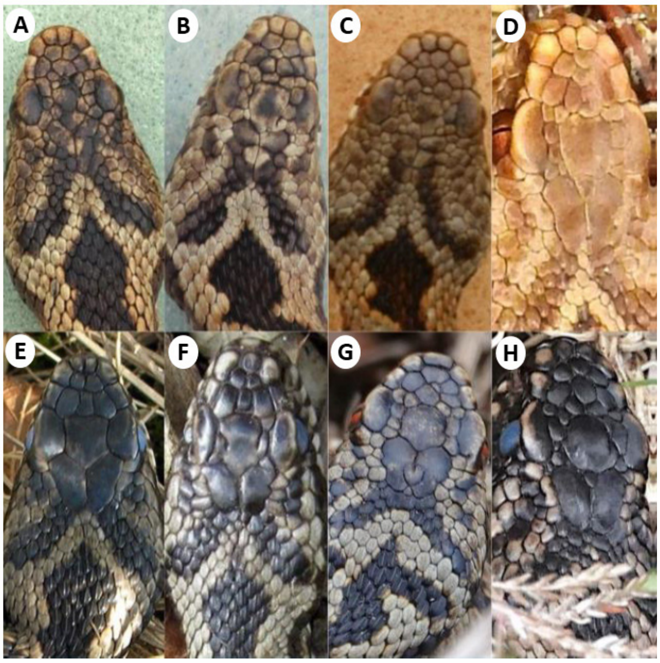

Figure 2. Examples of variation in scalation between individuals, top row are females, bottom row are males. From left to right, top row images **A.**–**C.** Kings Wood, **D.** Turbary Common, **E.** & **F.** Fackenden Down, **G.** & **H.** Talbot Heath

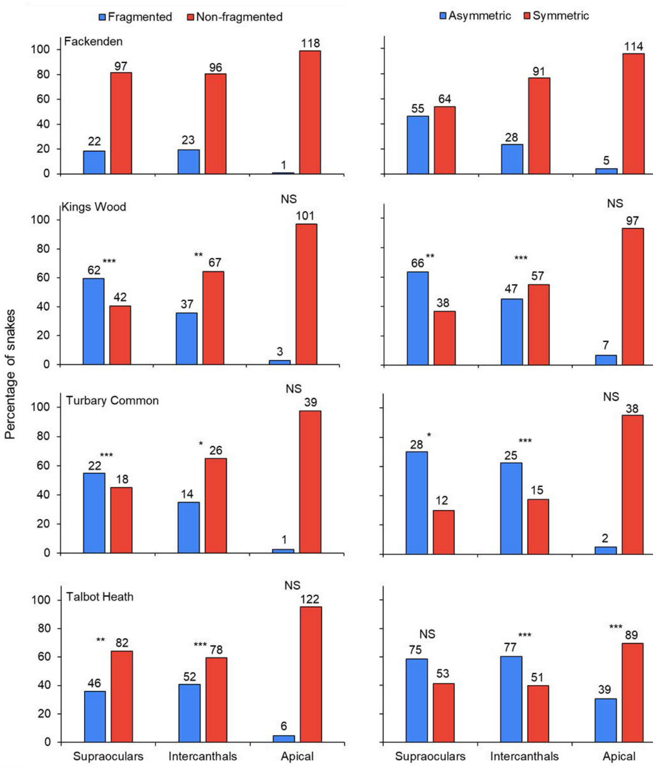

Figure 3. Comparison of (left) degree of scale fragmentation and (right) asymmetry in northern vipers at four sites in southern England. Numbers of vipers showing that character are given above the bars. A binomial GLM compared Kings Wood, Turbary Common and Talbot Heath to Fackenden Down; NS P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001. Further details of the GLM are provided as Supplementary Material.

RESULTS

Over half of the vipers displayed some degree of scale fragmentation or asymmetry. Asymmetry comprised either additional intercanthals or supraoculars on one side, or fragmented scales split into extremely small fragments tucked between scales within the scale pattern. Additionally, larger scales such as the frontal were often apparently fused with supraoculars (seen in Fig. 2 B, C & F) and intercanthals were frequently fused to form large kidney shaped scales.

There were clear differences between the sites in degree of scale fragmentation and asymmetry. While only 33% of vipers at Fackenden displayed some fragmentation, over 63% of vipers at the other three sites had fragmented scales (Fig. 3 and Supplementary Material). Likewise, whereas 57% of vipers at Fackenden had some form of scale asymmetry, the incidence of asymmetry at the other three sites was at least 79%. With the exception of the supraoculars of vipers at Talbot Heath, the vipers at Kings Wood, Turbary Common and Talbot Heath all had more fragmentation and asymmetry of supraoculars and intercanthals than those at Fackenden. Between the sites, patterns of fragmentation and symmetry in the apical scales were less clear with the only difference being that vipers at Talbot Heath had more asymmetry than Fackenden (Fig. 3 and Supplementary Material).

When data were pooled across all the sites, over half of the snakes displayed both scale fragmentation and scale asymmetry, and there was a significant association between the two conditions ($\chi 2 = 57.3$, df = 1, $P < 0.001$).

DISCUSSION

Although more snakes displayed scale asymmetry than scale fragmentation, those snakes showing fragmentation also tended to show asymmetry. Additionally, the intercanthals and supraoculars showed more fragmentation and asymmetry than the apicals. These findings are consistent with previous studies of single populations of vipers that also found variation between individual vipers in the incidence of scale fragmentation and asymmetry (Hodges & Seabrook, 2014; Bauwens et al., 2018). As observed by Bauwens et al. (2018), recaptures of individuals indicated that head scalation was stable over time in the majority of snakes.

The degree of scale fragmentation and asymmetry in vipers varied between the populations. Indeed, the viper population at Fackenden Down displayed a lower degree of fragmentation and asymmetry than the other three populations, particularly with respect to the intercanthals and supraoculars. The vipers at Kings Wood, Talbot Heath and Turbary Common occupy sites that are either small or isolated by surrounding urban or suburban development. Equally, surveys at these sites have indicated that the populations are either historically small or have declined in recent years. In contrast, although the population at Fackenden Down may also have declined, it is a historically large population occupying a site that is interconnected to different habitat types including woodland, scrub and rough grassland which promote a variety of habitat corridors, facilitating ecological edge effects. Although a road runs along the edge of Fackenden Down and may be a potential barrier, vipers also occur on the field margins across the road from the main population (Hodges et al., 2023).

A previous study by Hodges & Seabrook (2014) commented on the apparent instability of the head scales of some individuals at Fackenden and provided evidence that a scale anomaly on the frontal scale may be heritable. In slatey-grey snakes in Australia, Brown et al. (2017) showed that although scale asymmetry was associated with reduced heterozygosity, this trait was not heritable. In contrast, in the same species scale fragmentation appeared to be heritable. Forsman et al. (1994) found that vipers with anomalies in the midbody ventral scales probably had reduced survival, but there was no evidence that the scale anomalies were heritable. Löwenborg & Hagman (2016) showed a link between ventral scale asymmetry and rib deformity in *Natrix natrix*, and that scale asymmetry impacted terrestrial locomotion of hatchings. Clearly, more research is needed to establish the relationships between loss of genetic diversity, the heritability of scale anomalies and their impact on survival and fitness in snakes.

Although the reasons for the differences between the populations are unclear, it is well-known that asymmetry and fragmentation may reflect potential stressors on populations. Such stressors may be environmental or genetic, or a combination of both. For example, in addition to a link between asymmetry and lower heterozygosity in slatey-grey snakes, both asymmetry and fragmentation was higher in snakes that developed faster (Brown et al., 2017). Herczeg et al. (2005) showed that two populations of dice snakes *Natrix tessellata* varied in the degree of fluctuating asymmetry and suggested that these differences may be due to genetic or environmental stressors. Likewise, wall lizards *Podarcis muralis* display more fluctuating asymmetry in disturbed urban populations than in rural populations (Lazić et al., 2013). There may also be links between head scale symmetry and some measures of fitness in pygmy bluetongue lizards (Shamiminoori & Bull, 2015). We speculate that the levels of asymmetry and fragmentation shown in our populations could be related to historically small populations occupying habitats that have been increasingly subjected to fragmentation, isolation, disturbance and habitat change in recent years. Indeed, those populations showing the highest levels of asymmetry and fragmentation may be suffering higher levels of such impacts than those at Fackenden Down. Although the genetic diversity of vipers may be maintained despite historic population bottlenecks (Madsen et al., 2023), further genetic analyses may help clarify the relationship between scale anomalies and population stressors in vipers. If such a relationship is established, measuring scale asymmetry and fragmentation could prove to be a useful early warning of viper populations at risk from wider landscape change that may be driving declines.

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SHORT COMMUNICATION

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Indigenous beliefs benefit freshwater turtle conservation in West Bengal, India

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Cultural and traditional beliefs can influence the attitudes of humans towards other species and their habitats, and may determine whether these species are protected or threatened by over-exploitation (Uyeda et al., 2016). Ancient civilisations from across the world have held beliefs that value some integration of certain wild species with human society; for instance, chelonians have played important roles in human culture for at least 400,000 years (Stanford et al., 2020). In South India, the presence of turtles in households is viewed as a negative omen, while in North and Northeast India, it is considered to bring good luck (Pratihar et al., 2014). In West Bengal, people generally have a notion that turtles possess a distinct spiritual essence, for example, the second avatar of Lord Vishnu, the God of preservation, appears as half tortoise and half human (Purwanto, 2020). Certain groups practice traditional beliefs such as placing turtle carapaces on cowshed walls to protect cattle from ill health and bring prosperity, put turtle amulets around the necks of cattle to ward off 'evil eyes', and the use of turtle carapaces as toys for village children pulled along on ropes like toy cars (Mandal et al., 2024).

A particular example of traditional customs benefitting the conservation of turtles is the case of sacred temple ponds, which date back to ancient times (Kakati & Tamuly, 2020). Temple ponds hosting turtles are notably prevalent in the north-eastern states of India (Kakati & Tamuly, 2020). In this area, there exists a unique cultural practice where devotees donate turtles to temple ponds when a child is born in their family, believing this act ensures the child attains longevity akin to that of the turtle (Purkayastha et al., 2013). Despite facing numerous threats, many endangered turtle species seem to thrive in these unique habitats, as they are free from pressures such as hunting and human disturbance. For instance, the black soft-shelled turtle *Nilssonia nigricans* (Anderson, 1875), once declared Extinct by the IUCN in 2002, has been rediscovered thriving in temple ponds across West Bengal, Assam, and Tripura in India (Das et al., 2020).

In a recent socio-cultural research study focusing on the dynamic interaction of indigenous communities with freshwater chelonians, harvesting of threatened turtle species was strongly associated with traditional customs and the cultural use of turtle shells (Mandal et al., 2024). Interviews within Joyram Chak village (22° 31′50″ N, 87° 47′31″ E, Paschim Medinipur district, West Bengal) (Fig. 1) revealed that during the year spanning 2016–2017, "certain persons in the village had killed and consumed a unique species of turtle, previously not found in this area". The authors attempted to identify the species based on the given description, which mentioned 'circular markings on its carapace', the description of which resembled those of the endangered Indian peacock

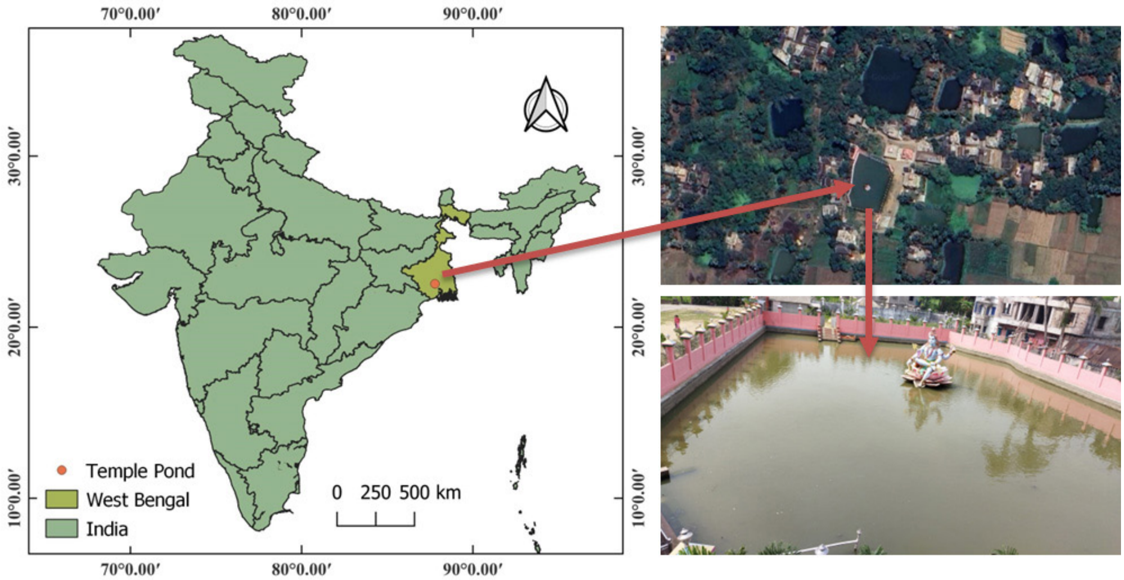

Figure 1. Map of India with marked location of Paschim Medinipur district, West Bengal having the sacred temple pond at Joyram Chak village

Figure 2. Villagers liken the circular markings **A.** on the turtle's *Nilssonia hurum* carapace to **B.** the sacred iconic chakram, the circular weapon (top left), held by Lord Vishnu in his second incarnation as a turtle

softshell turtle *Nilssonia hurum* (Gray, 1831). Co-incidentally, within a couple of years, those people involved in hunting the turtles had either died accidentally, or experienced health issues. As a consequence, villagers believed that "the turtle was an incarnation of Lord Vishnu", because the circular markings on the turtle's carapace were likened to Lord Vishnu's iconic chakram (circular weapon) (Fig. 2). This belief led them to attribute these adverse consequences to actions against the sacred turtle. Since then, there has been a notable transformation in the villagers' attitudes towards this threatened animal. They now refrain from harming turtles, and actively participate in safeguarding them by various means, especially by releasing encountered turtles into the temple pond (established in 2019) or nearby village ponds. During the survey period, unhatched eggs and hatchlings of the Indian flapshell turtle *Lissemys punctata* (Bonnaterre, 1789) were also observed in the village, which indicated some wider restraint on human interference of turtles, and that the turtles were healthy enough to breed. This would be of particular benefit to *L. punctata* as it is listed by IUCN as Vulnerable (Rahman et al., 2021) and since the early 1990s, in West Bengal, has been the subject of massive illegal exploitation for both local and international trade (Mendiratta et al., 2017).

In West Bengal and other parts of India, certain traditional beliefs and taboos can have a positive impact on the conservation of endangered chelonians and other herpetofauna (Bhattacharya & Koch, 2018). Similarly, such practices have been found to be effective in conserving and reviving marine turtle populations in Ghana (Alexander et al., 2017). The efforts of local communities complement the more conventional approaches to chelonian conservation being made by scientists, government agencies and NGOs.

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SHORT COMMUNICATION

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Activity of the weasel skink *Saproscincus mustelinus* under variable weather conditions

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Shade skinks (*Saproscincus*: Eugongylini) inhabit forests and sheltered suburban gardens in eastern Australia (Wilson, 2012). The weasel skink *Saproscincus mustelinus* is surface active, dwelling among leaf litter (Swan et al., 2017; Wilson, 2012; Sazima, 2023a). Its daily activity pattern is variable: this skink is reported as diurnal (Downes & Shine, 1999; Wilson, 2012); diurnal and under warm weather active at night (Robertson & Coventry, 2019); active at dusk or after dark on warm nights (Swan et al., 2017). Recent revisions of traits within Scincidae categorise the weasel skink as cathemeral, i.e. not strictly diurnal, nocturnal or crepuscular (Meiri, 2018; Slavenko et al., 2022), a classification supported by my previous observations on its habits (Sazima, 2023a).

Here I describe and illustrate the activity of the weasel skink under variable weather conditions, including its basking in sunlight (Downes & Shine, 1999), diurnal activity during drizzle and light rain, dawn hunting for winged termites and its predation and scavenging by a diurnal bird species.

I recorded the skinks from October 2023 to January 2024, incidental to my strolls on the Louise Sauvage Pathway (33° 49′51″ S, 151° 04′15″ E, 7 m a.s.l. to 33° 50′30″ S, 151° 03′56″ E, 6 m a.s.l.), Newington, New South Wales, Australia. This path is bordered by woodlots and paved with bitumen and small stone chips, and used by people for walking, running or cycling. I walked round trips of about 600 m at a steady pace and looked for skinks on the path and the adjacent ground with leaf litter and sparse vegetation. I recorded active skinks in the morning (about 08:30–09:30 h), in the afternoon (about 16:30–17:30 h) and after sunset (about 19:00–19:30 h) under sunny, cloudy and rainy weather. I photographed the skinks with a digital camera and a 55–250 mm telephoto lens from a distance of 1.5–3 m. Additionally, I recorded road kills during the same periods of the day, and preying or scavenging on the skink by ants and birds. I describe and illustrate first the events observed in the morning, then those in the afternoon, and finally those observed after sunset and at dawn.

I recorded an adult weasel skink basking on a sunlight and shade mosaic in the morning. The skink was under direct sunlight on the paved path (Fig. 1A) and remained basking for about 1 min after first sighted, retreating to the vegetation afterwards. An individual was recorded on a similar sunlight and shade mosaic five days before, but was basking on a shaded spot, and another adult individual was recorded active, possibly foraging, on a shaded patch of a sunlight and shade mosaic in the morning.

I recorded an adult individual moving during light drizzle in the morning. The skink was in close contact with the collected water (Fig. 1B) and rain drops were visible on its body. It moved across the path and retreated among the vegetation bordering the path. On the following morning, under the same weather conditions, I found a juvenile individual sitting in a shallow puddle (about 0.5 cm). The skink remained motionless there for about two minutes, then crossed the path and retreated among the leaf litter at the path margin.

One fresh road kill skink found on the morning in December was scavenged by small unidentified black ants (Fig. 1C), when it caught the attention of a foraging Australian magpie *Cracticus tibicen* male on the path. The bird took the dead skink full of ants in the bill, released it on the ground once, and thrashed it a little. Afterwards, it consumed the skink almost free of ants (Fig. 1D). Besides the small black ants and the Australian magpie, fresh weasel skink road kills were scavenged in the morning by the large southern meat ants *Iridomyrmex purpureus*.

I observed two predation events on the weasel skink by the Australian magpie at late sunny afternoon. The birds were foraging on the path and spotted the basking skinks, which were caught by the magpies at the mid-body and chewed before being swallowed whole. The events occurred on two consecutive days.

An adult skink individual was recorded active on a cloudy afternoon, about to jump 110 cm from the wall of a residence to the ground (Fig. 1E). On the ground, it began to forage on small unidentified insects (possibly termites or ants). I observed a juvenile individual active under a light rain shower in the afternoon, behaving like the one described above in the part about morning activities. A juvenile individual was recorded active in late afternoon at the onset of a heavy rain, but when a large raindrop fell on its back, it retreated to the vegetation bordering the path.

On one occasion I recorded weasel skinks hunting for swarming winged termites at dawn. There were about four to five skink individuals at a spot of the path, actively searching for termites on the ground, walking with the head raised up (Fig. 1F). Once spotted, a termite was grabbed quickly, chewed and swallowed. A garden skink *Lampropholis*

Figure 1. Activity and death of the weasel skink *Saproscincus mustelinus* under variable weather on a paved pathway in south-eastern Australia - **A.** An adult individual basks on a sunlit patch of a sunlight and shade mosaic in the morning, **B.** An adult moves under a light drizzle in the morning (note rain drops on the skink and its close contact with the water on the ground), **C.** A road kill adult scavenged by unidentified black ants in the morning, **D.** An Australian Magpie *Cracticus tibicen* male briefly grasps the same road kill, before thrashing the carcass on the ground and then swallowing it almost free of ants, **E.** An adult active on a cloudy afternoon, about to jump 110 cm to the ground, **F.** An adult actively forages for winged termites on the ground after sunset.

delicata was also hunting the winged termites on this occasion. Adult and juvenile weasel skinks warming on the paved path after sunset were a common sight for the whole observation period.

The observations reported herein document the activity of the weasel skink in three distinct periods during the day and under variable weather conditions. There is no doubt that this skink species qualify as cathemeral (Meiri, 2018; Slavenko et al., 2022) and that it occasionally behaves as a heliothermic lizard basking in direct sunlight (Downes & Shine, 1999), which I failed to record in a previous study (Sazima, 2023a). Still, I observed thigmothermy more often than heliothermy for the weasel skink, possibly due to it using leaf litter during the day (Wilson, 2012) and warming on open surfaces mostly after sunset (Sazima, 2023a; present study).

The ability of the weasel skink to jump from heights greater than 1 m seems to be a novelty, as well as the ‘gathering’ of a small number of individuals to hunt winged termites. The reported bird predators of the weasel skink now numbers at least three passerine species, the pied currawong *Strepera graculina*, the grey butcherbird *Cracticus torquatus* and the Australian magpie (Rose, 1999; Sazima, 2023a; present study). I suppose that there are more potential predators among bird species that forage on the ground or from a hunting perch and are quick enough to catch this agile lizard (Sazima, 2023b).

What came as a surprise during my observations was the weasel skink activity during light rain, which may be unusual for such a small lizard which, however, avoided heavier rain. A shade skink *Saproscincus* sp. resting on a leaf, with what seem water drops on its body is illustrated in Wilson (2012), but no information on the source of the drops is given.

It seems that the weasel skink is active mostly in the warm season (Sazima, 2023a), and presumably brumates during the coldest months, which remains to be verified with further observations (Swan et al., 2017; Wilson, 2012).

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SHORT COMMUNICATION

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The advertisement call of the Santa Marta robber frog *Serranobatrachus sanctaemartae*

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The recently erected genus *Serranobatrachus* Arroyo et al. (2022) — superfamily Brachycephaloidea — is a group of frogs that undergo direct development, and currently comprises seven species (Frost, 2024). All these species are endemic to the Sierra Nevada de Santa Marta in Colombia, a unique mountain range with high rates of endemism (Arroyo et al., 2022; Rada et al., 2019; Sánchez-Pacheco et al., 2017). One of these endemics, the Santa Marta robber frog, *Serranobatrachus sanctaemartae* (Ruthvens, 1917), is a nocturnal species that occurs in the cloud forest of the north-western flank of the Sierra Nevada de Santa Marta between 1100–2450 m a.s.l. (Arroyo et al., 2022; Ruthven, 1917), but little more is known about the natural history of this species.

The advertisement call is the most widely studied acoustic signal of frogs. It is broadly recognised as a prezygotic isolation barrier and thus a tool for species recognition (Köhler et al., 2017; Rivera-Correa et al., 2021; Toledo et al., 2015). To date there are no detailed descriptions of the advertisement calls of any *Serranobatrachus* spp. Herein, I describe the advertisement call of *S. sanctaemartae*.

Advertisement call recordings were made at a sampling rate of 48 kHz and 16-bit resolution with a Marantz PMD661MKII digital recorder with a coupled Sennheiser ME 66/k6 shotgun microphone and a Xiaomi Redmi Note 8 mobile phone using the default recording app set in WAV format. No temperature and humidity data were taken. The recording devices were positioned about one metre from the calling males. 'Voucher' recordings of the original calls have been deposited in the Banco de Sonidos OcainaCua (BSOC) from the Museo de Ciencias Naturales de La Salle (BSOC204–206).

All measurements of the call traits were made using the Raven Pro Software Program 1.6 (Lisa Yang Centre for Conservation Bioacoustics, 2023) and follow standard terminology and methodology (Köhler et al., 2017; Zollinger et al., 2012). Based upon the call type of *S. sanctaemartae*, the note-centred approach proposed by Köhler et al. (2017) was adopted. The temporal features of the call (call/note duration and note/call interval) were measured in oscillograms, and power spectra diagrams were used to obtain the spectral features of the call (bandwidth, dominant frequency, low frequency and high frequency). Spectrograms were only used for visual support, as suggested by Zollinger et al. (2012). Recordings were analysed using a Hann window function, a DFT of 512 points and overlap of 50%. A 20 dB threshold from the peak amplitude was used to select the sound of interest (Fig. 1B). The Seewave 2.2.3 (Sueur et al., 2008) package in R 4.4.1 (R Core Team, 2023) was used to create spectrographic depictions.

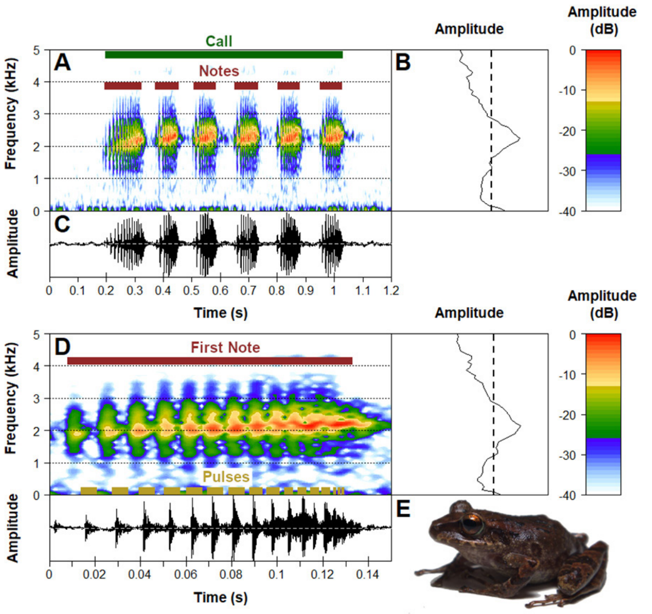

Figure 1. The advertisement call of *Serranobatrachus sanctaemartae* - **A.** Spectrogram, **B.** Power spectrum diagram, **C.** Oscillogram, and **D.** Zoom of the first note of the six-note call, **E.** *Serranobatrachus sanctaemartae*. Sound recording was from male EAFIT-Am 0658, archived in Banco de Sonidos OcainaCua (BSOC206). Dotted line in the power spectrum diagrams represents the 20 dB threshold from the peak amplitude.

For the statistical description, the recorded males were defined as the sampling unit for analysis. In other words, the mean of the spectral and temporal parameters of the call and note for each male was calculated, and then a new weighed mean and standard deviation were calculated for the population. In the text, measurements were given as mean ± standard deviation (min–max).

Given that this is the first report of the advertisement call of this species, voucher specimens that complement the voucher recordings have been deposited for the purpose of taxonomic traceability (Obrist et al., 2010). Specimens were sacrificed with an overdose of 5% xylocaine, fixed in 10% formalin and preserved in 70% ethanol. The specimens

Table 1. Descriptive statistics of advertisement call parameters of two specimens *Serranobatrachus sanctaemartae*. mean ± standard deviation (min–max). Unless otherwise noted n = 5.

Parameters	Pulses per note	Call/note duration (s)	Call/note interval (s)	Low freq (Hz)	High freq (Hz)	Bandwidth (Hz)	Dominant freq (Hz)
Call	77.8±20.62 (69–98)	0.828±0.213 (0.668–1.014)	90.877±16.069 (79.514–102.240; n = 2)	1793.9±178.9 (1671.9–1916.3)	3003.9±420.6 (2720.9–3303.3)	1209.9±265.8 (1027.2–1386.9)	2266.6±63.9 (2239.4–2343.7)
First note	16.6±3.04 (15–19)	0.133±0.008 (0.126–0.140)	0.036±0.012 (0.024–0.043)	1512.6±373.2 (1242.3–1699.2)	3020.3±366.1 (2764.7–3303.3)	1507.7±294.8 (1218.4–1639.9)	2193.1±80.4 (2153.3–2250)
Second note	10.6±0.93 (10–11)	0.079±0.014 (0.069–0.091)	0.051±0.005 (0.048–0.055)	1874.7±229.7 (1721.5–2051.5)	2994.9±390.6 (2742.8–3299.4)	1120.2±222.3 (967.1–1321.3)	2304.1±89.9 (2239.4–2343.7)
Third note	12.8±5.24 (10–18)	0.083±0.008 (0.074–0.088)	0.057±0.00 6(0.052–0.063)	1892.5±240.8 (1714.4–2055.4)	2981.8±415.4 (2688.2–3276.2)	1089.2±236.4 (917.9–1244.0)	2285.4±82.8 (2239.4–2343.7)
Fourth note	12.2±4.11 (10–16)	0.086±0.006 (0.081–0.090)	0.060±0.006 (0.054–0.063)	1862.9±242.9 (1693.1–2032.2)	2997.2±424.4 (2704.6–3291.7)	1134.2±234 (972.5–1278.8)	2285.4±82.8 (2239.4–2343.7)
Fifth note	13.2±4.96 (11–18)	0.085±0.010 (0.079–0.094)	0.070±0.017 (0.063–0.091)	1864.9±242.4 (1693.1–2020.6)	2995.7±441.9 (2688.2–3318.7)	1130.8±264.4 (956.1–1325.2)	2286.9±141.6 (2153.3–2343.7)
Sixth note	12.4±3.59 (11–16)	0.085±0.016 (0.071–0.097; n = 4)		1880.6±195.9 (1717.9–2009.0)	3038.8±433.7 (2818.3–3376.7)	1158.1±271.5 (988.9–1367.7)	2247.9±8 (2239.4–2250)

were prepared following Heyer et al. (1994) and identified following Arroyo et al. (2022), Lynch & Ruiz-Carranza (1985) and Ruthven (1917). Specimens were deposited at the Amphibian Collection of Universidad EAFIT (EAFIT-Am). Snout-vent length (SVL) was measured with a Mitutoyo digital caliper (precision ± 0.01 mm).

From the 6–9 May 2024 at the Reserva las Nubes, Santa Marta, Magdalena (11.15365°, -74.06770°; 1,486 m a.s.l.), a locality at 5.67 km in a straight line from the type locality, a population of actively calling males of *S. sanctaemartae* was found around a river canyon. Individuals started calling from approximately 19:00 h onwards. On 8 May 2024 at around 21:00 h, a total of five calls were recorded from two collected males (EAFIT-Am 0657, SVL:32.78 mm, two calls and EAFIT-Am 0658, SVL:32.88 mm, three calls). Both males were found in a riparian forest, perched 30 cm above ground on the edge of a 3 m long cleft leading to a creek.

The advertisement call of *S. sanctaemartae* consists of five to seven (mode = 6) loud, non-frequency modulated, rapidly repeated, pulsed notes (Fig. 1). The call duration was 0.828 ± 0.213 s (0.668–1.014 s; n = 5; Fig. 1C; Table 1), and the call interval was 90.877 ± 16.069 s (79.514–102.239 s; n = 2). The dominant frequency of the call when the energy reached peaks in the band was 2266 ± 63.9 Hz (2239–2343 Hz; n = 5; Fig. 1B; Table 1). The duration of the first note appears to be longer than the remaining notes (Table 1). The silent interval between notes appears to be shorter at the beginning and longer at the end of the call (Table 1). A complete report of the spectral and temporal parameters is given in Table 1.

Although the lack of formal descriptions for any other species of the genus *Serranobatrachus* prevents comparisons, there are descriptions of the advertisement calls for a couple of species of the genus *Tachiramantis* — *T. douglasi* and *T. lassoalcalai*, the sister clade of *Serranoatrachus*. The advertisement call of *S. sanctaemartae* differs from *T. douglasi* by the lower number of notes (5–7 vs 7–9) and the shorter duration (0.82 s vs 1.66 s). From *T. lassoalcalai*, it differs in call structure, since the call of *T. lassoalcalai* is composed of a single note (or call, see Köhler et al., 2017), while the call of *S. sanctaemartae* is composed of several notes (5–7 vs 1). Also, the call of *S. sanctaemartae* has a longer note duration (0.828 s vs 0.72 s) and lower dominant frequency (2.26 kHz vs 2.88 kHz). However, it should be noted that in the case of the advertisement call of *T. douglasi*, it was difficult to understand the methodology and approach employed in its description by Mendoza-Roldán et al. (2019). As for example, they do not state which approach they took for the designation of the main unit of "call", and thus, in their description, the distinctions between call, notes and pulses are not clear; moreover, their definition of peak frequency is not clear. In that sense, comparisons were only made in temporal characteristics, as these were less ambiguous.

As stated by Köhler et al. (2017) and Rivera-Correa et al. (2021), the acoustic signals are powerful tools to study anuran diversity, and there is still a lot of acoustic diversity to discover. The current report represents the first description of an advertisement call for a *Serranobatrachus* species, which represents an advance in elucidating the natural history of the enigmatic species of the Sierra Nevada de Santa Marta, especially those that are part of the superfamily Brachycephaloidea, as well as a step towards the conservation of the Santa Marta robber frog.

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SHORT COMMUNICATION

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Partial neoteny in the northern spectacled salamander *Salamandrina perspicillata*

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We report here potential cases of partial neoteny in a population of the northern spectacled salamander *Salamandrina perspicillata*. The term neoteny was introduced by Kollmann (1885) to describe the retardation of physiological development during the larval stage of amphibians (Gould, 1977). According to Lanza et al. (2007) there are at least three different forms of neoteny: i) full - larvae never undergo metamorphosis; ii) almost full - larvae can reach a rather large size and can also reach sexual maturity, remaining more or less as such for a long time; iii) partial - partial neoteny occurs when metamorphosis is delayed due to temporary ecological changes in the environment, without larvae developing sexual maturity.

Salamandrina perspicillata is a tiny salamander (total length: 70–100 mm) endemic to the central and northern areas of the Italian Peninsula (Amgelini et al., 2007; Di Nicola et al., 2019). At hatching, the length of larvae is 7–13 mm (Vanni, 1981) and metamorphosis is completed in 2–4 months, depending on water temperature, food availability and other ecological factors (Della Rocca et al., 2005; Angelini et al., 2008).

During a field survey of the herpetological fauna of the Reatini mountains (north-eastern Latium, central Italy) ten large larvae of *S. perspicillata* in an advanced stage of development (four limbs, evident dorsal pattern, well developed spot in the shape of 'spectacles' on head typical for the species, Fig. 1A) were observed during two research visits (11 and 19 July 2024), in a large drinking trough fed by a spring located at 1,135 m a.s.l., in the municipality of Vazia (Province of Rieti, Latium, central Italy). The total length of these larvae ranged from 34 to 48 mm. This exceeds the total length normally attained by pre-metamorphic larvae of the species (17.7–23.4 mm according to Angelini et al., 2008) by a large margin, and would even be considered long when compared to metamorphosed larvae at the end of their first year of terrestrial life, which may measure 25–30 mm length (Vanni, 1981; Angelini et al., 2008). The remarkable size of the observed larvae indicates that they have had a long larval stage, and that they probably hatched in early autumn 2023, thus far exceeding the time normally required for metamorphosis in the species (2–4 months). In our opinion this can be considered as a case of partial neoteny. To date the only known case of neoteny (partial neoteny) for the species is that recorded by Filippi et al. (2020) in a drinking trough sited in the Valle del Treja Regional Park (Province of Viterbo, Latium, central Italy). However, Filippi et al. (2020) observed only one neotenic individual, a leucistic larva with a total length of ca. 50 mm. Cases of full neoteny or almost full neoteny have never been recorded for the species.

Figure 1. Larvae of *Salamandrina perspicillata* from the study site - **A.** Two large-sized neotenic larvae, **B.** Medium-sized normal larva (scale bars- 1 cm)

At the same study site and on the same dates, 25 small larvae (10–12 mm, first stage of growth, no limbs) and 16 medium sized larvae (18–20 mm, well developed fore and hind limbs, Fig. 1B) of *S. perspicillata* were also observed. This suggests to us that the local population of *S. perspicillata* is characterised by different breeding periods, and that females living in the study site can lay eggs either in autumn or in winter and springtime. After collection, all individuals were measured, photographed and immediately released where they were collected.

In central Italy, the oviposition period of *S. perspicillata* normally extends from February to May (Della Rocca et al., 2005), though adult females of some populations from southern Latium (Lepini mountains) are known to lay eggs also in autumn and winter (Corsettti, 1999). However, it must be stressed that climate, altitude and habitat characteristics of the study site are very different from those of the southen Latium sites, which are about 200 km from the Reatini mountains. In fact, the altitude of the study site is one of the highest among those known for *S. perspicillata* in central Italy (Bologna et al., 2000). The study site lies in a mountainous area of the central Apennines characterised by temperate climate and beech forests, while the southern Latium sites lie at lower altitudes and are characterised by a Mediterranean climate, Mediterranen scrub and holm oak woods. It seems likely that the wide egg-laying period is linked to still unknown ecological and population biology factors rather than to either climatic factors (e.g. late spring-summer drought, maximum rainfall in autumn and winter) or altitude as previously suggested by Corsetti (1999) and Angelini et al. (2007; 2008).

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SHORT COMMUNICATION

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Hooding in the dice snake *Natrix tessellata* may backdate the origin of such behaviour in the genus *Natrix*

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Hooding is a very well-known defensive display of snakes, the snake raises its head and the anterior part of the body from the ground, and the neck is dorsoventrally compressed by lateral expansion of the cervical ribs (Greene, 1979; Young et al., 2010). This aposematic signal is intended to intimidate potential predators or threats, and has been made famous in popular culture by cobras (family Elapidae) although its use is not restricted to elapid snakes (Pope, 1935; Gharpurey, 1954; Greene, 1979). Among others, it has been recorded in three natricid snakes of the genus *Natrix*: *Natrix natrix*, *Natrix helvetica* and *Natrix astreptophora* (Kabisch, 1978; Pokrant et al., 2017; Paterna, 2019). In 2017, Pokrant et al. concluded that this defensive mechanism is a 'fossil' behaviour, inherited by the grass snakes from an ancestor that lived in Europe until the Miocene–Plio-Pleistocene, which had evolved hooding as Batesian mimicry of sympatric elapids. Besides apparently sharing this defensive display with cobras, *Natrix* species also visually imitate sympatric viperids in both dorsal pattern, defensive behaviour and by head triangulation (Valkonen et al., 2011; Paterna, 2019).

In this study we describe hooding in two dice snakes *Natrix tessellata* (Laurenti, 1768). The first observation was on 3 May 2006 along the Chienti river, in the locality of Bistocco di Camerino, in the Marche region, Italy, where there is a well-established population of dice snakes. On this occasion, a specimen of a total length of about 105 cm, raised its neck and spread a hood while the posterior portion of its body was being held by one hand (Fig. 1A & B). This performance lasted long enough for the snake to be photographed from several different angles. The maximum width reached by the lateral expansion of the neck was approximately equal to the length of the specimen's head, and stretching of the neck revealed dark-pigmented skin between the dorsal scales. This manoeuvre also accentuates the head triangulation of the specimen, in a similar manner to that observed in congeneric and sympatric *N. helvetica* (Paterna, 2019). The second episode occurred on 1 April 2008 in the Colfiorito swamp, in the province of Foligno, also in the Marche region, where on the lakeside, a dice snake displayed a hood (Fig. 1C & D) in a similar manner to the previous observation. Furthermore, in both cases the snakes maintained the defensive display even after their necks were returned to the ground and they were in the process of fleeing (Fig. 1B & D). In both episodes there was a marked dorsal plane variation between vertebrae and ribs (Fig. 1A & C), i.e. the neck didn't appear completely flattened dorsally as the vertebrae were more 'elevated' or 'externally pronounced'. Despite descriptions of hooding in the grass snakes being rare in literature, the externally pronounced vertebrae does not seem to be so evident in the reported case of *N. helvetica* (Paterna, 2019), but clearly present in *N. astreptophora* which is featured in many photographs on online platforms, to the extent that it seems that hooding could even be frequent in this species.

It seems likely that hooding has a common evolutionary origin in those four *Natrix* species that have been recorded displaying it. The fact that such display has been observed in *N. tessellata*, a sister taxon of *N. astreptophora*, *N. helvetica* and *N. natrix*, but has not been documented in the most basal species *Natrix maura*, allows us to connect the origin of this behaviour into a more precise time range between the divergence of *N. maura* and the last common ancestor for the remaining species. On the basis of recent phylogenetic studies, the development of such behaviour could be placed in the early-middle Miocene (Guicking et al., 2006; Kindler et al., 2018; Schöneberg et al., 2023). This is consistent with the hypothesis that this defensive mechanism may have been evolved as emulation of sympatric elapids in central Europe (Pokrant et al., 2017).

The oldest elapid fossils found in Europe belong to the species *Naja romani*, of which there is an abundance from the early German Miocene (Szyndlar & Schleich, 1993), middle French Miocene and late Austrian Miocene (Szyndlar & Rage, 1990), to a second species, *Naja iberica*, and indeterminate remains of a *Naja* sp. of which records date back to the middle–late Spanish Miocene (Szyndlar, 1985; Villa et al., 2024). To date, the known European fossil records of the genus *Natrix* from the mid–late Miocene belong exclusively to extinct species, such as *Natrix sansaniensis* from France (Rage, 1988; Rage & Auge, 1993; Ivanov, 2002), and *Natrix longivertebrata* in Austria (Bachmayer & Szyndlar, 1985) and eastern Europe (Szyndlar, 1984, 1991), while fossils attributed to the contemporary *N. tessellata* and *N. natrix* have origins no earlier than the late Pliocene and Pleistocene (Markert, 1976; Zerova & Chkjikvadze, 1984; Ivanon, 1999). However, the oldest fossil records of the genus *Natrix* are restricted to central Europe, and date back to the late Eocene and early Oligocene, with

Figure 1. Adult dice snakes *Natrix tessellata* performing the hooding display - **A.** Specimen from river Chienti hooding in erected position, **B.** Same specimen as **A.** hooding while its neck ascends to the ground, **C.** Specimen from the Colfiorito swamp hooding in erected position, **D.** Same specimen as **C.** hooding parallel to the ground.

the species *Natrix mlynarskii* found in France (Rage, 1988; Ivanov, 2001). It is therefore plausible that such defensive mechanism has a central European origin, back-dated to the ancestors of *N. tessellata* prior to the fragmentation in which ancestral populations expanded eastwards, and from which *N. tessellata* evolved in south-western Asia (Rögl & Steininger, 1984; Guicking et al., 2006).

An alternative hypothesis on the origin of this defensive display might not connect this phenomenon to the previous coexistence in the same territory with cobras, but with another defensive mechanism involved in the imitation of viperids. It is noteworthy how hooding in *Natrix* is linked to head triangulation and contributes to its definition and extension through the lateral expansion of the first cervical ribs (Paterna, 2019). In this genus hooding seems to be generally performed while raising the neck, while other species of colubroids, e.g. *Dasypeltis* and *Heterodon*, usually perform hooding with their neck and head close or parallel to the ground. European viperids, in defensive posture raise their heads from the ground and hiss loudly, while larger allopatric viperids, such as *Bitis* spp. and *Crotalus* spp., in addition to this further triangulate their heads. It is of interest that hooding has not been observed (yet?) in *N. maura*, instead this species, as its common name ‘viperine water snake’ suggests, is very well documented as a viper mimic (Valkonen et al., 2011).

Further investigation of hooding in natricids is required to fully understand its function and origin. If hooding was to be subsequently recorded in *N. maura*, the more ancient of the species considered, then this would influence the temporal and spatial explanations of its origin.

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NATURAL HISTORY NOTE

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Predation of Madeiran wall lizards *Teira dugesii* by the banded garden spider *Argiope trifasciata*

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Spiders are primarily invertebrate predators but some larger species can occasionally capture small vertebrates such as lizards, bats, birds and frogs (Valdez et al., 2020). For example, in 1987, the banded garden spider *Argiope trifasciata* was observed in two cases of predation, preying upon *Anolis porcatus* and *Anolis sagrei*, lizards endemic to Cuba (De Armas & Aláyon, 1987). Another example is some adults of *Hogna ingens* were observed feeding on juveniles of *Teira dugesii mauli* on the Desertas Islands in the Madeira Archipelago (Cardoso et al., 2016).

Figure 1. *Argiope trifasciata* feeding on *Teira dugesii dugesii*, Miradouro Trigo de Negreiros or Moledos, Madeira

Figure 2. *Teira dugesii dugesii* secured by *Argiope trifasciata* using the wrapping technique, Escola EB123/PE Bartolomeu Perestrelo, Madeira

The Madeiran wall lizard *Teira dugesii* is endemic to the Madeira and Selvagens archipelagos, with a wide distribution in both archipelagos, occurring in a variety of habitats (Jesus et al., 2009). The banded garden spider *Argiope trifasciata* is of medium-size (4–25 mm) and is widely distributed on the island of Madeira, especially at lower altitudes (Araújo, 2009). There have been suggestions that this spider is an occasional predator of *T. dugesii* (Araújo, 2009), but until now this has not been documented.

This note is the first documented account of the predation of Madeiran wall lizards by banded garden spiders. The first observation was on 7 February 2023 in the Miradouro Trigo de Negreiros or Moledos (32° 42.3999’ N, 17° 8.3595’ W), where the spider was seen feeding on a wall lizard caught in a web (Fig. 1). The second observation was on the morning of 10 February 2024 in the Gardens of Garajau (32° 38.77194’ N, 16° 50.85282’ W), where the spider was on its web by a clump of heather *Erica platycodon*, when a wall lizard having sighted insects in the web went towards them and became entangled (Diogo Ladeira, pers. comm.). The spider used the prey wrapping technique to secure the lizard (Barrantes & Eberhard, 2007). The third observation was on the morning of 16 February 2024 at Escola EB123/PE Bartolomeu Perestrelo (32° 39.52032’ N, 16° 54.91362’ W) by a pot of *Dimorphotheca* spp., where a wall lizard was observed caught in the web of a banded garden spider (Fig. 2).

Although these are the first documented records of the banded garden spider preying upon the Madeiran wall lizard, this interaction may be frequent since both species seems to be widespread, sympatric and common throughout the Madeiran archipelago, especially at lower altitudes.

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Evidence of sympatry between the endangered Réunion Island day gecko *Phelsuma borbonica* and the introduced gold dust day gecko *Phelsuma laticauda* on Réunion Island

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The gold dust day gecko *Phelsuma laticauda* (Boettger, 1880), a species with a conservation status of Least Concern in its native Madagascar, has been introduced into several oceanic islands in the Indian and Pacific Oceans (Ota & Ineich, 2006; Gerlach et al., 2011; Goldberg & Kraus, 2011; Augros et al., 2017; Moutou, 1995). The species is considered a competitor (Lund, 2015), a predator (Gehring et al., 2010) of other Gekkonidae, and a potential source of endoparasites (Goldberg et al., 2003). Introduced in the west of Réunion Island (Indian Ocean) in 1975 (Moutou, 1995), *P. laticauda* is now found all around the island, up to 600 m a.s.l. (Sanchez, 2016; Sanchez & Caceres, 2019). To this day, only two of the seven known native reptile species of the island remain (Arnold & Bour, 2008; Cheke & Hume, 2008; Cole & Sanchez, 2021). These are two are species of day gecko: *Phelsuma borbonica* Mertens, 1966, classified by the IUCN as Endangered (Sanchez, 2021a) and *Phelsuma inexpectata* Mertens, 1966, classified as Critically Endangered (Sanchez, 2021b). These two threatened native day geckos represent a significant conservation challenge. The endemic *P. inexpectata* was observed in sympatry with the exotic *P. laticauda* and they seemed to be in competition (Deso et al., 2023). Here we report observations of sympatry between the introduced *P. laticauda* and the other native day gecko of Réunion Island, *P. borbonica*, in a natural environment.

On the morning of 28 April 2024, during a diurnal wild photography session in Bois Blanc (east of Réunion Island, Sainte-Rose, 10 m a.s.l.), we observed evidence of sympatry between the native *P. borbonica* and the introduced *P. laticauda* (Fig. 1). *Phelsuma borbonica* is characterised by its brown and blue body colours, and *P. laticauda* by a golden yellow dorsal colouration with three red stripes on the lower part of the back (Sanchez & Probst, 2012). The event took place on the fleshy rachis of the native *Pandanus utilis*, which is part of the habitat of the native gecko. Several individuals of both species were observed feeding at the same time on the same infructescence.

Figure 1. Adults *Phelsuma laticauda* (left) and *Phelsuma borbonica* (right) feeding on the same fleshy rachis of *Pandanus utilis*

This observation is the first description of sympatry between *P. borbonica* and a species of the same genus. Cases of sympatry between exotic and native species of the genus *Phelsuma* on Mauritius have led to the extinction of native populations (Buckland et al., 2014). In its native range, *P. borbonica* does not live in sympatry with other species of the genus *Phelsuma*, unlike *P. laticauda*. Therefore, *P. borbonica* may be less well predisposed to interspecific competition, in particular competition for food resources and basking sites. Further studies are needed to better understand this competition, which may include direct physical interference as in other cases of interspecific competition with geckos of the genus *Phelsuma* (Lund, 2015; Deso et al., 2023). This native population of Bois Blanc is one of the last remaining along the coast and is therefore a conservation and heritage issue of concern. This relict coastal population also exhibits a distinctive brown colour pattern on the head, which appears to be characteristic of the Sainte-Rose coastal area (Sanchez & Caceres, 2019; Cornuault et al., 2024). However, the Bois Blanc population is not currently considered by site managers (ONF, 2016), a situation that poses a serious threat to the preservation of the species, especially in a habitat that is increasingly degraded for the gecko.

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Attempted predation by the grass snake *Natrix natrix* of the great ramshorn snail *Planorbarius corneus* in Hungary

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Colubrid snakes feed on a wide spectrum of vertebrate and invertebrate prey, nevertheless, several colubrids are known to be dietary specialists. For example, snail- or slug-eating has evolved in at least four groups. However, attempts to consume (mostly) aquatic snails by non-snail specialists have occasionally been reported.

The grass snake *Natrix natrix* (L., 1758) is a widely distributed water snake (subfamily Natricinae) across central Europe, with its range extending far into central Asia (Speybroeck et al., 2016). As a semi-aquatic species, it primarily inhabits wetlands, though it is not as aquatic as other European water snakes. *Natrix natrix* is considered a generalist feeder, preying predominantly on anurans but also frequently consuming small mammals, and occasionally fish, lizards and birds (Luiselli & Capula, 1997).

On 26 July 2024, at the Botankerti-tó, a small artificial pond in the Botanical Garden of Sopron University (NW Hungary, 47.680408° N, 16.573403° E, WGS 84), the first author (KK) observed a subadult *N. natrix* (approximately 40 cm in length) with an aquatic great ramshorn snail *Planorbarius corneus* (L., 1758) (family Planorbidae) in its mouth. The botanical garden is located in a suburban area on the northern slopes of the Sopron Mountains. The pond itself is richly vegetated, with the water surface almost entirely covered by *Lemna minor* during the summer months. It hosts a healthy population of water frogs *Phelophylax* sp. and newts *Lissotriton vulgaris*. Additionally, various aquatic invertebrates (i.e. aquatic beetles, water bugs and aquatic snails) can be found in high numbers. When discovered, the snake was floating motionless on the surface and was initially thought to be dead. However, when picked up, it became evident that the snake was alive, as it attempted to escape, though it did not show any antipredator behaviour typical of the species. Upon closer examination, it was apparent that the snake's upper jaw, including most of its head up to the eyes, had disappeared into the snail's shell, while its lower jaw was grasping the whorl of the snail from the outside (Fig. 1). Despite being handled, the snake did not release its prey, and it was not clear whether it was stuck. It appeared to still be trying to swallow the snail.

Figure 1. Subadult *Natrix natrix* attempting to eat a *Planorbarius corneus* (Sopron, NW Hungary)

The specimen was not collected but instead released in a concealed part of the pond; therefore, we have no information on whether it managed to ingest its unusual prey. To our knowledge, this is the fifth documented observation of an attempted predation of an aquatic snail by *N. natrix* and the first such observation in Hungary. Examination of previous cases suggest that attempts to consume aquatic snails, particularly species with an operculum (i.e. a calcareous anatomical structure attached to the foot that functions like a trapdoor) are likely fatal to snakes that lack specific adaptations for consuming these molluscs (Kornilev et al., 2023). Recently, Balogh et al. (2024) reported that a *N. natrix*, attempting to feed on a Lister's river snails *Viviparus contectus* was trapped by the snail's operculum, leading to its subsequent death, likely by suffocation. These trapped snakes are also prone to predation and may become road traffic victims. Fuchs et al. (2020) reported an adult southern watersnake *Nerodia fasciata* crossing a paved road while its head was trapped by a spotted apple snail *Pomacea maculata*. Similarly, Young & Boyarski (2012) reported an adult northern Mexican garter snake *Thamnophis eques megalops* being run over by a vehicle while attempting to eat a Chinese mystery snail *Cipangopaludina chinensis*.

Although *P. corneus* lacks an operculum, this does not necessarily make it a less dangerous prey. Previously, Consul et al. (2009) and Zimić & Klisura (2016) described feeding attempts on *P. corneus* very similar to ours. In all cases, the snouts and eyes of the snakes were entirely covered by the snail's shell, likely incapacitating their main sensory organs. Wood & Hollingsworth (2005) described a subadult Baja California gartersnake *Thamnophis validus celaeano* whose lower jaw was stuck inside the shell of a *Planorbella subcrenata* and died of exhaustion and/or starvation. The fact that our *N. natrix* specimen was found floating on the water suggests that residual air in its prey's shell likely hindered it from submerging. Repeated and failed attempts to dive could lead to fatigue and, eventually, predation, given that both the Eurasian goshawk *Accipiter gentilis* and the European kestrel *Falco tinnunculus* are very common in the area.

These observations indicate that even non-specialist water snakes, such as *N. natrix*, occasionally attempt to prey on aquatic snails, particularly *P. corneus*. However, it remains a question whether *P. corneus* is a common prey item for *N. natrix*. If it is, these observations might represent sporadic events where the snake erred. Otherwise, all attempts could be doomed to failure from the outset due to the specific characteristics of *P. corneus*. Since *P. corneus* is a widespread species across various aquatic habitats in Europe, Asia and, more recently, North America (Seddon & Van Damme, 2011), more observations of water snakes attempting to feed on these snails are likely.

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Potential predation of a barred grass snake *Natrix helvetica* by a brown rat *Rattus norvegicus*, with a link to video evidence

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The barred grass snake *Natrix helvetica* is a medium-sized snake species typically growing to 150 cm in length and found throughout north-western Europe (Speybroeck et al., 2016). The brown rat *Rattus norvegicus* is a very widespread and highly adaptable mammal exploiting a wide range of food resources, especially cereals and their products. However, *R. norvegicus* may consume meat opportunistically and has been observed eating live snakes (Swanson, 1952; Fitch, 1963; Hummer & Tolley, 2008; Reynolds et al., 2023).

On the afternoon of 16 August 2024 at approximately 15:50 h, a subadult *N. helvetica* was seen crossing a slightly shaded footpath at Culford Park, West Suffolk (52° 18’11” N, 000° 40’13” E). Shortly afterwards, an adult *R. norvegicus* attempted to grab the snake by the tail (Fig. 1). The rat came from the same direction that the snake had travelled from, suggesting that it was following the snake. After initially attacking the snake, the rat grabbed it roughly 20 cm further up the body and started chewing on it. At this point, the snake feigned death before it was carried off into the undergrowth, at which point the view of both animals was lost (BHS Video, 2025). At the time of the observation the *N. helvetica* was alert and the weather was still bright and sunny with an air temperature of 23–25°C.

Figure 1. A still from the video where a brown rat *Rattus norvegicus* can be seen predating upon a barred grass snake *Natrix helvetica*, later it disappeared with the snake into the undergrowth

Our observation demonstrates the brown rat’s adaptability and willingness to exploit a wide range of food sources, although there may be potential risks involved if the snake had been larger or venomous. As seen in the video, the rat initially grabbed the snake by the tail end in a way that would be very risky if it had been a venomous snake and was apparently not distracted by the snake’s foul-smelling and tasting cloacal gland secretions. This behaviour might also indicate a scarcity of typical food sources for rats. Observing this rat preying on a grass snake could signal changes in food availability, forcing the rats to seek alternative food sources, or may just indicate opportunistic behaviour.

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Arboreal and nocturnal activity of the snake-eyed skink *Ablepharus kitaibelii* in Greece, with a link to video evidence

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The snake-eyed skink, *Ablepharus kitaibelii* Bibron & Bory de St-Vincent, 1833, is a diurnal species distributed widely in the central and southern Balkan peninsula, north-western Turkey, as well as on several Ionian and Aegean Islands (Vergilov et al., 2016). It inhabits a variety of biotopes from sea level up to highly elevated regions (Speybroeck et al., 2016) and is well known as a terrestrial, semi-fossorial species found frequently in moist leaf litter in sparse to densely vegetated habitats throughout its native range (Gruber, 1981).

On the night of 9 September 2024 (20:25 h), during a faunistic survey of the Strofylia forest, north-western Peloponnese (38.123° N, 21.375° E, WGS 84), we observed an adult snake-eyed skink moving on the ground. After a few minutes it moved up the vertical trunk of a eucalyptus tree (Fig. 1, BHS video, 2024) to a height of about 2 metres and then hid itself under the bark. The observation took place in a stand of eucalyptus trees situated in the middle of an extensive forest of *Quercus macrolepis* surrounded by wetlands and adjacent clearings.

Nocturnal activity has not been reported previously in this species. It is known that *A. kitaibelii* usually shows a bimodal activity pattern, being mostly active during the first hours after sunrise and the last hours before sunset (Pasulević, 1965; Gruber, 1974). However, its activity can extend into dusk (Fuhn, 1970), while during summer months the species is considered crepuscular (Speybroeck et al., 2016; Vergilov, 2017). Other authors have suggested that the species is not active during the summer and the hot months of the year (Rotter, 1962; Herczeg et al., 2007). Our observation of nocturnal activity was made one hour after sunset, in dark conditions when the individual was observed under artificial light. Arboreal activity has apparently not been reported previously and neither has it been seen to climb vertical surfaces (Fejérváry, 1912). Our note shows that the snake-eyed skink has a more diverse behavioural repertoire than reported previously.

Figure 1. *Ablepharus kitaibelii* climbing an eucalyptus tree after dark

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BOOK REVIEW

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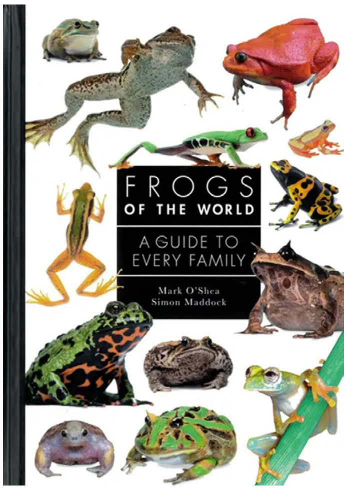
Frogs of the World: A guide to every family

Mark O'Shea & Simon Maddock
Princeton University Press, 2024
978-0-691-24830-1, 240 pp.



With over 7,600 species of anurans known to science (Frost, 2024), trying to fit all of them into a single book would be impractical given the high rate of species discovery that would render such a guide obsolete within a year or two. The next best thing is to deal with anurans family by family, giving examples of interesting species in each family and this is what 'Frogs of the World: A guide to every family' has done, offering a clear and beautifully illustrated account of this group of amphibians.

Billed as an in-depth introduction to each family with profiles of specific species to illustrate their specific characteristics and adaptations, 'Frogs of the World' would seem to be a valuable companion to anyone with an interest in anurans. The title of the book is slightly misleading as it does include toads too, although most readers with an interest in frogs would likely suspect this.

The book opens with a comprehensive introduction to the natural history, evolutionary biology, anatomy, ecological importance, and conservation of frogs and toads (to name but a few such topics). There are a number of illustrations and brilliant photographs throughout this section that put the information into context and make it accessible. This information is a good general introduction to the clade so that it applies to the majority of anuran species, which is important given their diversity and the fact that over 40% of species are threatened with extinction.

The first four families of anurans that are featured belong to the suborder Archaeobatrachia, featuring the Ascaphidae, Leiopelmatidae, Alytidae and Bombinatoridae in that order. The introductory page to this suborder describes where the species that belong to it can be found, when they evolved, and the primitive features that differentiate them from other anurans. Next comes the Mesobatrachia with Megophryidae, Pelobatidae, Pelodytidae, Pipidae, Rhinophrynidae and Scaphiopodidae. With added complexity comes additional species features, with the two subfamilies within Pipidae (Dactylethrinae and Pipinae) being treated separately from one another. Where necessary, this format of layout and information is continued throughout the rest of the book. Finally, the book considers the Neobatrachia which encompasses 46 families – so the majority of known species and so rightfully comprises the majority of the book.

Each family summary contains a number of beautiful photographs along with information on their ecology and conservation status, as well as additional information such as a distribution map, size, diet, habitats and modes of reproduction. Given how varied frogs and toads are, it is this information that the general reader is likely to be most captivated by, if not the photos. These clear summaries allow readers to see how each family is differentiated from the others. This, I feel, is one of this book's strongest features allowing side-by-side comparisons of anurans throughout the book.

The information presented about each family or subfamily is well written, concise and accessible. For those families that are particularly large such as Microhylidae, there are double-page spreads dedicated to each subfamily to aid with the representation of both the volume and diversity of species found within them. The authors have worked hard to ensure that readers with widely differing degrees of interest in amphibians would all be able to make use of the information presented to them. The paragraphs are not overly technical or complicated, often with relevant photographs alongside the text. Furthermore, given that the book is relatively short for such a large subject, the pages are not overflowing with text, which prevents the reader from becoming overwhelmed, and instead maintains their engagement with the information throughout.

While not all anuran species are covered, which is particularly true for the larger families, the information that is present is highly relevant, and the highlighted species have been carefully chosen. For example, if a species is listed as being Endangered by the IUCN Red List, this is highlighted along with the threats to that species' conservation. Another useful feature is a summary of the species in each family or subfamily that are listed in each of the Red List threat categories, with a percentage telling the reader how many of those species are in trouble. This helps readers visualise that risk, especially when a whole family may be endangered in similar ways.

At the end of the book is an extremely helpful glossary and although most of the sections I have just described are generally free of scientific terminology, jargon is occasionally unavoidable. There are also more definitions here than included in some more technical books, which I feel adds to the accessibility of this quick overview of the world's frogs and toads.

Given its very wide-ranging content, in a volume of only 240 pages, this book can only act as a quick guide to the anurans of the world, and so some further books and websites are suggested to help readers find additional relevant information. The book list is arranged geographically but does miss some key texts as North America, South America and Europe are only represented by a single book each. If I had a criticism it would be that the contents page at the front only lists the suborders and not the families themselves, so these need to be located by using the index at the back.

To my knowledge, this is the first book of its kind that aims to include information about every family of anuran, with up-to-date and accurate scientific information. Previously, Chris Mattison has produced at least two books with a similar title that have been published in a number of different editions on both sides of the Atlantic (Mattison 1987; 2011) and which some may argue are the spiritual precursors to the latest book. More recently, Halliday (2016) covered 600 species of anurans in depth with life-size photos and the relevant information about each that you would expect in such a tome.

Finally, given that 'Frogs of the World: A Guide to Every Family' retails for at most about £25, it is an affordable guide for anyone interested in, or working with, amphibians. It may not contain all of the information some readers or researchers may be looking for, but it provides a solid foundation that then allows for further research. The authors have succeeded in balancing accessibility with scientific depth, making complex information understandable without oversimplifying it.

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HERPETOLOGICAL BULLETIN REPORT 2024

In 2024, issues 167–170 of The Bulletin were uploaded to the BHS website on the first day of each quarter and the hardcopy versions posted to subscribers within the following four weeks. For the year there were 105 manuscripts submitted of which 57 were published giving an overall acceptance rate of 54%, down from 60% in 2023 but still within the typical acceptance range of 50–60% for The Bulletin. There were 9 fewer manuscript published in 2024 than 2023 and given that The Bulletin page count is fixed at 48 pages, this indicates a modest rise in the length of articles (from 3.0 to 3.4 pages).

Table 1. Submission and acceptance rates for Bulletin issues published in 2024 (2023)

	No. submitted	No. accepted	% accepted
Full Papers	16 (15)	10 (11)	63 (73%)
Short Notes	9 (13)	6 (6)	67 (46%)
Short Communications & Natural History Notes	80 (82)	41 (49)	51 (60%)
Totals	**105 (110)**	**57 (66)**	**54 (60%)**

The time from acceptance to the publication of a manuscript is now typically 4 to 6 months. Of the published articles, only 5% were illustrated with an accompanying video, this is down from 11% in 2023. Authors are encouraged to submit accompanying videos as these are posted on the BHS YouTube site and hyperlinked to their articles. A further advantage of this is that such articles are open access from the time of release, as are all articles published by BHS members, rather than being subscriber-only for one year. One year after publication all remaining articles in The Bulletin are fully open access.

The BHS is very grateful to the following people who gave their time and expertise reviewing manuscripts for The Bulletin in 2024: Lauren Augustine, Roger Avery, João Luiz Gasparini, Richard Griffiths, Rick Hodges, Marcelo Kokubum, Simon Loader, Anita Malhotra, Charlie Manolis, Miguel Mendez, Chris Michaels, Cristiane Palmeira, Leandro Sabagh, Rodolfo Santos, Walter Schargel, Ben Tapley, Luciano Verdade and Catherine Whatley.

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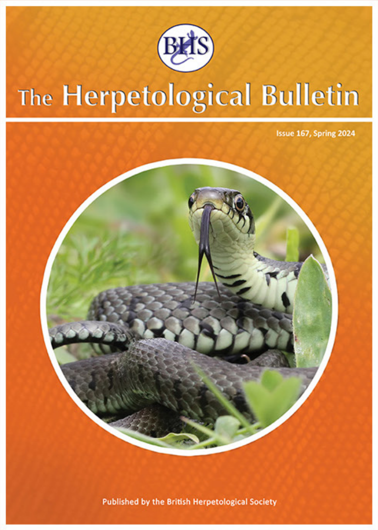
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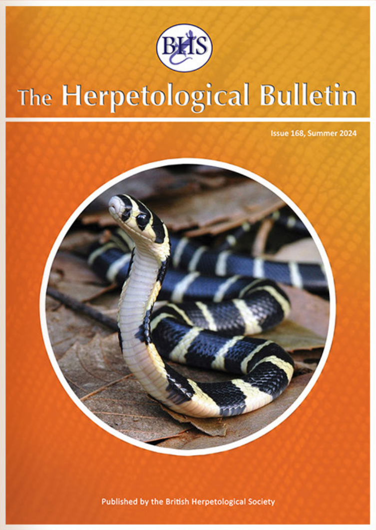
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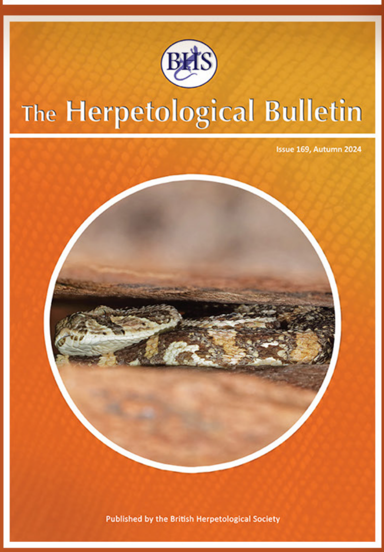
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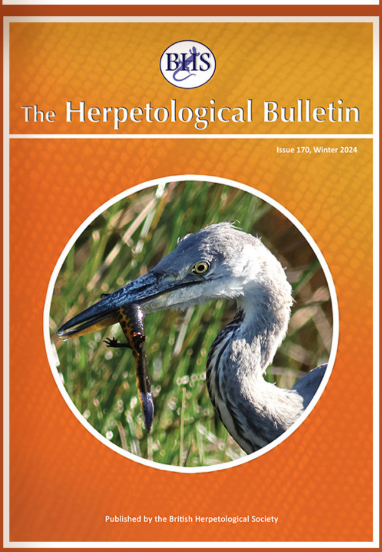
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