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Front Cover: *Chlorosoma laticeps* photographed by Otavio A.V. Marques in the Atlantic Forest (Sooretama Reserve), Brazil, see article on p.26.

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RESEARCH ARTICLE

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Annual spawn clump losses in a population of the agile frog *Rana dalmatina* in western France

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ABSTRACT – The agile frog *Rana dalmatina* is a common and frequently studied species in Europe including long-term studies of population change. Several have employed spawn clump counts to give estimates of annual presence of reproductive females. Spawn clumps are also subject to predation but little is known of the extent of losses but it could impact population densities significantly. The objective of the present study was to assess the extent of spawn clump losses due to predation in a population of *R. dalmatina* in western France using data from numbers of spawn clumps recorded over a four-year period (2019–2022). Spawn counts were greatest during 2019 but numbers declined with a zero count in 2022. Predation was greatest in 2021, the year total counts were lowest, but there were no statistical difference between annual losses, which varied from 22.9–41.6 %. Most spawn was deposited in a series of ditches rather than in the two available ponds but statistically predation levels were in agreement, with ditches ranging from 26.1–40 % versus ponds 28.6–40 %. Observed predators were ducks and alien crayfish *Procambarus clarkii*. The latter consumes all stages of frog development and hence likely represents the greater threat, especially since it has the capacity for population increase to very high numbers.

INTRODUCTION

Pond breeding amphibians face a series of decisions when selecting areas for oviposition, these include pond temperature, probability of ponds drying out, the presence of food for both their larvae and adults and for predator presence, the latter in both the aquatic and surrounding terrestrial environments. The agile frog *Rana dalmatina* (Fig. 1) breeds in ponds throughout Europe where it is listed as of Least Concern by the IUCN in Appendix II of the Bern Convention (Kaya et al., 2009). Various aspects of its ecology have been studied (e.g. Gollman et al., 2002; Hartel, 2003; 2004; Puky et al., 2006; Bartoń & Rafiński, 2006; Meek, 2012; 2018; Combes et al., 2018; Jovanović & Crnobrnja-Isailović, 2019). Among these is a long-term population study of *R. dalmatina* in Vendee, western France based on counts of spawn clumps and road mortalities. This showed that although numbers fluctuated widely they were, in general, stable over the long-term (Meek, 2018). Reproductive activity in *R. dalmatina* begins when the adults arrive at ponds in late winter, when females lay one clutch of around 500–2000 eggs (e.g. Gollman et al., 2002; Hartel, 2003; 2004; 2008; Bartoń & Rafiński, 2006; Puky et al., 2006; Meek, 2012). These are usually initially fixed to underwater twigs or plants that slowly float to the pond surface. The benefits of surface floating spawn is probably increased heat from warm sunshine and hence more rapid rates of larvae development, but the potential costs are increased risk of predation due to high visibility on water surfaces and also risk of the spawn being encased in ice during extreme winter conditions (Meek, 2012).

Survival of spawn masses is a critical aspect of pond breeding amphibian population dynamics influencing numbers of larvae and ultimately breeding adults. Therefore when spawn mass predation levels are high, and if they remain high especially over several years, there is a potential for population collapse. In this study numbers of missing spawn clumps, assumed due to predation, were recorded during the 4-year period from 2019 through to 2022 in Vendee, western France. Two main questions were addressed:-

1. Were there differences in annual proportions of spawn clumps lost as a proportion of the total numbers laid? This is important because survivorship of larvae is one critical aspect of long-term population stability.
2. Were there differences in spawn mass lost in ditches compared to ponds? This is important because differences in predation pressure between ponds and ditches could also impact on long-term population stability especially if there are major differences in numbers of spawn deposited.

Figure 1. Example of *Rana dalmatina* found in the study area

METHODS

The study area is a fragmented landscape dominated by agriculture in Vendee, western France (46° 27` N; 1° 53` W). The first spawn clumps were usually sighted in early to mid February and counts were made by a single observer along the edges of three ditches and two ponds. Photographs of the spawn clumps were usually made alongside habitat features to enhance count accuracy, especially when concentrations were large.

Sampling periods were for 13 days between 10 February and 14 March (2019), 12 days between 2 to 23 March (2020) and 12 days from 23 February to 9 March 2021. During 2022, sampling began 1 February and continued into late April, due to the need to confirm a zero count. Figure 2 shows a map of the study area and Figure 3 photographic examples. Clutches of *R. dalmatina* were counted in ditches situated alongside a hedgerow abutted by farmland, alongside roads, a New Pond excavated in 2009 and a long established pond (Old Pond).

To compare missing spawn clumps as proportions of total numbers deposited, *z*-tests for two independent proportions were used. Comparisons were between years and between ponds and ditches. The null hypothesis in both data sets is that annual losses were in approximate agreement, H_o: $p_1 = p_2$ where p_1 is the proportion of the first sample and p_2 the proportion of the second sample. The resulting P-values are based on two-tailed tests. Sample precision tests for the amount of variation around the *z*-scores derived from sample sizes for each of the three-year data sets were ±11.6 (2019), ±16.6 (2020) and ±20% (2021).

RESULTS

Spawn mass numbers declined during the 4-year sampling period. In 2019 a total of 74 spawn clumps were counted with 17 of these recorded missing (22.9 %). For 2020 spawn count was 35, with 10 recorded missing (28.6 %). During 2021 total spawn mass was 24 with 10 recorded missing (41 %). However, the proportional losses were not significantly different between years; 2019 versus 2020, $z = 0.62$, P = 0.53; 2019 versus 2021 $z = 1.59$, P = 0.11 and 2020 versus 2021, $z = 0.96$, P = 0.33. Hence despite the differences in sample sizes the proportional losses were in statistical agreement (Fig. 4). The majority of spawn clumps were deposited in the three ditches. To improve sample sizes for comparisons the data from ponds and ditches were each pooled. Spawn counts in the three ditches formed 87.5 % (n = 91) of total spawn counts from 2019 to 2021, with the remainder deposited in the two ponds (n = 13; C & D in Fig. 3). Most spawn losses were recorded for rue de Bourneau and in the ditch alongside open fields (A in Fig. 2). The proportional losses for the pooled 3 year total counts between ponds and ditches were not significant $z = 0.32$, P = 0.74.

DISCUSSION

The results of this study have indicated that, irrespective of total annual counts, spawn mass losses in each of the surveyed areas were approximately similar (question 1) with proportional losses also in agreement between ponds and ditches (question 2, and thus the null hypothesis is confirmed in both questions). This was perhaps an unexpected result given the habitat difference between pond and ditches.

Figure 2. Map of the study areas showing sections sampled. Marked are **A** ditch running alongside agricultural fields, **B** and **C** roadside ditches along with the location of the two ponds. See Fig. 3 for photographs of these features.

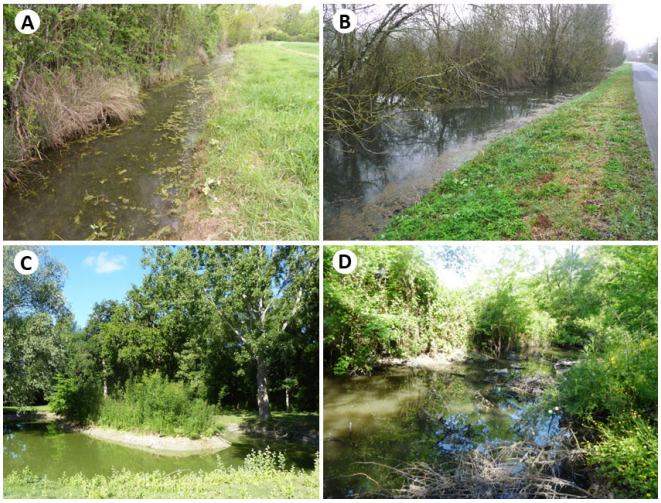

Figure 3. Water bodies in which *Rana dalmatina* spawn clumps were counted – **A.** & **B.** ditches, **C.** New Pond, and **D.** Old Pond

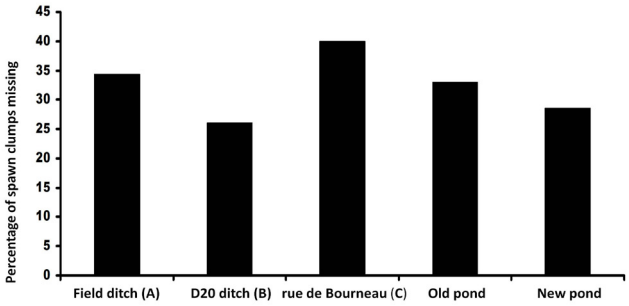

Figure 4. % of spawn clumps missing, assumed predated, during the study period. Data shown are pooled losses for the 3-year study period. Labeling on the x-axis refers to Fig. 2.

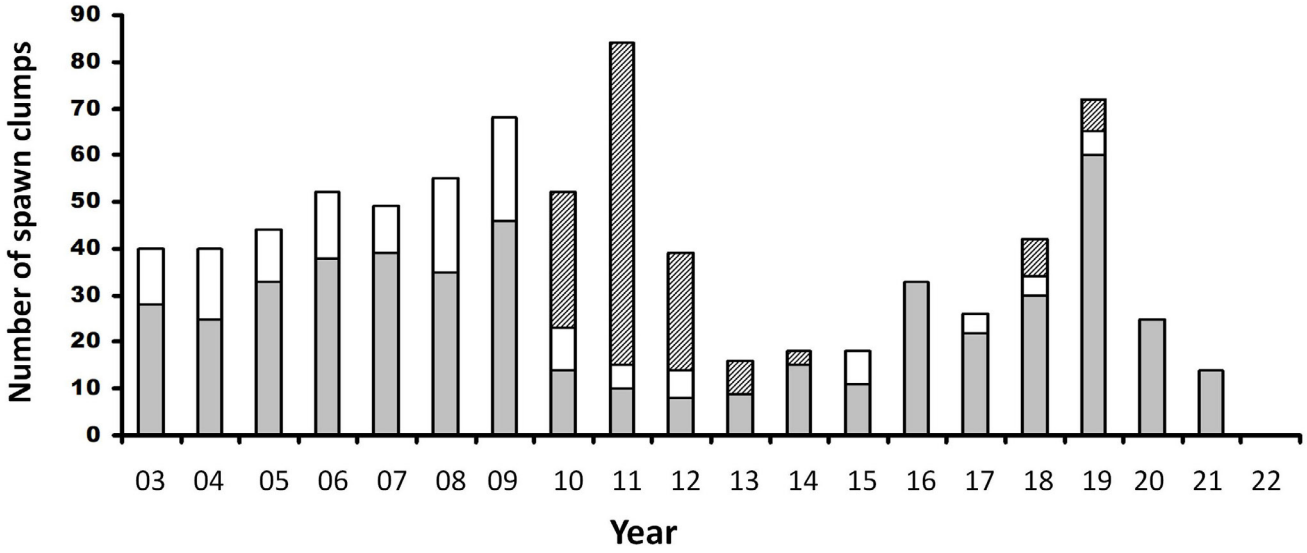

Figure 5. Spawn mass numbers deposited in the study area between 2003 and 2022. The histograms show final counts at the finish of the spawning period and hence do not include missing spawn counts. Grey bars represent pooled data for ditches; open bars Old Pond and crosshatched bars New Pond. Data from 2003 to 2018 are taken from Meek (2018), and 2019 to 2022, this study.

However, during the four-year study period spawn counts were mostly in ditches with smaller quantities in ponds and hence data comparison was potentially less reliable. The results suggest however that, despite total annual spawn mass counts, predation pressures were essentially similar, although further data are needed to support this finding.

The zero count recorded in 2022 was also unexpected but it should be kept in mind that spawn clumps represent only a proxy for numbers of reproductive females and hence does not necessarily indicate a local extinction, since males and smaller non-reproductive females may still be present in the locality. This followed a period of declining numbers beginning 2019 but was similar to the trend observed from 2011 when numbers declined from a 20 year high to low counts between 2013 and 2015 (Fig. 5). This trend was inversely mirrored by increases in numbers of alien crayfish *Procambarus clarkii* (Ficetola et al., 2011) a species well known to consume all stages of amphibian development, including adults. Crayfish numbers also fluctuate widely and in the study locality experience regular population crashes followed by increases. In the present study *P. clarkii* numbers began to increase from around 2018/19 and in 2021 were seen for the first time in areas B and C (Fig. 2). High numbers have been recently reported 6 km from the study area, for example in large garden ponds in the village of St Denys du Payre and in Deux-Sèvres region (e.g. Xavier Bonnet pers. com.). In addition to consuming spawn and larvae they effectively eliminate macrophyte cover, one of the key pond requirements for *R. dalmatina* breeding pond selection (Pavignano et al., 1990; Puky et al., 2006). Absence of macrophyte cover is normal in the New Pond when *P. clarkii* is present.

Several species of European amphibian are apparently able to detect *P. clarkii* presence. For example, Nunes et al. (2013) found that five of nine European species of anurans changed their behaviour when *P. clarkii* was present, apparently chemical cues emitted from predated or injured conspecifics is the means of detection. High numbers of spawn were being deposited in the New Pond during 2010 and 2011 after it was created in 2009 but declined with the arrival of crayfish, becoming absent by 2015. *Rana dalmatina* forages widely across the landscape and appears not to be faithful to particular breeding sites. Consequently, if it is capable of detecting *P. clarkii* presence then it may simply migrate to other breeding areas (Blab, 1986; Puky et al., 2002; Gollmann et al., 2002). A second observed predator is ducks that feed on spawn although they will not necessarily consume a whole spawn mass leaving perhaps 10 % or so.

Previous amphibian research has shown that many, including European species, may occasionally miss breeding in certain years due to some ecological disturbance (Renoirt et al., 2021; Meek, 2021; 2022); pond drying is a good example (e.g. Lomas & Anderson, 2006). In the case of the green frog *Pelophylax lessonae* numbers in the study locality declined from high annual counts to a 4-year total absence followed by population recovery (Meek, 2021). A zero count was also recorded in a population of sympatric *Bufo spinosus* followed by a limited recovery (Meek, 2022). The recoveries were attributed to immigration from adjacent populations in both species. Absence of breeding female *B. spinosus* has been observed in other areas of western France (Renoirt et al., 2021). These species are classed as highly fecund pond-breeding amphibians with wide annual variations in population size. This renders them susceptible to population crashes along with capacity for recovery (Green, 2003). The present results have relevance in that they suggest predation pressure on *R. dalmatina* spawn clumps persist at an almost constant rate. If a major ecological disturbance occurs when

spawn mass numbers are at a lower bound during a period of population oscillation, for example, during high numbers of *P. clarkii*, the potential for a zero count increases. Continual monitoring of spawn mass counts is therefore needed to understand not only general long-term population trends and relationship with numbers of *P. clarkii* and other predators, but also the frequency of population zero counts.

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RESEARCH ARTICLE

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Investigating the behaviour and enclosure use of zoo-housed Cuvier's dwarf caiman *Paleosuchus palpebrosus*

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ABSTRACT - The welfare needs of reptiles in zoological collections are generally less well understood than those of other taxa. Crocodilians represent an excellent opportunity to study a commonly-housed, conservation-dependant zoo animal. We studied the behaviour and enclosure use of five dwarf caimans *Paleosuchus palpebrosus* through day and night, at two British zoological collections; the enclosures had either six or seven identifiable zones. Time of day, mean temperature and collection were used as predictors of behaviour and enclosure usage. Camera traps recorded the position and behaviour of caimans at 30-second intervals. At each zoo, 80 hours of data were collected from which we constructed activity budgets and calculate a relativised Electivity Index of enclosure usage. The results identified that water-based perching, floating, swimming, immobile water behaviour and underwater behaviour were significantly affected by time of day, and that collection and temperature were good predictors of some behaviours. As for enclosure use, zone 3 (shallow water) was slightly overutilised in both collections, while all other zones were underutilised. Time was a significant predictor of the use of zones 3, 5, 6 and 7. There is considerable scope for future research on crocodilians in zoos.

INTRODUCTION

Modern zoological collections are united in their aim to champion both conservation and education (Hosey, 2005). It is important to ensure that animal welfare is optimised, both for the zoo and the general public (Melfi, 2009; Moss & Esson, 2010). However, not all zoo-housed species are equally well studied. Despite being frequently housed in zoological collections, reptiles have been researched less frequently (Brereton & Brereton, 2020; Moszuti et al., 2017). Reptiles are cognitively more complex than is often acknowledged, with many reptile species showing evidence of problem-solving ability, personality and social learning (Learmonth et al., 2021). Applying an evidence-based approach to future studies could help develop the husbandry and welfare of unrepresented taxa such as reptiles (Melfi, 2009).

One Order of reptiles that is well-represented in zoos is the Crocodilia (Ziegler et al., 2017) and there have been several zoo-based studies on crocodilian welfare. Enrichment has been shown to have a positive impact on the behaviour of African dwarf crocodiles *Osteolaemus tetraspis* (Uwakaneme et al., 2004) and in the case of broad-snouted caimans *Caiman latirostris* it was found that only 53 % of their husbandry requirements were being met in captivity (Prystupczuk et al., 2019; Verdade et al., 2006). Studies of the behaviour and enclosure use of Nile crocodiles *Crocodylus niloticus* in the presence of visitors demonstrated no effect from the visitors (Riley et al., 2021) but visitors significantly increased inactive behaviours in dwarf caiman (Hamilton et al., 2022). These studies show the feasibility of welfare research for zoo-housed crocodilians and the opportunity for further research. It is in this context that we investigated the behaviour and enclosure use of dwarf caimans in two zoological collections

METHODS

Study subjects and location

Following University Centre Sparsholt ethical approval (UCS050520) observations were made on two Cuvier's dwarf caiman *Paleosuchus palpebrosus* at The Living Rainforest and three individuals at Crocodiles of the World (see Table 1). The enclosures at Crocodiles of the World (Fig. 1A) and The Living Rainforest (Fig. 1B) are similar in design and contain a basking area, bank area, open water and a zone out of sight of the public. At Crocodiles of the World the caimans shared their enclosure with two male South American river turtles *Podocnemis expansa*.

Behaviour

The objective of the study was to build an understanding of dwarf caiman behaviour and enclosure usage at different times of the day (Plowman, 2008). Data collection was completed between 23 August 2020 and 5 October 2020, making up 160 hours; 80 hours per collection (for five whole days). Camera traps (Bluesmart trail camera, 4K 20MP IP66) (wide angle, infrared, set at 20MP) allowed the behaviour to be recorded without the observer effect, which has been reported in reptiles (Riley et al., 2021). Two camera traps were placed in each enclosure to ensure animals were always visible. Eight time codes were used to define different times of the day and to reduce pseudo replication effects (Plowman, 2008) (see Table 2). Time of day and

Table 1. Dwarf caiman sex, hatch date, and origin from two zoo collections

Collection	Sex	Hatch date	Origin
Crocodiles of the World	Male	Between April 2005 and April 2006	Undetermined
	Female	Between April 2000 and April 2001	Private British collection
	Female	15 September 1987	Cologne Zoo
The Living Rainforest	Male	17 August 2014	Undetermined
	Male	16 July 2015	Undetermined

Table 2. Eight time codes altered from Chapman et al. (2018)

Day/Night	Time code	Time of observation
Night	Late night	01:00-03:00
Night	Dawn	04:00-06:00
Day	Morning	07:00-09:00
Day	Late morning	10:00-12:00
Day	Afternoon	13:00-15:00
Day	Evening	16:00-18:00
Night	Dusk	19:00-21:00
Night	Early night	22:00-00:00

Table 3. Ethogram of state behaviours

Behaviour	Description
Terrestrial locomotion	Travelling taking place on land
Immobile land behaviour	The individual is not in the pool and is resting, thermoregulating or gaping
Surface swimming	Swimming on the surface of the water. Eyes and back above the water surface.
Floating	Individual immobile on the surface of the water or only the top of the head is showing
Perched in water	Individual head above the water with limbs resting on a surface/object under the water; absence of any other behaviour
Underwater	Completely immersed under the water
Out of sight	Unable to identify the location of the individual or determine the behaviour accurately

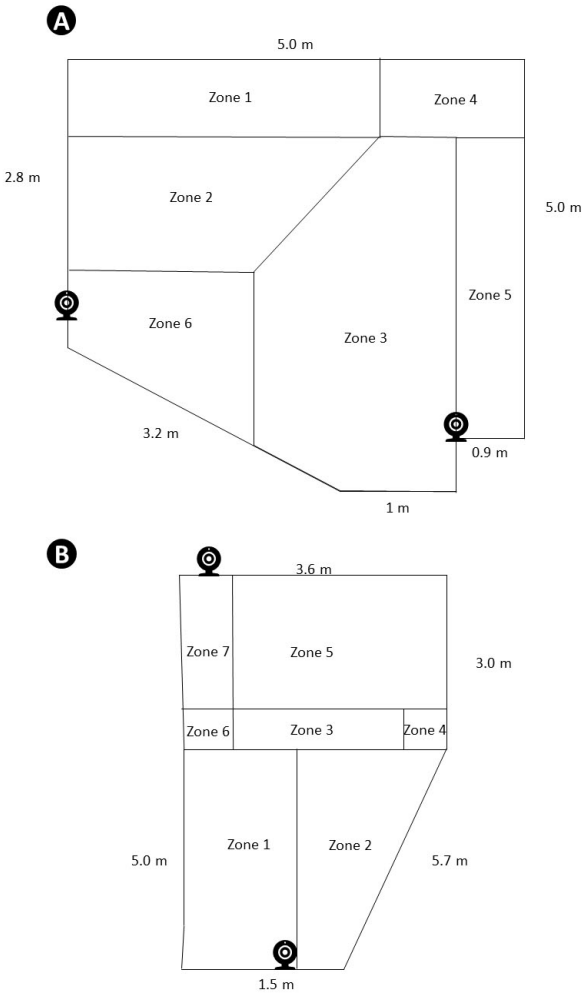

Figure 1. Dwarf caiman enclosures with zones, measurements, and camera trap positions (camera symbols) - **A.** Crocodiles of the World, basking is available in zone 2, and **B.** The Living Rainforest, basking is available in zone 1

mean environmental temperature (from the camera trap's internal temperature gauge) were recorded as predictors of behaviour and enclosure usage. The cameras were set to take photographs automatically every 30 seconds (categorised as instantaneous scan sampling). When an individual was located between two or more zones, the location of the head and forelimbs was used to identify the zone occupied. An ethogram was devised to describe the various behaviours displayed by the caimans (Bateson & Martin, 2021) (Table 3) and the observed behaviours were subsequently summarised in activity budgets (Fig. 2).

Enclosure use

The enclosures were divided into zones based on the useable space for the caimans and the biological use of each area (Fig. 1 A & B, Table 4). Both zoos were open to the public seven days week, with visitor hours for The Living Rainforest being 09:30–16:00 and for Crocodiles of the World from 10:00–17:00. The two exhibits were indoors with limited natural lighting. Both collections controlled their heating provisions through a thermostat, and the UV lighting provisions were on a timer. For the Living Rainforest they turned on at 08:00

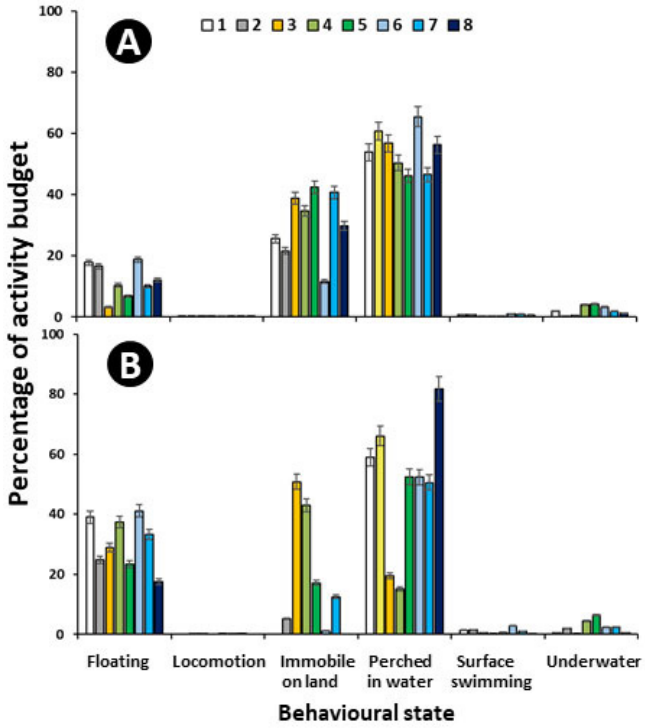

Figure 2. Activity budget for caimans housed at - **A.** Crocodiles of the World, **B.** The Living Rainforest, across all time periods (+/- standard error), Key: 1. Late night, 2. Dawn, 3. Morning, 4. Late morning, 5. Afternoon, 6. Evening, 7. Dusk, 8. Early night

h and off at 16:30 h, and for Crocodiles of the World turned on at 08:00 h and off at 17:00 h. Sizes of each respective zone were calculated based on exhibit blueprints.

Once data were collected, a relativised Electivity index (E*) was used to express the relative zone usage (Brereton, 2020). Electivity index values range between a maximum of 1 (overutilisation) and a minimum of -1 (underutilisation) of each zone. A value of 0 indicates use that an animal is neither overutilising nor underutilising a zone (i.e. the zone makes up 50 % of the exhibit, and the animal spends 50 % of its time in that area). The values were estimated from the formulae below taken from Vanderploeg & Scavia (1979), where ri refers to the observed use of a zone, pi refers to the expected use of a given zone (generated using the proportional size of the zone in comparison to the total available space). The letter n denotes the total number of zones or resources available to the study species.

$$E^* = (Wi - (1/n))/(Wi + (1/n)) \quad \text{where} \quad Wi = (ri/pi)/\sum(ri/pi)$$

Data analysis

Behavioural and enclosure use data were collated in a Microsoft Excel 2019© spreadsheet and statistical analyses undertaken using Minitab® 19. Prior to testing of behaviour and enclosure use data, normality of the residual errors was confirmed. Checks were also made using general linear models to determine whether the collection (as a random effect) had high standard deviation (SD) values. As the SD values were consistently low, collection was discounted as a random effect. Behavioural data were analysed using a series

Table 4. Zone sizes and their definitions for dwarf caiman enclosures at The Living Rainforest and Crocodiles of the World

The Living Rainforest	Definition
Zone 1 (5.92m²)	Open land with a basking area
Zone 2 (3.6m²)	Capture cage area
Zone 3 (1.04m²)	Shallow bank water area
Zone 4(0.37m²)	Deepwater with cover
Zone 5 (4.89m²)	Open water
Zone 6 (0.46m²)	Water/land combination with cover
Zone 7 (1.5m²)	Waterfall
Crocodiles of the World	**Definition**
Zone 1 (2.04m²)	Land with cover
Zone 2 (3.35m²)	Open land basking area
Zone 3 (5.48m²)	Open water with a shallow bank and perching areas
Zone 4 (0.82m²)	Open water with land access
Zone 5 (1.93m²)	Open water with access to the shallow bank
Zone 6 (1.55m²)	Open water with land access

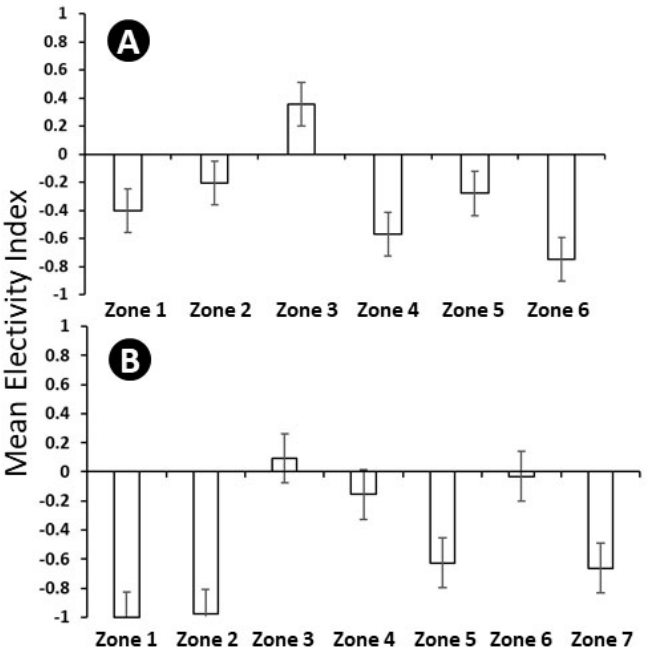

Figure 3. Mean Electivity Index values for enclosure zones - **A.** Crocodiles of the World, and **B.** The Living Rainforest, across all time periods (+/- SE)

of Poisson regressions (not general linear models), in which the variables of mean environmental temperature, collection and time of day (categorical) were inputted as predictors. For enclosure use data, regressions were run on the Electivity

Index values, with mean environmental temperature, collection and time of day (categorical) inputted as predictors. A Bonferroni correction factor was applied to account for the use of three predictors in the Poisson regression and regression models, meaning that the new, corrected alpha value was 0.016. The results of statistical modelling are presented in Supplementary material, available from the BHS website.

RESULTS

Behaviour

The activity budgets for the caimans housed at Crocodiles of the World and the Living Rainforest are shown in Fig. 2A & B respectively. Poisson regressions revealed that floating, immobile land behaviour, perched, surface swimming and underwater were impacted by time of day (See Table 1S, Supplementary Material). Collection and temperature were significant predictors for some, but not all tests.

Enclosure use

Electivity graphs were generated for Crocodiles of the World (Fig. 3A) and the Living Rainforest (Fig. 3B). Except for zone 3 in both collections, on average, all zones were underutilised.

The mean Electivity index was then calculated for each time period for each collection (Fig. 4A & B). This revealed that for both collections, zone use was not consistent throughout the day, with several zones in both collections being overutilised during specific time periods and underutilised during others. Time of day was a significant predictor of Electivity Index value for zones 3, 5, 6 and 7 (Table 2S, Supplementary Material).

DISCUSSION

Behaviour

The results of this study suggest that the behaviour and enclosure use of zoo-housed dwarf caimans can be affected by the time of day, collection, and temperature. Apart from locomotion, dwarf caimans from both collections showed differences in all other behaviours (Table 1S). Model predictive power ranged from as high as 36.37 % for immobile land behaviour, to as low as 2.68 % for terrestrial locomotion.

For both collections, immobile land behaviour and a range of water-based behaviours were observed at different times the day. Recent studies on Nile crocodiles have found comparable results, with the time of day and temperature being predicates of behaviour (Riley et al., 2021). A significant increase in immobile land behaviour during the daytime hours is expected, as this has been noted in previous studies on crocodilians (Verdale et al., 2006) and would be expected, as the dwarf caiman needs to thermoregulate during daylight hours (Lopes et al., 2021; Somaweera et al., 2020). Previous observations on broad-snouted caimans noted that they only left the water to bask in the sun during the hottest time of day (Prystupczuk et al., 2019). Little is known about the nocturnal behaviour of dwarf caimans (Campos & Magnusson, 2016). Our results indicate that the caimans display water-based

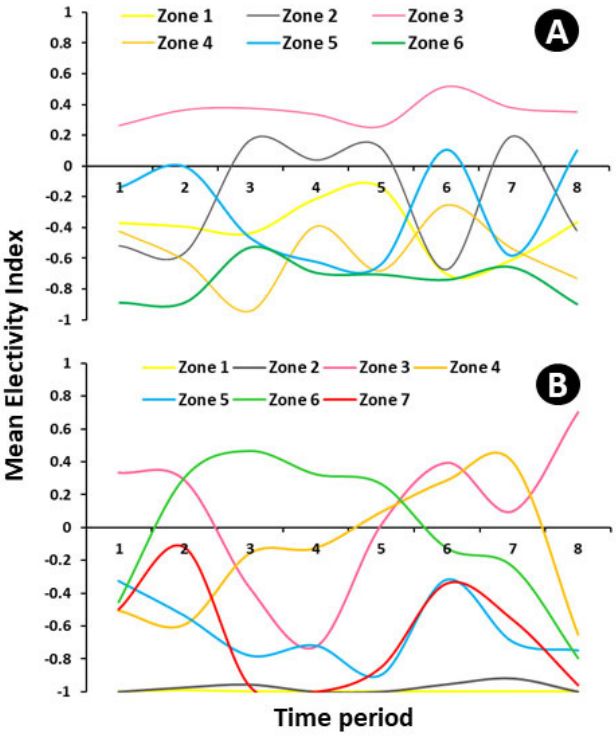

Figure 4. Mean Electivity Index in each time period for enclosure zones by time period - **A.** Crocodiles of the World, **B.** Living Rainforest, Key: 1. Late night, 2. Dawn, 3. Morning, 4. Late morning, 5. Afternoon, 6. Evening, 7. Dusk, 8. Early night

behaviours during the night; similar behaviour has been reported for captive broad-snouted caimans (Filogonio et al., 2014).

The differences between the two collections are particularly interesting, and they may reflect differences in husbandry, environmental conditions or individual background of the dwarf caiman (Marshall et al., 2016). The behaviour of zoo animals is significantly influenced by exhibit design. Increasing environmental complexity may help promote the expression of natural behaviours in zoo-housed dwarf caiman by providing different thermal zones or opportunities to express natural behaviours (Lawrence et al., 2021; Devlin & Ogle, 2022). Future research should focus on how these extraneous variables influence crocodilian behaviour in captivity.

Enclosure use

Except zones 2 and 4, regression models revealed significant predictors for the other enclosure zones. For exhibit zones 3, 5, 6 and 7, time was a significant predictor (Table 2S). The enclosure usage of the exhibits from both collections indicates a predominant use of water-based environment. Zones 3, 5, 6 and 7 are located around the margins of the water's edge in both exhibits. By using the margins, the dwarf caiman could both thermoregulate and have a sense of security by locating themselves within the water (Reber et al., 2021). This resource was apparently not scarce as agonistic interactions between individuals were not observed which could have been displayed if scarcity had resulted in competition between individuals.

In both enclosures, many zones were underutilised which may reflect the innate behaviour of dwarf caimans. Reber et al. (2021) compared the behavioural differences between two Alligatoridae species towards novel objects and found behavioural predispositional differences. They suggested that the differences were because of the species' life history. Spectacled caiman *caiman crocodilus* mothers do not protect the young, making them less bold. This safeguarding behaviour may be the case with the dwarf caiman, as they are the smallest crocodilian species and would be vulnerable to an array of natural predators in the wild (Campos et al., 2012). However, the regression outputs suggest that there are differences between collection management or temperature, which could explain why certain zones were underutilised at different times of day.

The ability of the model to account zone usage was low in the case of zone 3 (R^2 = 16.95 %) and zone 4 (R^2 = 26.94 %) where significant differences were found, suggesting other variables may be predictors for behaviour or enclosure use (Rose et al., 2021a). Among the variables in zoo studies, the presence of visitors is a factor to consider in zoo research (Hosey, 2005). The Visitor Effect (VE) has been regraded to have an overall negative effect on zoo species, associated with an increase in stereotypical and avoidance behaviours (Chiew et al., 2021). A recent study on dwarf caimans suggested they were affected by VE, as behavioural diversity was significantly reduced when visitors were present (Hamilton et al., 2022). This is an issue, as space use is a major component of crocodilians thermoregulatory behaviour, and if VE is influencing the behaviour then it may have consequences on welfare (Riley et al., 2021b). Future research should focus on how the negative impacts of VE on dwarf caiman behaviour can be mitigated.

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RESEARCH ARTICLE

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An information sign changes the way the public perceives exotic pond sliders *Trachemys scripta* in the Altrhein of Kehl (Germany)

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ABSTRACT - Invasive pond sliders *Trachemys scripta* have been released in thousands of fresh waters within Europe and reproduce in the southern states and even in warm areas of Germany. All member states of the European Union must have an action plan on how to fight this invasive species. The German action plan focusses on informing the public, but to date no study investigated the impact of such actions. Instead, NGOs setting up information signs, report that this has not stopped the release of exotic pond turtles but have provided no quantitative data to back this up. In 2019, we put up an information sign at the Althrein of Kehl, an oxbow lake where for the first time it had been shown that *T. scripta* is breeding in Germany. I interviewed people walking along the oxbow lake before the sign was put up in 2019, and again in 2022. Counts of exotic pond turtles still increased, but this was mainly due to an increased number of small pond turtles, while the number of very large turtles did not increase. This suggests that the increase in peak counts might be rather due to local reproduction than additional release. After the information sign was set up, more people responded that the presence of exotic pond turtles is problematic for nature conservation and animal welfare, that it is illegal to release them, and that they should be removed. This response was especially strong in people who had read the information sign. Independent of the information sign, most interviewed people stated that one should not release pond turtles into the wild, but bring them to animal shelters. While the data here only represent one single case study, it is the first study showing that putting up information signs can be effective in changing the attitudes of people. This indicates that investment in informing the public is worthwhile, but also that at the same time evaluations of the impact of the measures are important. National action plans should focus on a combination of informing the public and removing the exotic pond turtles, but also on providing keepers of these animals the option to leave the animals at an animal shelter instead of releasing them into the wild.

INTRODUCTION

Invasive species, i.e. exotic species introduced by humans that establish themselves outside their natural distribution range, are threatening native biodiversity worldwide (Geiger & Waitzmann, 1996; Wilson et al., 2009). One reptile species that has become invasive in Central and South America, Africa, Asia, and Europe is the north American slider *Trachemys scripta* (Böhm 2013; Standfuss et al., 2016; Mo, 2019). This species is now widely distributed in Europe, where it has been released by pet owners into thousands of fresh water habitats (Cadi et al., 2004; Prevot et al., 2007; Kopecký et al., 2013; Standfuss et al., 2016).

The European Union has identified *T. scripta* as an invasive species (European_Commission, 2016) against which the member states must take action to prohibit the import, breeding and release (European_Parliament, 2014). As environmental conditions differ between member states, the national action plans also differ. In southern European countries like Spain and France, where this species breeds and spreads very fast, removal of exotic pond turtles is one main action. In Germany, the main proposed action against *T. scripta* is to increase public awareness (StA_„Arten-_und_Biotopschutz“, 2018), which is also part of the general actions proposed by the European Union (European_Parliament, 2014). However, I am not aware of any study investigating the effects of such actions; one study exists that evaluated the impact of public awareness actions including signs to protect endangered reptiles (Başkale & Kaska, 2005). While signs not to release exotic pond turtles have been set up at some localities, for example in Munich by the Reptilienauffangstation (https://www.reptilienauffangstation.de), effects of these public awareness actions have not been measured. Instead, as the release of exotic pond turtles seems to have continued, there has been considerable frustration at the low efficacy of interventions (anonymous communication by different NGOs). However, it was never measured, only assumed, that releases continued and that public awareness actions were ineffective. So far, it is unknown whether such information signs influence the awareness of the public and lead to a decrease in the release of exotic pond turtles.

Here I present a case study conducted at an oxbow lake, the Altrhein of Kehl, the only location in Germany where successful reproduction of *T. scripta* has so far been reported (Schradin, 2020). I have personally monitored this population since 2016. In 2019, I set up an information sign for the public and I continued monitoring the population, to determine

whether it was still increasing. Before the information sign was set up, I conducted interviews with people walking along the oxbow lake, asking them about how they evaluate the presence of exotic pond turtles. These interviews were repeated in 2022. If the information sign had a positive impact in educating the public, I predicted that in 2022:

1. More people would regard the presence of exotic pond turtles as problematic,
2. There would be increased awareness of the problems posed for both nature conservation and animal welfare, and
3. There would be increased understanding of why the release of exotic pond turtles is illegal, and why they should not be released or removed from the oxbow lake.

MATERIALS & METHODS

Study area and study period

The study was conducted from 2016 to 2022 at the Altrhein of the city Kehl (48° 34'1.95" N, 7° 48'35.41" E), which is a 90 m long and 25–80 m wide oxbow lake formed over 100 years ago from the River Rhine. Kehl is in the Upper Rhine Valley, the warmest area of Germany. A community of six different species of exotic pond turtles exists in the Altrhein, the populations of which have been growing continuously from 2016 to 2020 (Schradin, 2020). Of these, *T. scripta* is the most common species, and both clutches and hatchlings have been found in several years, proving for the first time that the species reproduces successful in Germany (Schradin, 2020).

Monitoring

In the years 2016–2020, the population of exotic pond turtles was monitored on six to eight afternoons per year, during April to July. These data are already reported in a previous publication (Schradin, 2020), but included here as a baseline to determine whether further releases of exotic pond turtles occurred after the information sign was put up. Due to the Coronavirus lockdown in 2021, and the focus on getting more data from interviews in 2022, during these two years turtles were counted only during four afternoons per year. In all cases, observations were made using binoculars at five locations along the eastern shore of the lake, that were previously determined to have a high abundance of pond turtles. In addition, any pond turtle observed between these locations was recorded. For every individual, the carapace length was estimated to be in one of the following categories: 5 cm (hatchlings), 10 cm, 20 cm or 30 cm.

Installing an information sign

An information sign was created by me during a course I deliver at the Hector Akademie Kehl. In this course, I teach highly gifted school children 8–9 years old about ecology and nature conservation, using the exotic pond turtles as a case study. The information sign and its translation into English are shown in Figure 1. The sign provides information about the origins of the animals, how many species there are, that one species is invasive, and why releasing exotic pond turtles is a problem for nature conservation and animal welfare, and thus illegal. As the German city of Kehl is next to the French

Figure 1. **Top**: The information sign put up in July 2019 **Bottom**: English translation of the information sign

city of Strasbourg, it is visited by many French citizens and has several thousand French residents, and so the information sign also includes a summary in French (Fig. 1).

The city of Kehl installed the information sign at a bridge over the Altrhein next to the communal hospital on 16 July 2019. This spot was chosen as, from here people often observe the exotic pond turtles sun-basking on some branches of a dead tree lying in the water.

Interviews

Interviews were conducted during 5 afternoons in June and July 2019 and 4 afternoons in May and June 2022. In 2019, 28 people were interviewed, in 2022, 30 people, 13 of whom had read the information sign. Nobody had read the sign immediately before the interview, though many interviewees read the sign afterwards. Interviews were conducted by myself and by pupils from the Hector course that I was teaching. The pupils were trained in interview technique, and observed me performing the initial interview. I was present while students performed their interviews. As the interviews were anonymous, no ethical clearance was needed under French or German law and CNRS administration.

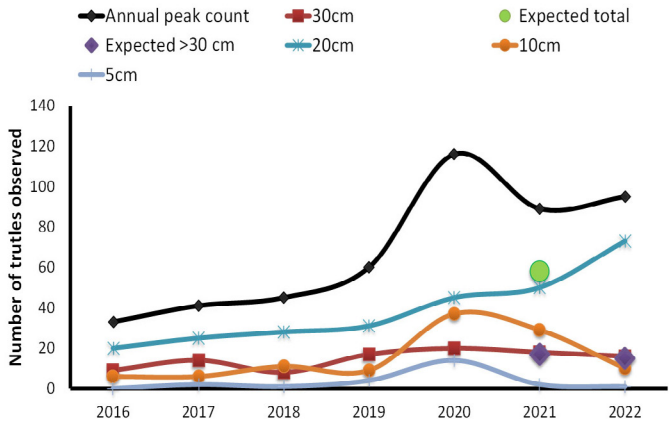


Figure 2. Annual peak count for all turtle species in the Althrein of Kehl. In black, total numbers. In red, the very large turtles which are most likely to be released, in other colours smaller turtles. After the count in 2020, a total of 58 turtles were removed, indicated by the green spot as expected value for 2021 (count 2020-58). Of these 58 turtles, one was very large; additionally, two large turtles caught on land were removed in 2021. The purple diamonds indicate the number of expected large turtles if no new ones were released.

First the pupils introduced themselves and explained that they are from the Hector Kinderakademie undertaking a survey about the exotic pond turtles. They made it clear that it was not a test, but the aim was to find out what people know about these animals. We only used the term 'pond turtles', without 'exotic' during the interviews. The questions were identical in both years. In 2022, we added a final question about whether the interviewee had read the information sign or not. After the interview was concluded the interviewees could ask questions and obtain more information, if they requested it.

The questions and the possible categorised answers (in brackets) were:

1. Do you find it problematic that there are pond turtles in the Altrhein? (yes / no).
2. Why do you think it is problematic? (not problematic / animal welfare / nature conservation / other / don't know).
3. Is it legal to release pond turtles here? (is illegal / is legal / don't know).
4. In your opinion, what should someone do who has a pond turtle as a pet but can no longer keep it, e.g. because they no longer have time or are moving away? (animal shelter / sell / keep/ release / don't know).
5. What should happen to the pond turtles in the Altrhein? (leave them and don't disturb / trap and remove / kill / don't know).

Data analysis

Data on pond turtle abundance are expressed as peak counts, the maximum number of live individuals observed in one survey afternoon of a particular year. Data from the interviews were analysed by comparing the ratios of correct answers vs. unknown plus wrong answers, comparing between years or between people who read / did not read the sign, using the Fisher`s Exact test.

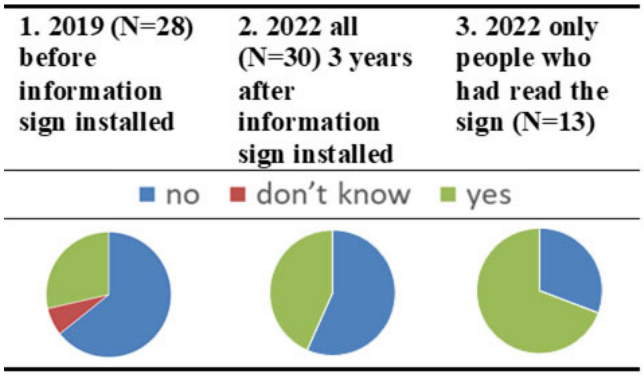
RESULTS

Turtle numbers

The annual peak counts increased continuously from 2016 to 2020, even after the information sign was put up (Fig. 2). After the count in 2020 (May–July), 58 turtles were trapped and removed by the end of July 2020. This influenced the expected pond turtle numbers for the following year: if there was no recruitment in turtle numbers, I expected the peak count in 2021 to be similar to that in 2020 minus 58; instead, it was higher in 2021 and further increased in 2022 (Fig. 2). The number of very large pond turtles (carapace size 30 cm) did not increase after the information sign was put up and was not higher than the expected peak number. However, the number of large pond turtles (carapace size 20 cm) increased, which could be due to medium sized pond turtles (carapace size 10 cm) growing, as their numbers decreased (Fig. 2).

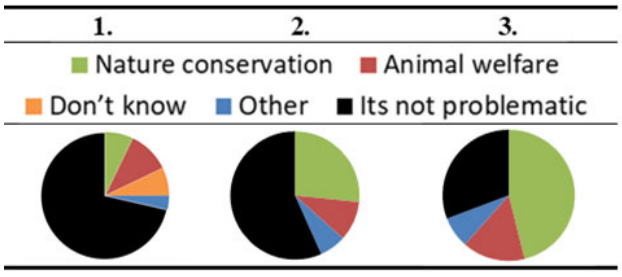
Responses to interview questions

1. Do you find it problematic that there are pond turtles in the Altrhein?



There was no statistical difference in the proportion of people reporting the pond turtles to be a problem or to be unproblematic between 2019 and 2022 (p=0.28). However, when only the people that had read the sign are considered then significantly more regarded the pond turtles as problematic, both compared to 2019 (p=0.02) and to the people in 2022 who had not read the sign (p=0.02).

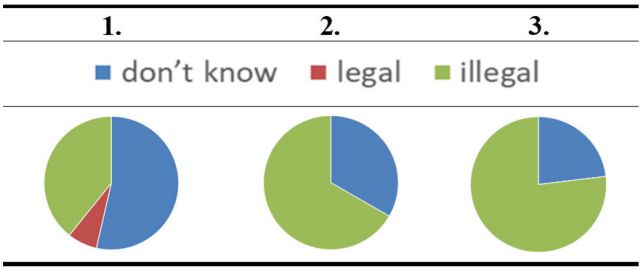
2. Why do you think it is problematic?



In 2022, no more people reported nature conservation and/ or animal welfare to be a problem than in 2019 (p=0.15). However, when only considering the people that had read the sign, then significantly more identified nature conservation and/or animal welfare to be the problem, both compared to

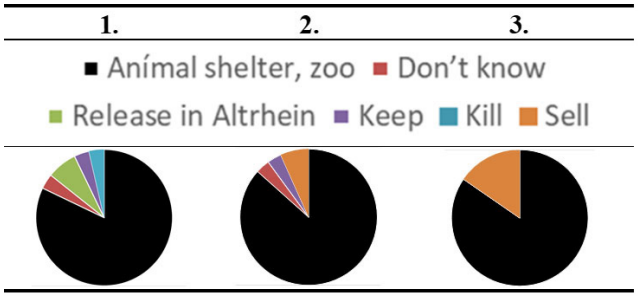
2019 (p=0.02) and to the people in 2022 who had not read the sign (p=0.01).

3. Is it legal to release pond turtles here?



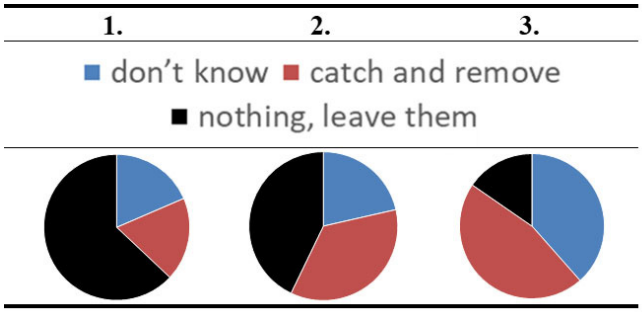
In 2022, more people assumed it to be illegal to release pond turtles than in 2019, though the difference was not significant (p=0.06). Considering only the people that had read the sign, the difference was significant (p=0.04), while the difference between people who had and who had not read the sign in 2022 was not statistically significant (p=0.44).

4. In your opinion, what should someone do who has a pond turtle as a pet but can no longer keep it, e.g. because they no longer have time or are moving away?



Asked what somebody should do with a pond turtle they have as a pet if they cannot keep it anymore, in both years most people suggested that the pet owner should bring the pond turtle to an animal shelter or a zoo. Only in 2019 did two people suggest releasing them in the Altrhein.

5. What should happen to the pond turtles in the Altrhein?



In 2022, no more people suggested trapping and removing the exotic pond turtles from the Altrhein than in 2019 (p=0.23). Considering only the people who had read the sign, the difference was more pronounced, but did not reach statistical significance (p=0.07).

DISCUSSION

In this case study, I found that the awareness of the general public to the problem of invasive *T. scripta* increased significantly three years after an information sign was put up. At the same time, there was no evidence that a large number of additional pond turtles were released. Therefore, this study gives the first empirical support that informing the public may be a suitable tool of the action plan against invasive exotic pond turtles.

The current study has several shortcomings reducing its general significance. First, the findings are based on a small sample of interviewees and evaluated a single sign at one location. How the results would apply at different localities and with different information signs is unknown. But this study indicates that it is worthwhile to try informing the public using such signs and then to evaluate whether it raises awareness and knowledge. Second, the monitoring of the population was done without individual identification or reliable population estimates, and was carried out during very few afternoons. As such, the data do not allow me to unequivocally conclude that no pond turtles were released after the sign was put up. Nevertheless, the data do not provide evidence that a large number of additional exotic pond turtles have been released. If the pond turtles could be identified individually, for example via photos and the use of artificial intelligence (AI), it would be possible to identify which animals are recruited from year to year into the population, and whether they are small (possibly due to reproduction) or large (possible releases).

Comparing responses to the interview questions before and three years after the information sign was put up indicated a clear change in public awareness: after the information sign was installed, many more people were aware that the exotic pond turtles represent a problem for nature conservation and/or animal welfare, that it is illegal to release them, and that it would be appropriate to remove them. Theoretically, this response could have been influenced by many co-factors and not the information sign alone, for example by reports in the local newspaper about the problem, or by people having had more time to walk along the Altrhein during the Coronavirus pandemic and then inform themselves at home about the exotic pond turtles they had seen. However, the difference in awareness was most obvious in people that are reported to have read the information sign, and these were also significantly better informed than people interviewed during the same period in 2022 that had not read it. Thus, the most parsimonious explanation is that reading the information sign increased the awareness about the problem of exotic pond turtles.

The answer to one question did not differ between years and was not dependent on whether or not the interviewees had read the information sign: what should somebody do with a pond turtle pet if they cannot keep it anymore? Few respondents suggested releasing a pet into the Altrhein, but the large majority suggested depositing them in a zoo or an animal shelter. The problem is that, usually, neither zoos nor animal shelters are interested in taking exotic pond turtles, as they do not have the facilities or resources to care for them

indefinitely. This can explain why ten thousands of these pets have been released within Europe, as there are few alternatives to place them elsewhere. This means that if the release of exotic pond turtles is to be reduced, then potential animal shelters need supporting. In Germany, private organisations exist, such as the Reptilienauffangstation in Munich (https://www.reptilienauffangstation.de), but these are very underfunded. National and regional authorities interested in reducing the number of releases should provide funding for such organisations, and funding to local animal shelters to provide facilities to keep exotic pond turtles.

The European Union demands that all member states should take action against invasive species (European_ Parliament, 2014) including *T. scripta* (European_ Commission, 2016). In Germany, it has been believed that the climate is too cold for released individuals to survive for long periods or to reproduce (Geiger & Waitzmann, 1996; Pieh & Laufer, 2006; Laufer, 2007; Nehring, 2016). The German action plan focusses on increasing public awareness (StA_„Arten-_und_Biotopschutz“, 2018), but this is neither enforced nor evaluated. A previous study, demonstrating successful reproduction of *T. scripta* at the Altrhein of Kehl (Schradin, 2020) and the ever increasing population size there (Fig. 2), makes it clear that public awareness has to be increased, and that additional actions are required (Teillac-Deschamps et al., 2009).

In conclusion, the information sign was successful in increasing public awareness. The national action plan (for Germany and all other countries) for *T. scripta* should include three main components:

1. Increasing public awareness by providing information, and evaluating the effectiveness of these actions,
2. Providing funding to animal shelters to take in exotic pond turtles, and
3. Removing *T. scripta* from natural habitats to avoid them establishing viable and spreading populations (Cadi et al., 2004; Sancho & Lacomba, 2016).

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SHORT COMMUNICATION

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Eastern green lizard *Lacerta viridis* predation on adult wall lizard *Podarcis muralis* - another reason for tail loss in small lacertids?

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The green or eastern green lizard, *Lacerta viridis* (Laurenti, 1768), is a large and adaptable species of Lacertidae from south-eastern Europe and a sister taxon of the western green lizard *Lacerta bilineata* (Daudin, 1802). In Romania, which sits in the core distribution area, *L. viridis* is abundant and widespread and occupies a broad range of forest edges, clearings, and scrub habitats in lowland and hilly areas, extending generally to 400 m in altitude and with isolated records at 1000 m or more (Cogalniceanu et al., 2013). It can colonise sites impacted by roads or forestry and some recent observations suggest it might be expanding its altitudinal range in parts of the country due to climate change. *Lacerta viridis* can share habitat with most other lizard species from Romania, yet adults are rarely seen in the immediate vicinity of smaller lizard species or even small individuals of the same species, reportedly because it can be aggressively territorial and can predate small reptiles, birds or mice (Nettman & Rykena, 1984). Equally, they exhibit niche partitioning and can coexist with other species due to differences in fine spatial scale habitat choice such as the observed *L. viridis* preference for areas with shrubs compared to *L. agilis* selecting for more open, grass-dominated areas within the same urban habitat (Heltai et al., 2015).

The diet of *L. bilineata* is composed primarily of invertebrates, but small vertebrates and especially juvenile lizards such as young slow worms *Anguis fragilis*, are occasionally reported as prey (Vacher & Wendling, 2019), yet these are considered rare events (Angelici et al., 1997). The diet of *L. viridis* has not been studied extensively but reviewing records from different European countries Nettman & Rykena (1984) describe it as varied, opportunist and based on invertebrates, particularly Coleoptera, but sometimes including young vertebrates, such as a juvenile *L. agilis* in Germany (Peters, 1970). In a recent study from Greece, *L. viridis* diet comprised mainly arthropods, with Coleoptera, Orthoptera and various insect larvae as the most common prey; larger prey size was consumed by adults compared to juveniles and adult males consumed harder and more diverse prey items than females (Sagonas et al., 2018). In Bulgaria, stomach content of 110 individuals of *L. viridis* comprised mainly Orthoptera, Coleoptera and Hymenoptera, with the highest number of prey items recorded in July–August and a somewhat different diet composition in May and September (Mollov et al., 2012). Finally, 41 road-killed *L. viridis* analysed from Romania had consumed primarily Orthoptera, Araneida and undetermined Coleoptera (Maier et al., 2020). Fragments of vegetation and inorganic elements were also recorded, but it was unclear if these might have been ingested accidentally during feeding. None of the studies of stomach contents identified vertebrate prey, suggesting that, as for *L. bilineata*, vertebrates are indeed rare in the diet of *L. viridis*.

On 24 April 2022 we recorded an instance of predation by an eastern green lizard on an adult wall lizard *Podarcis muralis* (Laurenti, 1768) on the edge of Semenic-Caras Gorges National Park (45° 15´36´´ N, 21° 57´16´´ E, 431 m a.s.l.), south-western Romania, in a clearing created by a clay embankment for an unpaved forestry road in mixed deciduous forest with *Betula* spp., *Fagus sylvatica* and some *Pinus nigra*. This is a common habitat type in the region and where both species are regularly found, often in sunny patches with bramble *Rubus* spp. vegetation, although wall lizards preferentially select such areas if the substrate is rocky. An adult male *L. viridis* was observed at 13:50 h, in sunny weather, 22 °C, as it was holding in its mouth a live adult male *P. muralis* which had a large part of its tail missing, with a fresh and still bleeding wound. The wall lizard had grabbed part of the head of the green lizard in defense (Fig. 1A) but both lizards were still and were observed from a distance for over 2 minutes until the wall lizard violently shook its own head and body for about 4 seconds, and then released the snout of the green lizard, followed by 2 minutes of rest and then another 3–4 seconds of shaking and biting the head of the green lizard. The *L. viridis* male released, then grabbed the tail stump and severed about 5 mm of the remaining wall lizard tail and swallowed it (Fig. 1B; Fig. 2); this allowed the wall lizard to escape to a nearby hole in the ground. The green lizard followed it rapidly into the hole only to reappear after a few seconds, with its head emerging from the hole. After about 5 minutes it went back into the burrow and neither lizard re-emerged in the following 15 minutes, at which time the observation was stopped. It is unclear if the green lizard managed to corner, kill, and consume the wall lizard inside the burrow but it seems plausible. Even if the wall lizard was not killed, the *L. viridis* male certainly consumed a part and probably its entire tail. This is a rare observation but instances of predation by this species, and lizards more generally, are inherently uncommon and, in the absence of additional studies, it remains unknown how frequent or not is such predatory behavior in areas where *L. viridis* coexist with other lizard species. The observed *L. viridis* had several visible parasites attached (tick nymphs) and this might be relevant as

Figure 1. A. and **B.** Adult male *Lacerta viridis* predation of adult *Podarcis muralis*

large or ectoparasite-free adult males were shown to be faster explorers than smaller or parasitised males during behavioral trials (Bajer et al., 2015). Both lizard species involved in this predation event are habitat generalists and opportunists, with wide spatial niche breadth (Vacheva et al., 2020), and as such the opportunities for direct interaction are probably higher than for other species. Sites where green lizards appear to be expanding into new areas due to habitat change or climate warming, where they might encroach the territory of other lizard species, could present interesting future research focus. Some European lizard populations have high percentages of individuals with missing or regenerating tails, something typically blamed on avian predators. In habitat where they coexist, predation from adult green lizards, particularly males, could represent another reason for tail loss.

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Figure 2. Adult male *Lacerta viridis* ingesting the tail stump of the *Podarcis muralis*. Several tick nymphs are visible on the green lizard head and body. Notice that plant matter (a dry leaf) was accidentally ingested during feeding.

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SHORT COMMUNICATION

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Amelanism in the Nicaraguan slider turtle *Trachemys grayi* in Costa Rica

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Species and subspecies definitions in *Trachemys* have had a complicated taxonomic history but *Trachemys grayi* was recovered by Parham et al. (2013) and endorsed by other authorities (e.g. Seidel & Ernst, 2017). *Trachemys grayi* is a large turtle with a relatively flattened carapace of 40 cm length in females, although males are smaller (Acuña-Mesén, 1998; Leenders, 2019). It has a greenish brown shell, with eye-shaped patterns formed by yellow, orange and black rounded lines (Acuña-Mesén, 1998). The plastron is light yellow, with complex dark patterns that fade with age, and the whole body gets darker as individuals age (Seidel & Ernst, 2017). The head, neck, limbs and tail are dark grey with yellow or olive stripes, including a prominent broad postorbital yellow or orange one on each side of the head (Acuña-Mesén, 1998). This stripe does not come into contact with the eyes. The eyes have round pupils with a yellow iris that has a dark horizontal stripe through its centre (Leenders, 2019). Humans hunt Nicaraguan sliders for meat and collect their eggs to eat; this has a negative impact on their populations (Mora & Ugalde, 1991).

A striking colour anomaly in vertebrates is the occurrence of white individuals. In many cases this is a result of albinism, a condition characterised by lack of retinal and integumentary pigmentation that results in white or pinkish skin and red eyes (Lamoreux et al., 2010). However, some animals are white due to amelanism; they also have red eyes due to the absence of melanin but have produced other pigments so that other colours may be present (Borteiro et al., 2021). In the literature, there are clear cases of confusion between these two anomalies (Borteiro et al., 2021). Here we present a case of amelanism in the Nicaraguan slider turtle at the Caño Negro wetlands in the northern plains of Costa Rica.

Caño Negro Wildlife Refuge (10° 53’ N, 84° 47’ W; 40 m a.s.l.), extends over 9,969 ha of a series of marshy, lacustrine and river-type wetlands, both temporary and permanent, with different types of plant associations (SINAC-ACAHN, 2013). Tour Pantanal is a private enterprise that offers ecotourism services and travel in Caño Negro. They informed us about a freshwater turtle incubation project, run by the ULIMA association, which over several years released thousands of turtles at Caño Negro (Table 1). They also sold many turtles to help support the low income families who had participated in the turtle project. Tour Pantanal maintains five white turtles in captivity that we photographed in November 2020 (Fig. 1).

Table 1. Estimated average clutches transferred from the field, number of turtles hatched, released and sold, and amelanistic turtles, managed during the period 1990-2013 by the ULIMA association at Caño Negro, Costa Rica

Year	Average no. of clutches	No. hatched	No. Released 30 %	No. Sold 70 %	No. amelanistic
1990	17	354	70	284	0
1994	15	305	91	214	0
1999	35	7,380	1,702	5,678	0
2000	784	16,468	3,800	12,668	1 female
2001	4	7,800	1,800	6,000	0
2002	8	16,900	3,900	13,000	1 male
2003	2,316	48,639	11,399	37,240	2 females
2004	1,735	36,445	10,791	25,089	1 female
2005				97	0
2006	185	3,893	1,168	2,725	0
2007	824	17,303	3,993	13,310	0
2008	479	10,067	1,292	7,744	0
2012	80	16,810	5,043	11,767	0
2013	486	10,198	10,198	0	0

Source: adapted from SINAC-ACAHN, 2013 and adjusted from data provided by Tour Pantanal

Colour aberrations in turtles range from the near-complete lack of color in albinos to very dark in melanistic individuals, but also other variations such as yellow, without yellow, and golden yellow ones (Jubber & Leyendecker, 2016; Cavalcante & Bruni, 2018; Devkota et al., 2020). We did not find any previously reported cases of amelanism or any other abnormal coloration in *T. grayi* under its current or previous

Figure 1. One of five *Trachemys grayi* with amelanism kept in captivity in Caño Negro, Alajuela, Costa Rica

scientific names. White turtles suffer higher mortality rates due to their lack of camouflage, greater disease susceptibility, and other health problems related to abnormal retinal pathways to the brain (Perrault & Coppenrath, 2019).

Although normally infrequent, the incidence of anomalous colouration in wild populations can increase due to environmental stress or inbreeding in isolated populations (Espinal et al., 2016). Colour anomalies may therefore signal issues that have negative impacts on turtle fitness (Krecsák, 2008). It would therefore be useful for researchers to continue collecting data on the incidence of colour anomalies at Caño Negro.

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SHORT COMMUNICATION

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New record of the green vine snake *Oxybelis fulgidus* in the Cerrado of Mato Grosso, central Brazil: A significant shift from forest to savannah?

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The Cerrado of Brazil is considered to be one of the world's biodiversity hotspot and one of the largest and most threatened tropical savannah in the world (Klink & Machado, 2005; Colli et al., 2020). Regarding snake species, owing to the fact that the Cerrado has contact with four other biomes (the Amazon Rain Forest, Atlantic Forest, Caatinga and Pantanal) it is the richest ecoregion in South America with 222 species of snakes (Guedes et al., 2017). Likewise, the Amazon is known as the world's largest tropical rain forest. It harbours approximately 150 species of snakes, with endemic and widespread species co-occurring (Fraga et al., 2013). The transition zone between the Cerrado and the Amazon forest ecoregions (CAT) extends over more than 6,000 km, crossing from the central-west to north-eastern Brazil, and is the largest savannah-forest transition on Earth (Torello-Raventos et al., 2013). There have been relatively few studies on snake assemblages in the CAT but these have found either a predominance of Amazonian species (Carvalho, 2006) or balanced number of species from Cerrado and Amazonia (Pinheiro et al., 2015). Snake species composition and distribution in savannah-forest zones have been explained as a matter of species-specific habitat preferences determining savannah species penetration within degraded forests (Freedman et al., 2009).

Herein we present a new record of the arboreal colubrid snake *Oxybelis fulgidus* (Daudin, 1803) at the limit of its distribution in Brazil. We collected a specimen (field tag AS018) (Fig. 1) on 2 October 2021 in Nova Xavantina municipality, Mato Grosso state, Brazil (14° 50'7.39" S, 52° 29'57.36" W, 420 m a.s.l.) (Fig. 2). It was a large female (SVL 1345 mm, TL 680 mm) found during an incidental encounter at 15:28 h inside a gallery forest and it was stretched out, without moving, on a tree branch approximately 1 m from the ground. We made a video at that moment of the find and this has been deposited at Fonoteca Neotropical Jacques Vielliard (see FONTECA, 2021). The species shows a broad distribution from Mexico to South American in forest habitats (Peters & Orejas-Miranda, 1970) and is considered as an opportunistic predator of birds and lizards, using both active hunting and sit-and-wait ambush behaviour (Scartozzoni et al., 2009). In South America, the species has been recorded in tropical forests, within fewer marginal records in the "dry diagonal" (Nogueira et al., 2019).

Figure 1. *Oxybelis fulgidus* (field tag AS0018) from Nova Xavantina municipality, Mato Grosso state, Brazil

The area under study is situated close to the north-west border of the Cerrado with presence of Cerrado sensu stricto (savannah woodland), Campo (grasslands), Cerradão (dense savannah woodland) and gallery forest (Marimon et al., 1998; Marimon-Junior & Haridasan, 2005; Marimon et al., 2010). The climate is Aw of the Köppen classification, with annual precipitation from 1300 to 1500 mm and a mean monthly temperature of 25 °C. The gallery forests at the boundaries of the CAT harbour several plant species in common with Amazon Forest (Marimon et al., 2010). The new locality is about 420 km north-west of the closest previous record site (Diamantino, Mato Grosso state) and 660 km from the closest north-east record site (Palmas, Tocantins state) (Nogueira et al., 2019). Most of the records in the South American "dry diagonal" lie in the CAT located in the central and north-east areas of Brazil (Marques et al., 2019). This ecosystem is situated in the transition area of southern Amazonia and comprises a mixture of savannahs and forests with an approximated area of 152,180 km^2 in central and northern of Brazil (Marques et al., 2019).

The occurrence of *Oxybelis fulgidus* in this ecosystem could be related to the natural expansion of the Amazon Rain Forest into the savannahs of the Cerrado during the Holocene

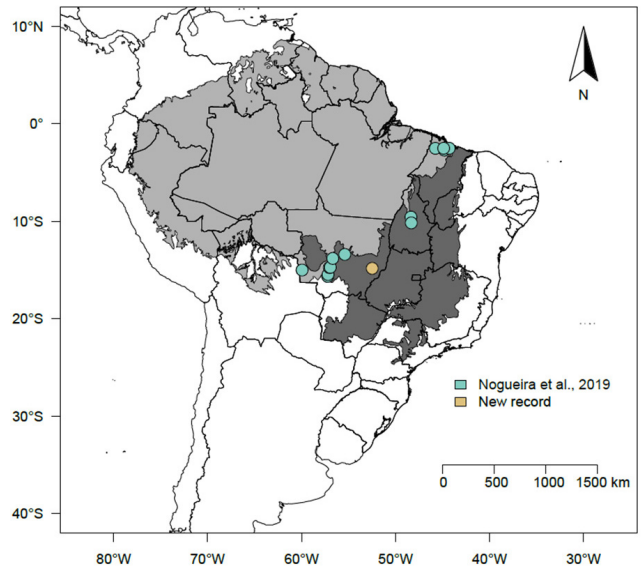

Figure 2. Previous records of *Oxybelis fulgidus* inside the limits of Cerrado within the range of Cerrado-Amazon ecotone (Marques et al., 2019; Nogueira et al., 2019) and the new record reported in this paper. Light grey is the limit of the Amazon Rain Forest and dark grey is the limit of Cerrado.

(Marques et al., 2019). Other forest snake species show similar patterns of distribution in the CAT, such as coral snake *Micrurus surinamensis* (Cuvier, 1817), which occurs mainly in the Amazon Rain Forest but has also been recorded in areas of the Cerrado (Morais et al., 2011). This suggests that the CAT seems to be a suitable area for occupation by species from both savannahs and forests habitats.

It should be noted that in the past the boundaries of CAT area were wrongly interpreted by the Brazilian Institute of Geography and Statistics (IBGE), being larger than expected (Marques et al., 2019). Currently, the region is suffering from extensive deforestation and is referred to as the "Arc of deforestation" (Marques et al., 2019). The current rate of deforestation is preventing the natural expansion of forests into the Cerrado savannahs due the convergence of forests habitats into large areas for cattle raising, logging and agriculture (Marques et al., 2019). Yet, the snake recorded herein was found in a fragment of gallery forest surrounded by large agricultural fields. Our record highlights the importance of CAT as a domain which supports species from both savannahs and forests habitats.

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Deaths of overwintering *Nanorana* spp. tadpoles due to desiccation during check dam maintenance in a western Himalayan stream, India

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All amphibian life stages may be directly or indirectly affected by drought, although many amphibians exhibit plasticity in the duration of tadpole development, in response to drought conditions, with trade-offs such as reduced body condition, survival etc. (Cayuela et al., 2016; Yeung, 2021). Droughts may be induced by human activity such as when check dams are drained for maintenance purposes. Check dams are important civil engineering structures built for soil conservation, groundwater recharging and water extraction, and are very common in Indian streams (Agoramoorthy et al., 2008). Here, we report the first observations of the deaths of overwintering tadpoles of two species of *Nanorana* during check dam maintenance. It is known that check dams have negative impacts on stream channel morphology (Fortugno et al., 2017), water quality, velocity, and substrate fineness (Kang & Kazama, 2012), cause loss of larval microhabitat (Thomas et al., 2019), and influence the breeding ecology of frogs (Lind et al., 1996). However the influence of check dam maintenance has been little documented.

Our observations concern two dicroglossid frog species. The small paa frog *Nanorana minica* (Dubois, 1975) that is associated with montane subtropical forests and streams distributed in Nepal, Uttarakhand and Himachal Pradesh at 1000–2400 m a.s.l. (Ohler et al., 2004; Bhattarai et al., 2020; Frost, 2021). It is listed as Vulnerable on the IUCN Red List and major conservation threats are waterway management and loss of habitat through forest clearance (Ohler et al., 2004). The other species is the Himalaya paa frog *Nanorana vicina* (Dubois, 1976) that is distributed in the Himalayan front of India, north-central Pakistan from Kashmir, Himachal Pradesh to Punjab, and Uttarakhand ranging from 2000–3000 m a.s.l. and is associated with montane streams, springs, fountains and other running water within open forest and grassland habitats (Ohler & Dutta, 2004; Frost 2021). Apart from morphological descriptions and other natural history records, little is known of the larval ecology of either species (Das & Dutta, 2007; Sircar, 2010; Banerjee et al., 2020; Gill et al., 2020; Jithin, 2021). Jithin (2021) reported overwintering tadpoles of *N. minica* and *N. vicina* from the western Himalaya, India. The overwintering tadpoles feed on periphyton growing on check dam walls, bedrocks, boulders, cobbles, leaf litter surface, submerged logs in the pool; humus and animal carcasses; the check dam pools also offer a temperature gradient (Boix-Fayos et al., 2007: Banerjee et al., 2020; Jithin, 2021).

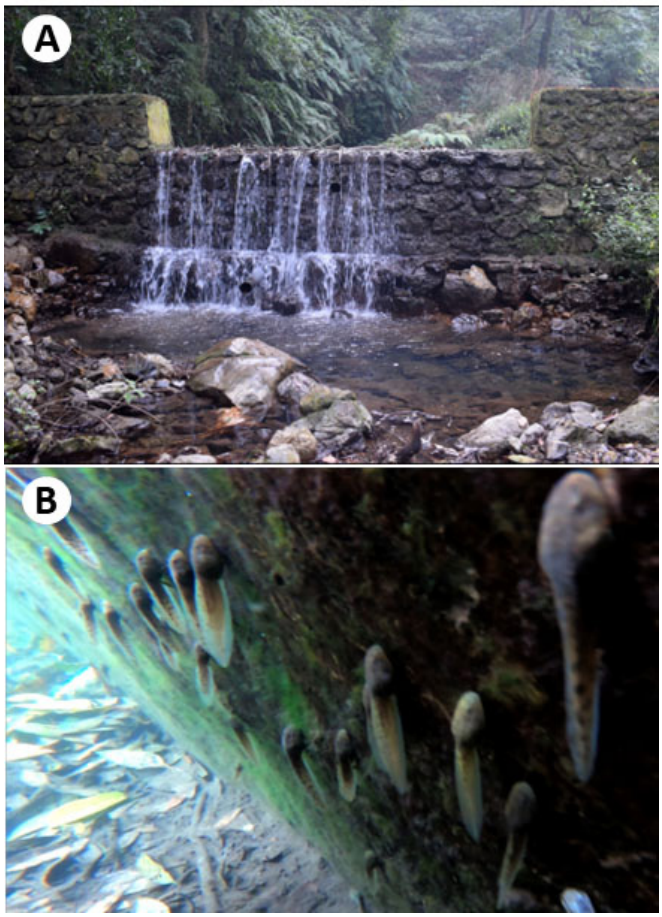

Figure 1. **A.** Example of a check dam pool with wider broad-crested weir, and **B.** Overwintering tadpoles feeding on the periphytons growing on the check dam walls in the Mussoorie Wildlife Sanctuary, Uttarakhand, India

We recorded tadpole mortality at two check dam maintenance events along the Dhobhighat (Ringali Gad) stream, flowing through the Mussoorie Wildlife Sanctuary. This second-order stream flows through private resorts,

agricultural fields, and human habitations and is dammed in several places creating large check dam pool habitats. These check dams were built during the 1960s and are presently used for torrent control, water recharge and extracting drinking water for Mussoorie town. The check dams are managed by the Uttarakhand Jal Sansthan and a pump house is situated in the sanctuary (Management Plan, 2010). Concrete or stone-mortar walls are present with wider broad-crested weirs in the check dam pools. Filtered water goes directly to a pump house via long underground pipes from the check dams. The dams are used by overwintering tadpoles, fish: snow trout (*Schizothorax richardsonii*; Cyprinidae), stone loach (*Schistura rupecula*; Nemacheilidae) and other aquatic organisms (Management Plan 2010; Jithin, 2021).

Annual maintenance of check dams is essential. Water is usually diverted from the original channel making the area dry apart from a few shallow pools that act as refugia for aquatic organisms. Then broken walls are renovated, pipes repaired and silt removed, after which the bottom is cleaned and pebbles and small boulders from other natural pools are deposited in the dam pool bed to facilitate water filtration. These maintenance activities usually take 2–3 days to finish and by this time, tadpoles, fish and crabs are either dead or are predated by birds. During one such maintenance event on 16 March 2021 at 12:32 h in a check dam pool (30° 28' 2.06" N, 78° 01'45.19" E, 1645 m a.s.l.) we quantified the death of overwintering tadpoles. There were a total of ~150 tadpoles in an area of 2.5 m^2 (Fig. 2 and Fig. 3D) of which 32 were found dead (21.3 %) and 61 alive, but stuck in the silt (40.7 %).

We collected a representative sample of the dead tadpoles and deposited it at the Wildlife Institute of India Herpetofauna Collection (N=14, WIIAD T-175-188). The size of these tadpoles ranged from 25.32 to 63.4 mm (total length) and stages from 26 to 36 (Gosner, 1960). We could not quantify the deaths of two species separately as field identification is difficult (Jithin, 2021). Apart from the mortality of tadpoles, newly laid egg strings of Himalayan toad *Duttaphrynus himalayanus* were also found in the dried stream bed.

On 20 March 2021 at 17:00 h, we recorded another maintenance event in another stream portion (30° 27'57.90" N; 78° 01'46.40" E; 1751 m a.s.l.) where the death of tadpoles resulted from deposition of silt, which was dug out while cleaning a cement-lined tank built near the stream for water filtration (Fig. 3A-C, BHS video, 2022). The silt flow made the water downstream turbid; we measured pH and Total Dissolved Solids (TDS) using a portable pH meter (Aquasole Digital Pen Type Meter, AM-P-PH) and a portable EC/TDS meter (Aquasole Digital Pen Type Meter, AM-P-EC) and Dissolved Oxygen (DO) levels using the modified iodometric (Winkler) method (Jithin, 2021). These values were compared with a dataset we generated from other check dams and natural pools in the same stream (Jithin, 2021). All parameters were above mean [± 95 % CI] values for the month of March (pH: 8.7 [8.356±0.063], DO: 10.145 [7.736±0.471] mg/L), but TDS showed a very high value (275 ppm), beyond the measurement range recorded (205–228 ppm) in the whole month from similar check dam pools.

Figure 2. Tadpole mortality during check dam maintenance and renovation - **A.** The pool before maintenance when the water flow had been stopped and diverted, circles showing close-up images of dead overwintering tadpoles, **B.** The check dam after maintenance

Figure 3. Tadpole mortalities in a stream clogged with silt from the maintenance of a check dam - **A.** Portion of stream clogged with silt deposition, **B.** The cement-lined tank from where the silt was removed, **C.** White circles showing dead overwintering tadpoles of *Nanorana* spp. embedded in silt, **D.** Image showing the size range among dead overwintering tadpoles of *Nanorana* spp. and a dead stone loach *Schistura rupecula* fish at bottom centre of the photograph

Considering the large-scale killing of overwintering tadpoles in check dam pools during maintenance, we discourage complete draining of pools as this could lead to the loss of refuge areas for the tadpoles. A reasonable water volume should be left in the pools in such cases. When complete draining operations are required during maintenance, aquatic organisms (including tadpoles, fish, crabs etc.) must be carefully transferred to nearby pools to avoid large-scale deaths due to desiccation. A standard protocol for this procedure should be developed in collaboration with the Forest Department and Water Supply Department.

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SHORT COMMUNICATION

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Dark gaping - presumed independent origin for a remarkable warning signal in four Neotropical snake species

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Mouth gaping is a widespread warning signal displayed by a great number of vertebrate species, from fish to mammals (Tickell, 1984; Brantley et al., 1994; Moon-Fanelli, 2011; Toledo et al., 2011). This behaviour is widespread among reptiles, especially among lizards and snakes (Hertz et al., 1982; Bramble et al., 1984; Greene, 1988; Combrink et al., 2016). In snakes, it usually is an effective warning signal, as cornered snakes may easily strike at, and even bite the potential predator (e.g. Sazima, 2000). However, some species mouth gape as a bluff and do not bite the opponent (Greene, 1988). In several colubroid snake lineages this mouth gaping may be either a pre-bite phase such as a threat, or a bluff (Greene, 1988; Martins et al., 2008). Mouth gaping is likely enough to discourage the attack of some but not all predators. Consequently, it is often a step within a more complex behavioural repertoire (e.g. Burghardt & Greene, 1988; Marques & Sazima, 2004). In addition, such behaviour may be complemented by other signals, such as tongue hanging, hissing, or teeth displaying (Burghardt & Greene, 1988) which possibly emphasise the threat posture.

Here we briefly describe and illustrate the mouth gaping behaviour in four species of Neotropical snakes that have dark oral lining, which indicates that this feature likely increases the threat signal of this display. Furthermore we present a simplified phylogenetic tree to show the distribution of mouth gaping among related snake lineages.

We gathered the data used herein during photography sessions of *Leptophis ahaetulla*, *Oxybelis aeneus*, *Chlorosoma laticeps* and *Tomodon dorsatus* individuals. We elicited this defensive behaviour by approaching our hand and occasionally gently touching the body of the snake. Mouth gaping is already recorded for *L. ahaetulla*, *O. aeneus*, and *T. dorsatus* (Greene, 1988; Martins et al., 2008; Marques et al., 2019) but until recently there was no information about this behaviour in *C. laticeps*. This latter snake is known only from the type specimen and a few preserved ones (Zaher et al., 2008). While handling an individual collected in the Atlantic Forest at the Sooretama Reserve in the state of Espírito Santo, we took a picture of mouth gaping by *C. laticeps* and presented it in a field guide (Marques et al., 2019). A simplified phylogenetic tree based on Zaher et al. (2009), Grazziotin et al. (2012), Jadin et al. (2014, 2019), Klaczko et al. (2014), Montigelli et al. (2019), and Albuquerque et al. (2022) was used to show the distribution of dark gape behaviour among related lineages of Neotropical snakes.

All four species cited below displayed a sigmoid (S-shaped) posture of the anterior portion of the body, gaped the mouths with head facing our hand, and stayed in this posture for a few seconds (Fig. 1). The simplified phylogenetic tree (Fig. 2) shows that dark gape arose independently, appearing in two lineages of Colubridae and Dipsadidae. All species reported here show a darkening of the inner portion of the mouth with almost black mucosa, except *L. ahaetulla*, in which the dark pigmentation surrounds the larynx and trachea. However, in this latter species, the dark lining is restricted to a few individuals, the remaining ones having light mouth lining.

Among colubrid snakes, mouth gaping is a widespread defensive behaviour, with the great majority of species displaying light oral mucosa (Greene, 1988). Light coloured mouth lining occurs in snakes phylogenetically related to *Leptophis* and *Oxybelis* with its sister group *Chironius* (Martins et al., 2008). The presence of light or dark pigment in the oral mucosa of *L. ahaetulla* together with the fact that related species have light oral mucosa and display mouth gaping suggests that the acquisition of dark pigment is evolutionarily more recent than this defensive gaping. Thus, it is possible that dark pigment in *Leptophis* evolved to enhance the warning effect of mouth gaping. A similar scenario (warning enhancement) could have occurred among *O. aeneus*, as related snakes that display mouth gaping (e.g., all *Chironius* species and most individuals of *Leptophis*) have light oral mucosa. The congener, *O. fulgidus*, displays mouth gaping but has no dark pigments in the oral mucosa (Mendes et al., 2019), which indicates that the dark mucosa in *O. aeneus* is likely a derived character that appeared after mouth gaping evolved as a warning signal.

Among dipsadid snakes, the independent origin of mouth gaping associated with blackish oral mucosa is even more evident when considering the phylogenetic relationships among the species within this clade. Mouth gaping appears to be uncommon among dipsadid species (Greene, 1988; Martins et al., 2008). There is no record of this defensive behaviour among the great majority of *Philodryas* species and *Xenoxybelis*, which are closely related to *Chlorosoma*. An exception is *P. aestiva*, which was recorded mouth gaping while attacked by birds in the wild (Banci et al., 2018). However, this latter species has light oral mucosa similar to its congeners and *Xenoxybelis*. *Chlorosoma* includes three species, *C. dunupyana*, *C. laticeps* and *C. viridissimum* (Melo-Sampaio et al., 2021). The last two species are green and both

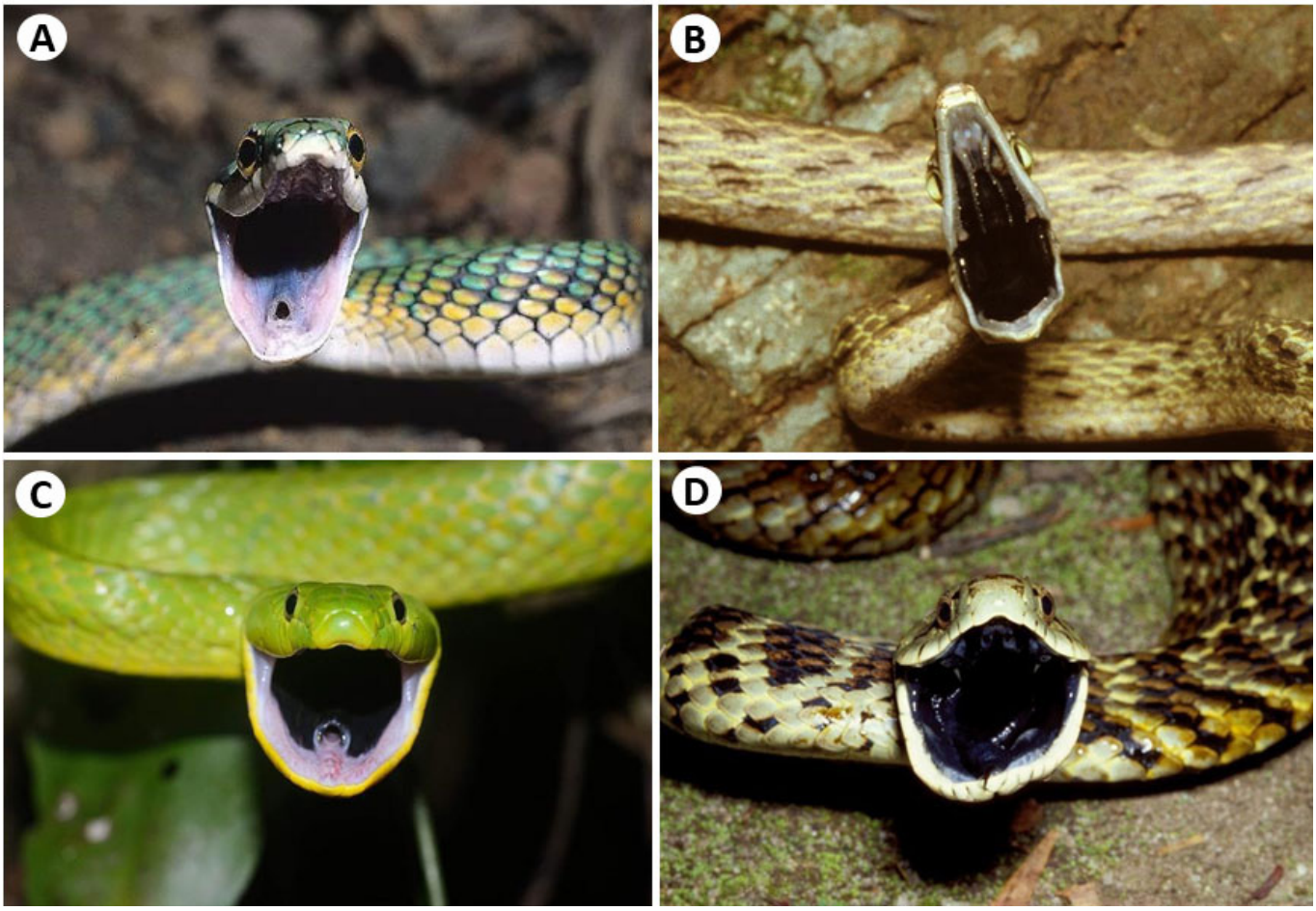

Figure 1. Dark mouth gaping and S-shaped posture recorded for - **A.** *Leptophis ahaetulla*, **B.** *Oxybelis aeneus*, **C.** *Chlorosoma laticeps*, and **D.** *Tomodon dorsatus*

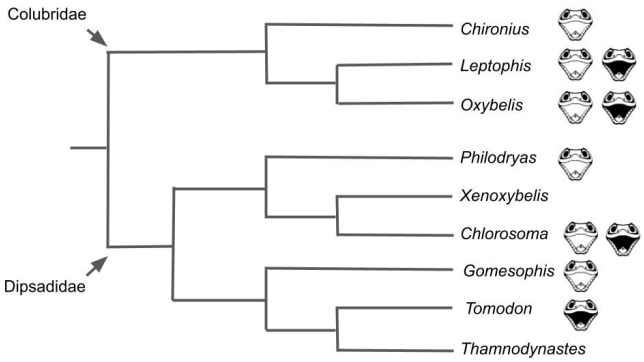

Figure 2. Simplified phylogenetic tree showing the position of the genera in which mouth gaping is recorded including those with light and dark mouth lining. Constructed trees based on phylogenies published in Zaher et al. (2009), Grazziotin et al. (2012), Jadin et al. (2014, 2019), Klaczko et al. (2014), Montigelli et al. (2019), and Albuquerque et al. (2022).

display lateral compression of the body, S-coil posture and mouth gaping (Marques et al., 1999, this study) but only *C. laticeps* has a dark oral mucosa. Thus, dark mucosa appears to be a derived condition in *C. laticeps*, as it is absent in other *Chlorosoma* and all related *Philodryas* and *Xenoxybelis* species (Zaher et al., 2008; Arredondo et al., 2020).

Tomodon dorsatus, which belongs in the Tachymenini, shares the dark oral mucosa and gaping display with other congeners (Harvey & Muñoz, 2004) and *Calamodontophis*, apparently a sister genus (Franco et al., 2006). The other two related genera in the Tachymenini, *Gomesophis* and *Thamnodynastes*, have light oral mucosa and at least the first displays mouth gaping (Menezes, 2017). Thus, the dark mouth lining and mouth gaping display appear to have arisen at least once (*Tomodon* + *Calamodontophis*) within this snake lineage.

The relationship between mouth gaping and dark oral mucosa is not restricted to these Neotropical species, as snakes from other lineages and other regions of the world gape with a dark mouth as well. Two unrelated examples are the North American rough green snake *Opheodrys aestiva* (Colubridae) and the African black mamba *Dendroaspis polylepis* (Elapidae) (Pitman, 1965; Walley & Plummer, 2000; Muller et al., 2012), which supports the suggestion that the black mouth lining is a feature that increases the warning signal of mouth gaping in snakes.

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On the geographic distribution of the caecilians *Caecilia goweri* and *Caecilia occidentalis*

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In the mid-1960s, Edward H. Taylor visited Colombia, hoping to examine the available caecilians (Amphibia: Gymnophonia) deposited at Museo La Salle (MLS), Universidad de La Salle, Bogotá, which, at the time, was the only herpetological collection in the country. During his visit, he was able to examine various Gymnophiona (*Caecilia*, *Microcaecilia*, *Oscaecilia*, *Siphonops* and *Typhlonectes*) to include them in his book 'The Caecilians of the World', published in 1968. Among the specimens that Taylor examined was a series (MLS 27, 45–46, 48–53) from Altiplano de Medellín, Antioquia, Colombia, that he identified as *Caecilia pachynema*, most likely based on colour pattern.

In 1998, the junior author undertook a taxonomic revision of the caecilians of Colombia, which required that he examined the material housed at MLS, which Taylor had studied during his brief stay in Bogotá. Prior to Lynch (2000), MLS 45 (Fig. 1) had been identified by Taylor as *C. pachynema*, but Lynch (2000) begged to differ, given its high counts of 179 primary grooves and 14 secondary grooves, which he considered to be diagnostic of *Caecilia occidentalis* instead. Thus, MLS 45 represented the first record of *C. occidentalis* from departamento Antioquia, and its distribution range was increased 428 km to the north (in a straight line), from Pance, Valle del Cauca (UVC 6567), to Yarumal, Antioquia. A re-examination of these caecilians led the senior author to re-consider the identification given by the junior author in 2000, and is now regarded as a conspecific of *Caecilia goweri*, (Fernández-Roldán & Lynch, 2021), and whose measurements and meristics are given in Table 1S (see Supplementary Material) along with those of the type series, which provides further insight into intraspecific variation.

Caecilia goweri (MLS 45) was examined under a Leica stereoscope using entomological pins to facilitate the counting of primary and secondary grooves. The total grooves count was made twice by the senior author in order to avoid miscalculation. A small incision to the commissure of the mouth was made in order to access dentition (i.e. the number of teeth per series), and all observed teeth were examined directly with the mouth opened. All dental counts were made clockwise from left to right postero-anteriorly; teeth that were not fully exposed outside the gums were not counted. A small, ventral longitudinal incision was made to search for sexual organs. All measurements were taken using a Neiko digital calliper rounded to the nearest 0.1 mm with the exception of total body length, which was determined using a measuring tape (in centimetres) and placed along the body length of the specimen.

Figure 1. General view of the body of *Caecilia goweri* (MLS 45) from Yarumal, Antioquia, Colombia

We identified MLS 45 as *C. goweri* because it has the following morphological characteristics, meristics, and measurements. An adult male with a total body length of 585 mm, body width of 6.2 mm at mid-body point, an attenuation index (i.e. length divided by width) of 94.3 times (the highest value known for the species), and in preservative (70 % ethanol) a mainly brown coloured body with a cream 'ventrolateral stripe' (see Fernández-Roldán & Lynch, 2021). This individual has 179 primary grooves and 14 secondary grooves, the last 12 fully encircled the posterior end of the body. Dentition and the number of teeth per series are arranged as follows: premaxillary-maxillaries 5-1-4, vomeropalatines 6-1-7, dentaries 8-8, and inner mandibulars 3-1. Dermal scales begin at the 37th primary groove and end at the last one, where these are circular in shape and thicker at the margin of inception with the pocket (Table 1S). Furthermore, this specimen (MLS 45) was collected in Yarumal, Antioquia at 1800 m a.s.l., in January 1963, not far from the previously known records of *C. goweri* (Fig. 2).

Caecilia occidentalis remains a poorly known species that has been found close to the towns of Moscopán and Popayán, in departamento del Cauca, (Pubenza Valley), and Pance, Valle del Cauca (i.e. on the eastern slopes of the Cordillera Occidental), from 1350–1800 m a.s.l. (Fig. 2). Recently, a photograph of a caecilian being eaten 'head first' by the coral snake *Micrurus mipartitus* was published by Vera-Pérez et al. (2019), depicting a black-ish caecilian - allegedly *C. occidentalis* - which would confirm Lynch's (2000)

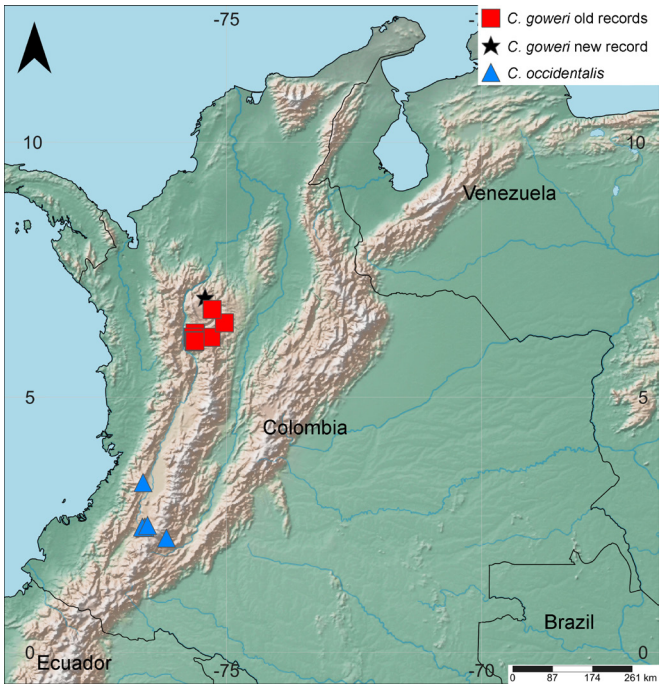

Figure 2. Map of Colombia showing the geographic distributions of *Caecilia goweri* (red squares), the new record of *C. goweri* from Yarumal (black star), and *C. occidentalis* (blue triangles)

observation of this species having a black colouration in life. Aside from a publication by Taylor (1969) commenting on the morphological variation, cranial osteology, and distribution of *C. occidentalis*, not much is left for us to report apart from the fact that three additional specimens (MHUC 93–95) - all from the town of Popayán (or from its surroundings) - are housed in the herpetological museum at Universidad del Cauca, Popayán, but only the most basics of meristics and measurements are available. Their primary grooves range from 186–201, their secondary grooves range from 2–7, their total length from 547–681 mm, and their attenuation index from 55–85 times.

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SHORT COMMUNICATION

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Egg laying and neonate morphology of the stout sand snake *Psammophis longifrons*

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The stout sand snake *Psammophis longifrons* Boulenger 1890, is a little known endemic snake species of the Indian subcontinent, reported from the states of Gujarat, Maharashtra, Madhya Pradesh, Andhra Pradesh, Telangana and Karnataka (Hussain et al., 2020). Apart from distribution and adult morphology, the natural history of this species remains largely unknown although oviposition has been reported in the month of April and in that case the clutch of eight eggs had average egg dimensions of 40 x 21 mm (Vyas & Patel, 2013). Here, we provide further information on reproductive biology and neonate morphology.

A female *P. longifrons*, measuring 170.2 cm in total length (SVL = 136.4 cm, tail length = 33.8 cm) was rescued at ca. 16:30 h on 25 February 2022, from a rural residence in Untdi village, Valsad, Gujarat, India (20.7045° N, 72.9289° E). Two hours after rescue, it laid 15 white leathery eggs (Fig. 1A).The egg dimensions were - mean (± standard deviation) length = 35.1 ± 1.3 mm, range = 33–38 mm; mean diameter = 22.9 ± 0.9 mm, range = 21–24 mm (for data see Table 1S, in Supplementary Material). The mean egg size noted in the present study is smaller than that of the earlier record by Vyas & Patel (2013), perhaps due to factors such as health and size of the female.

The eggs were marked and then incubated in a large plastic container, containing a vermiculate and sandy clay mixture moistened with 80–90 % by weight of water, placed in a cool and dark location at room temperature (22–28° C) (Vyas, 1988). On the night of 20 May 2022, after 84 days of incubation, the first hatchling started to emerge (Fig. 1B) followed by the rest in the next two days; full emergence from the egg took about eight hours. All hatchlings appeared healthy and were active. They were kept under observation for a couple of days, examined to collect data on bodily dimensions and scale counts (Table 1S), and released near the same location from where the female was rescued. Eggs dimensions and the scale counts of all 15 hatchlings are presented in Table 1S.

Based on the present study and previous observations (Vyas & Patel, 2013), the breeding season of *P. longifrons* is late winter; egg laying occurs at the end of February to April and hatchlings emerge from May to June.

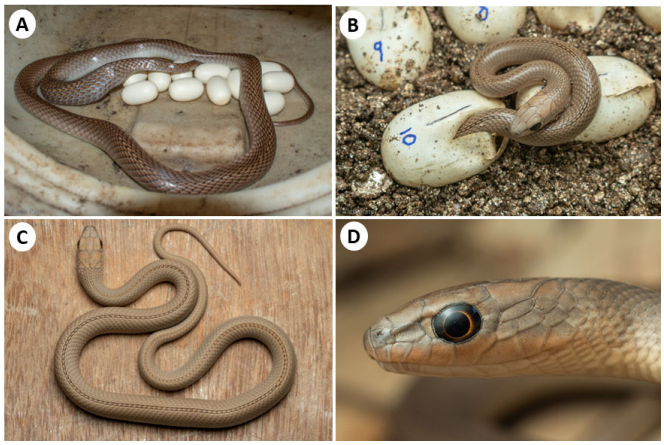

Figure 1. **A.** Female *Psammophis longifrons* laying eggs, **B.** Hatchling emerging from the egg, **C.** Dorsal view of a hatchling, **D.** Lateral view of the head of a hatchling

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The Herpetological Bulletin 162, 2022: 33–34
SHORT COMMUNICATION

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A female Amazon water snake *Hydrops martii* with an exceptionally large number of secondary follicles

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The genus *Hydrops* Wagler, 1830, comprises three species of neotropical snakes widely distributed in South America (Wallach et al., 2014). Species in this genus are small to medium sized, aquatic, diurnal and oviparous (Cunha & Nascimento, 1978; Albuquerque, 2000; Scrocchi et. al., 2005; Nunes, 2006; Fraga et al., 2014). *Hydrops martii* (Wagler in Spix, 1824) is widespread in the Amazon forest, being found in Brazil, Colombia, Ecuador, Guyana, Peru, and Venezuela (Nogueira et al., 2019). This species can be easily distinguished from the other congeners by its colour pattern (yellow body with black rings delimited by whitish spots arranged on each scale) and the presence of the 17 dorsal scale rows (versus 15 in *Hydrops caesurus* and *Hydrops triangularis*) (Fraga et al., 2014).

On 14 September 2019 we captured an individual of *Hydrops martii* (Fig. 1) at Mucajaí (2° 16'28.2" N, 60° 58'01.0" W), Roraima, Brazil, as a part of a field study. The snake was a large gravid female with a snout vent length (SVL) of 894 mm and tail length (TL) of 208 mm. The individual was captured (permit No. IBAMA 02001.004325/2015-71), euthanised with a 2 % lidocaine solution, fixed in a 10 % formaldehyde solution, stored in 70 % ethanol and is currently deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro, under the number MNRJ 27503. This specimen at a total length of 1102 mm would appear to be large for the species and is second only to a specimen of total length 1219 mm reported by Albuquerque (2000). On dissecting our specimen we revealed 26 secondary follicles (18 in the right ovary and eight in the left ovary) (Fig. 2). The largest follicle measured 20.1 mm. This record of 26 follicles in a female represents the maximum number of secondary follicles published so far, which may be related to the female's size, since large females tend to have larger fecundity rates (Shine, 1977).

Albuquerque & Camargo (2004) assessed the reproductive biology of the genus *Hydrops* and found 31 gravid specimens with fecundity ranging from 7 to 23 "eggs". Later, Braz et al. (2016) re-examined 18 of these specimens and found no female with oviductal eggs, but only with enlarged ovarian follicles. So far, only a single specimen of *Hydrops martii* has been classified as gravid, containing 13 oviductal eggs (Braz et al., 2016). Although in some species the number of secondary follicles and the number of eggs/embryos is similar (Maschio et al., 2007), in others the number of secondary follicles produced by a female is higher than the number of eggs/embryos observed in the oviduct (Shine, 1977; Mesquita et al., 2013).

Figure 1. Female *Hydrops martii* (MNRJ 27503) from Mucajaí, Roraima, northern Brazil

Figure 2. Dissected female (MNRJ 27503) with 26 secondary follicles in the ovaries

All species of *Hydrops* are considered to be oviparous but other Hydropsini genera may be either oviparous, viviparous

or in some species both (Nunes, 2006; Braz et al., 2016). Consequently, South American water snakes are considered to offer a suitable opportunity to study the evolution of viviparity. In that context, the reproduction of *Hydrops* still needs further research to include variations in clutch size, production of secondary follicles, and the relationship between female size and clutch sizes.

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NATURAL HISTORY NOTE

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First record of male-male combat in the Malagasy giant hognose snake *Leioheterodon madagascariensis*

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Male ritualistic combat has been widely documented in snakes and is considered to be an ancestral characteristic of the group (Senter et al., 2014). Rival males engage in combat in order to gain mating dominance or access to fertile females. Combat is generally non-lethal and combatants attempt to force the head of their competitor to the ground, often by entwining their bodies to exert a downward pressing force, known as topping. Herein we present the first known recorded observation of male combat in *Leioheterodon madagascariensis*, a Malagasy endemic now also found on the Comoros Islands (Meirte, 1993). It is a large and common lamprophiid species that may reach lengths of 1800 mm and is frequently encountered close to human habitation where it eats rats and eggs (Glaw & Vences, 2007). The species has an upturned rostral scale, which is referred to in the common name, that is used for foraging under leaf litter and sand (Glaw & Vences, 2007). Previously, the only reproductive behaviour described for *L. madagascariensis* has been courtship and mating between a captive male and female (Campbell & Murphy, 1977).

On 24 July 2019 at 11:28 h during a herpetofaunal survey in the north-west dry forests of the Mahamavo region of Madagascar (15° 28'30.7" S, 46° 41'43.4" E), two large adult male *L. madagascariensis* were observed engaged in combat (BHS video, 2022). The two males were found mid combat on a frequently used village path, with both snakes seemingly undisturbed by the presence of the two authors despite being only metres away. Their body and tail regions were entwined, and heads elevated (Fig. 1A). Both snakes attempted to pin the head of the other, whilst continuously coiling around each other without much forward travel. An individual would attempt to pin the head of the other to the ground with the underside of its own head, whilst the other would jerk its head to one side in an effort to not be pinned and to try to regain dominance. Combat was observed for approximately 8 minutes and the position of dominance switched repeatedly. Whilst combat in other species sometimes involves multiple phases (Guedes et al., 2019; Senter et al., 2014), combat in *L. madagascariensis* only seemed to have a 'topping' phase, but it must be noted that combat was not observed from the start. No biting was observed.

When combat was completed, the victor flared and raised its neck in a manner often exhibited in *L. madagascariensis*' defensive behaviour (Fig. 1B) and appeared to chase off the loser, which moved off the path into the undergrowth, shortly followed by the victor. We did not observe a female, however, a passing villager claimed to have seen another snake, which we assumed to be the female. The victor was later caught and biometric data recorded. The snake was the largest *L. madagascariensis* caught during the survey season (1500 mm long and weighing 1200 g); its large size may have played a role in victory (Glaudas et al., 2020; Schuett, 1997). Whilst handled, the snake convulsed its cloacal region, seemingly in response or sensitivity to touch, perhaps indicating sexual stimulation during combat. Despite obvious fatigue displayed whilst catching the snake,

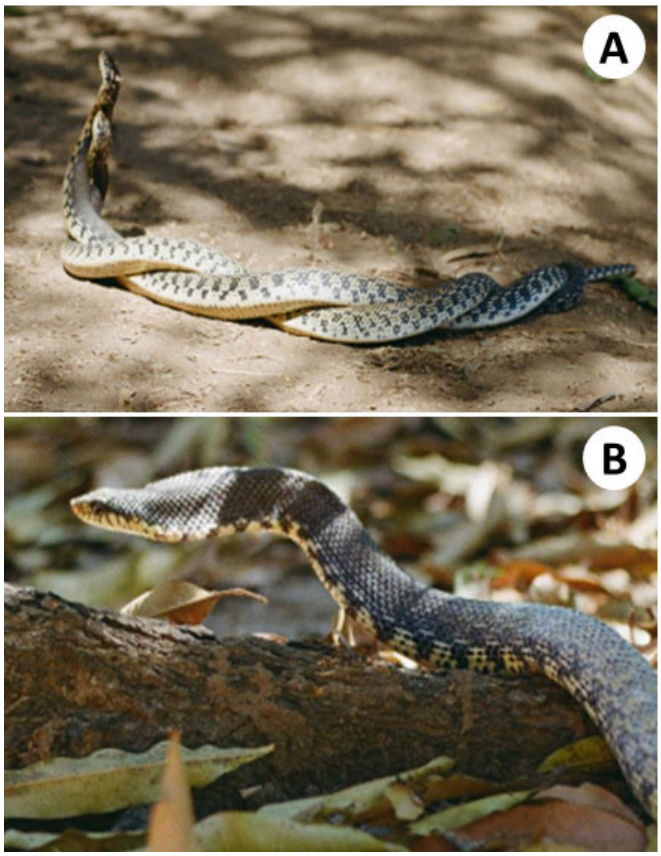

Figure 1. Male combat in *Leioheterodon madagascariensis* - **A.** Two males engaged in ritualistic combat, **B.** Threat behaviour exhibited by the victorious male

it was still capable of producing large amounts of force and at one point freeing itself from the grip of the handler.

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NATURAL HISTORY NOTE

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A case of nocturnal activity in the diurnal southwestern fence lizard *Sceloporus cowlesi*

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The southwestern fence lizard *Sceloporus cowlesi* Lowe & Norris 1956, belongs to the *Sceloporus undulatus* complex of species (Leaché & Reeder, 2002). It is distributed in south-western USA and north-central Mexico (Leaché & Reeder, 2002; Leaché, 2009) where it inhabits deserts, grasslands, shrublands, forests, rocky areas (Jones & Lovich, 2009; Leaché & Reeder, 2002) and anthropogenic biomes that include urban areas (pers. obs.). The species has been characterised as strictly diurnal (Jones & Lovich, 2009). Most reptiles are either diurnal or nocturnal but there are reports of typically diurnal reptiles being active at night (Baxter-Gilbert et al., 2021; Duncan et al., 2003).

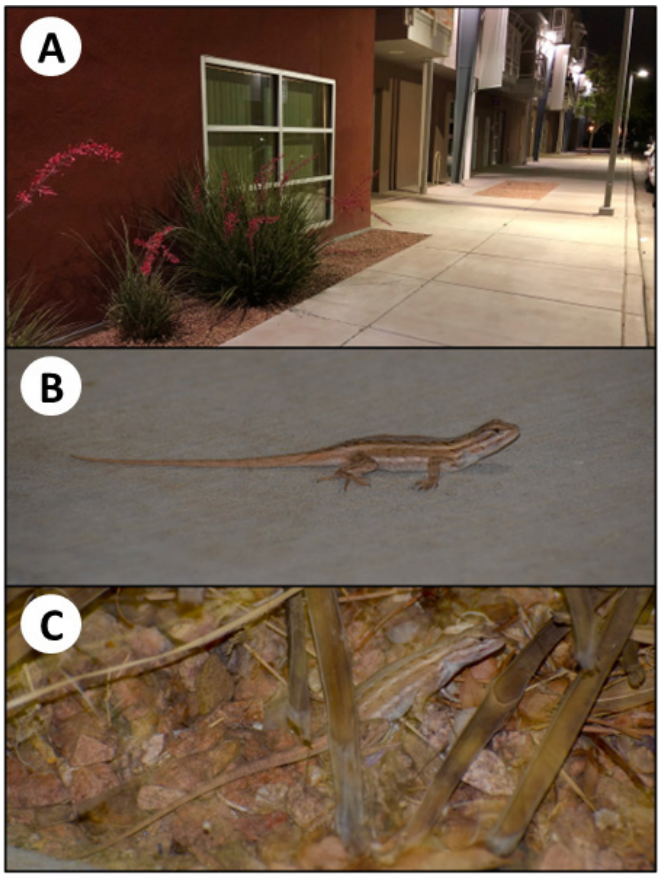

Figure 1. *Sceloporus cowlesi* individual displaying nocturnal activity in Albuquerque (New Mexico, USA) - **A.** General view of the location where the observations took place (25 May 2022), **B.** Basking on the pavement (26 May 2022), **C.** Seeking refuge in a Texas red yucca (*Hesperaloe parviflora*; 26 May 2022).

On four days in May 2022 (23, 25, 26, & 30), I observed a young adult *S. cowlesi* active at night in the city of Albuquerque, New Mexico, USA (35° 6'6.2712'' N, 106° 39'50.2524'' W) at a location illuminated by artificial light during the night (Fig. 1A). The time and air temperature of these four observations were 21:31 h - 23.3 °C, 21:58 h - 22.2 °C, 21:43 h - 22.8 °C, and at 20:44 h - 23.9 °C. On all occasions the individual was detected 'basking' on the pavement (Fig. 1B) and when approached it fled to a Texas red yucca (*Hesperaloe parviflora*; Fig. 1C). On the third day, 2 minutes after the individual fled, it was then observed returning to the pavement. Apparently, this is the first documented account of recurrent nocturnal activity in *S. cowlesi*, although *Sceloporus jarrovii* and *Sceloporus virgatus* exhibit crepuscular activity in south-eastern Arizona (Duncan et al., 2003).

The potential benefits associated with opportunistic nocturnal behaviour in lizards include extended activity in the evening in warm climates (Duncan et al., 2003), avoidance of diurnal predators, the availability of nocturnal prey items (Gordon et al., 2010), and night-light niche expansion (Amadi et al., 2021; Maurer et al., 2019). While it is relatively rare that the same species exhibit both diurnal and nocturnal behaviours, human activities and anthropogenic related environmental changes are providing an opportunity for animals to extend what may be crepuscular behaviour into the night time (Gaynor et al., 2018). However, studies evaluating patterns of nocturnality by predominantly diurnal species are still scarce.

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NATURAL HISTORY NOTE

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Skin secretions from the velvet swamp snake *Erythrolamprus typhlus*

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The velvet swamp snake *Erythrolamprus typhlus* is a diurnal and predominantly terrestrial species (Martins & Oliveira, 1998) that is distributed widely in South America (Wallach et al., 2014). It feeds mainly on frogs and toads (Pazmiño-Otamendi, 2020). When threatened the species usually compresses its body dorsoventrally (especially the anterior third of the body), and hides its head under its body, rolling it up; when manipulated it may twist the body and release a fetid discharge from its cloacal glands, and occasionally it may also bite (Martins & Oliveira, 1998). Here we report the first cases of skin secretions in *E. typhlus*.

At 10:00 h on 20 March 2022, in Miriti-Paraná, department of Amazonas, Colombia (1.20591° N, 69.88753° W; 102 m a.s.l.), during a local fauna observation walk with people from the community, we observed an adult *E. typhlus* crossing a forest path. We captured the specimen which was about 400 mm total length. At the moment of capture, the snake compressed its body dorsoventrally, it did not try to bite, but after two minutes of manipulation the individual began to twist its body and secreted very small white drops of fluid that emerged from between the scales (Fig. 1A). These white drops were observed mainly in the anterior and middle region of the body; after a few minutes this white substance became sticky, as if it were latex. We were surprised by this behaviour and so we placed the specimen in a plastic box so we could make further observations. After three hours, the individual was manipulated again, and after two minutes of manipulation the snake again secreted small white drops of fluid. The next day we released the snake in the forest area where it was captured.

Ten days later, at 10:43 h on 30 March 2022, in Miriti-Paraná, department of Amazonas, Colombia (1.19566° N, 69.89171° W; 103 m a.s.l.) we encountered a second individual of this species, which we captured. After a few minutes of manipulation this specimen also released white droplets of fluid mainly from the anterior and middle region of the body (Fig. 1B). This appears to confirm that this behaviour is typical of the species.

To the best of our knowledge, this is the first report of skin secretion from any species of the genus *Erythrolamprus*. Skin secretions are well known in some other species of snake and were first documented in the Asian natricine *Rhabdophis tigrinus*, where they are released from nuchal glands below the skin. These secretions are toxic, the toxins being sequestered from the parotid glands of toads that are their prey (Hutchinson, 2007) and are known to cause toxin ophthalmia (Chen et al., 2014). Subsequently, such secretions have been identified from a total of 17 other Asian natricine snakes (Takeuchi et al., 2018) and are referred to as toxungens, to distinguish them from venoms where there is a distinct delivery mechanism or poisons that lack a delivery mechanism (Nelsen et al., 2014). The status of the *E. typhlus* secretion as a toxungen has still to be confirmed. We propose future studies on the chemical composition of the secretion and the morphology of the glands that produce it.

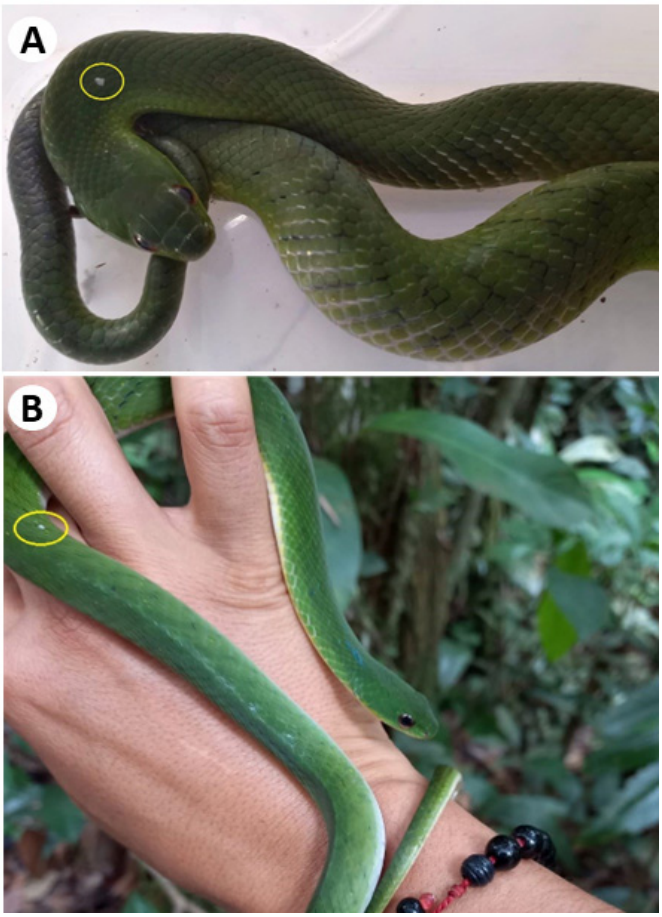

Figure 1. Cutaneous secretion in adult *Erythrolamprus typhlus* at Miritî-Paraná, department of Amazonas, Colombia, the yellow circles indicate secretion droplets - **A.** First specimen, **B.** Second specimen

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NATURAL HISTORY NOTE

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Smooth newts *Lissotriton vulgaris* observed hibernating in a waterfowl nest

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The smooth newt *Lissotriton vulgaris* occurs across most of Continental Europe as well as the British Isles, with a distribution that extends all the way into western Asia (Skorinov et al., 2008; Wielstra et al., 2018). Their presence indicates a healthy environment, which is why newts serve as bioindicators (Vershinin, 1996). Adult smooth newts are semi-aquatic and breed in stagnant or semi-flowing water during spring and early summer, favouring fish-free ponds, puddles and ditches as aquatic habitats. They generally spend the rest of the year on land, where they repose and hibernate (Sparreboom, 2014).

The length and start of the aquatic phase depends on the local climate and differs by region. In the Netherlands, for instance, the length of the breeding season is limited to four months and it generally starts around March (Creemers & van Delft, 2009). In warmer, south-western areas, however, the breeding season may already start around mid-February and the terrestrial phase is shorter, or even absent. The opposite is true for colder, north-eastern areas, where the aquatic phase is known to be shorter and will also start later, in summer or even autumn (Sparreboom, 2014).

The terrestrial phase is not well documented, with smooth newts being described as secretive animals that mostly live underground (Kaczmarek et al., 2018). They have a nocturnal existence, feeding on invertebrates nearby their overwintering shelter, the hibernaculum, in which they hibernate during the coldest period of the winter. They often overwinter in groups of several individuals (Creemers & van Delft, 2009; Sparreboom, 2014).

The hibernacula chosen by smooth newts are diverse, as they include wood piles, rotting tree logs, tree roots, compost heaps, stones, and even structures created by other animals, such as burrows of small mammals, or abandoned ant nests (Creemers & van Delft, 2009; Kaczmarek et al., 2018). Preferably, hibernacula are moist and frost-free. Basements, sheds, and wall cavities are also known to be used to hibernate within urban areas (Creemers & van Delft, 2009; Dervo et al., 2018).

Here we present, to our knowledge, the first documented account of a waterfowl nest being used as a smooth newt hibernaculum (Fig. 1A). After the bird's breeding season, on 5 October 2021, we collected the nest of a common coot (*Fulica atra*; Fig. 1B). We did so for a study on artificial nest material (Hiemstra et al., 2021), but the nest in question was fairly natural and was constructed mostly of twigs. The nest was located in a stagnant ditch that was about two metres wide and one metre deep, located in the suburbs of the city of Leiden, the Netherlands (52° 09.065' N, 004° 28.568' E). For several consecutive years, the nest was used by coots for breeding.

After pulling the top part of the nest aside, we noticed the presence of several clustered, lethargic smooth newts (Figs. 1 C & D). They were located under the top layer, but above the water level, on the side of the nest. After about a minute, the newts started to slowly crawl deeper into the nest, after which we decided not to disturb them further and we restored the nest to its old state. We do not know the exact number, but at a first glance and just in one corner, there were at least five congregated individuals, suggesting the nest was used as a hibernaculum (Creemers & van Delft, 2009; Sparreboom, 2014). It is unknown if hibernating in a nest increases the chances of being preyed upon by coots, but it is known that smooth newts can fall prey to waterfowl.

There are many examples of bird nests being used by other organisms than the host species (Kosicki et al., 2007; Silva et al., 2018; Krištofík et al., 2009) and nests are even referred to as 'biodiversity hotspots' (Maciorowski et al., 2021). Waterfowl nests are found to include a wide range of species among which are planarians, leeches, worms, gastropods, crabs, spiders, insects, frogs, water snakes, and water voles (Ceylan et al., 2021). Red mites *Dermanyssus gallinae* are also common in urban bird nests (Cafiero et al., 2013), and nests should therefore be frozen before doing a deconstruction (Hiemstra et al., 2021), but from now on preferably not before checking if hibernating newts are present, considering they are often protected by law.

Increased winter activity in the smooth newt has been linked to climate change, which causes winters to be milder (Kaczmarek et al., 2018). Although waterfowl nests may not be frost-free in winter, climate change may thus instigate a shift to other hibernacula. All in all, the nests of waterfowl may provide microhabitats for a range of species, with a waterfowl nest acting as a hibernaculum for smooth newts being a seemingly, newly discovered example. For the future, it is important that nest researchers, ditch or pond dredgers, and conservation managers are aware that abandoned waterfowl nests potentially house overwintering newts.

Figure 1. **A.** The nest of a common coot *Fulica atra* in which smooth newts were observed to hibernate, **B.** One of the common coots from Leiden, the Netherlands, **C.** Two hibernating smooth newts *Lissotriton vulgaris* from the nest, visible close to the wall and residing above the water level, **D.** One of the lethargic smooth newts under the nest cup after the top of the nest was pulled aside

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NATURAL HISTORY NOTE

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Panther chameleons *Furcifer pardalis* using aerial cables in urban habitats

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The panther chameleon *Furcifer pardalis* Cuvier 1829, was introduced from Madagascar to Réunion Island in 1750 and 1830 (Cheke, 1987; Probst, 1998). Despite being an introduced species, this chameleon is commonly considered a native, locally called 'endormi' for 'asleep', and is protected by French law. In its native range in Madagascar, the species is mainly observed in open areas such as forest edges, shrubby or semi-natural areas, where it benefits from a good overview of its environment (Andreone et al., 2005; Lutzmann, 2006). However, habitat use in anthropogenic environments has been poorly documented. Here we report observations of an adult male *F. pardalis* in an urban situation.

On 7 June 2022, during a survey along electric cables suspended about 10 m above ground, an adult male *F. pardalis* was observed resting above a busy road at 17:45 h (air temp. 20.7 °C) in the city of Le Tampon [21° 15'45'' S, 55° 30'17'' E] (Fig. 1A). We followed the chameleon until dusk at 18:02 h (20 °C). The next morning at 08:10 h (18.7 °C, Fig. 1B) the chameleon was still in the same position. Recognition of individuals uses the unique shape of the white lateral stripe, which is fixed in adult male *F. pardalis* (Bourgat, 1969). This early morning observation suggests that the chameleon spent the night on the aerial cable network. Later that same morning at 10:58 h (22 °C) we photographed the same chameleon catching prey that was on an adjacent cable (Fig. 1C).

Road traffic has increased considerably in recent years in Réunion Island and vehicle collisions with chameleons

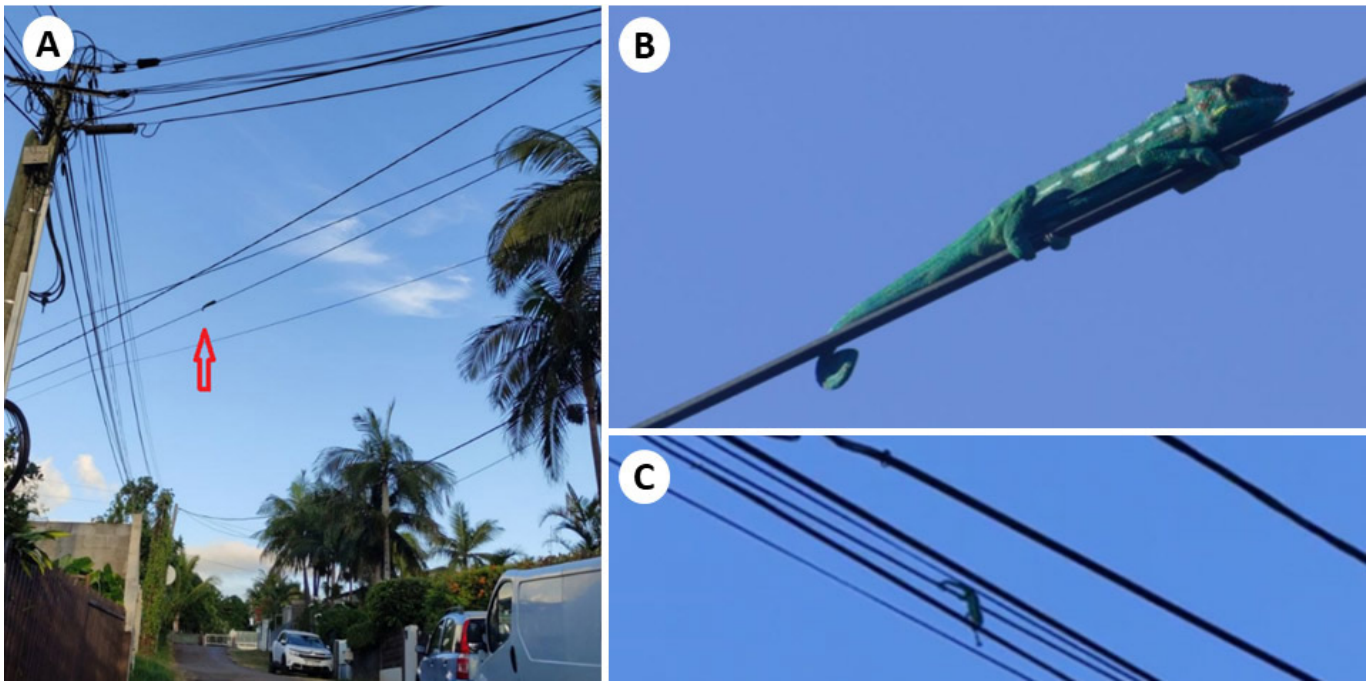

Figure 1. Adult male *Furcifer pardalis* on an aerial electrical cable in Le Tampon - **A.** In late afternoon (chameleon indicated by a red arrow), **B.** The same individual observed the next morning, and **C.** Showing the capture of prey

are numerous (author's personal observations). In its native range as well as in Réunion Island, many anecdotal accounts from naturalists show chameleons moving on the aerial cable network (Nečas, 2004; for one example see Labo SVT2 Canon 2017), presumably these chameleons are predisposed to walk on aerial cables given their similarity to natural branches and vines. Being out of reach of vehicles, humans and domestic animals, such as cats, while moving across roads and gardens provides better chances of survival and possibly facilitates dispersal, mate searching and foraging. Recent radio-tracking results show that in chameleons, males are more mobile than females (Gehring et al., 2008). This suggests that cable networks and ongoing land use change (urbanisation) may have some influence on chameleon population dynamics. Our observations reported here show the use of overhead wires by a male *F. pardalis* over busy city roads and gardens, for nocturnal resting, dispersal as well as feeding. New measures to bury overhead cable networks could have an impact on the population dynamics of urban and peri-urban chameleons. Further studies are needed to explore the spatial ecology of *F. pardalis*, notably to better understand how this species has successfully adapted to highly modified urban habitats.

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First record of male-male combat and courtship in the brown vine snake *Oxybelis aeneus*

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The brown vine snake *Oxybelis aeneus* Wagler 1824, occurs in the American tropics (Uetz et al., 2022). This opisthoglyphous colubrid has morphological traits typical of arboreal snakes, such as an extremely slender body, long tail, and elongated head (Jadin et al., 2020). In North America, vitellogenesis begins in March, and egg-laying occurs between June and August, followed by complete reproductive quiescence extending to the following March (Censky & McCoy, 1988). However, little is known about the reproductive biology of this species in Brazil.

Combat between male snakes has been described for several families (Senter, 2022) and has been associated with both dominance for ecological and reproductive resources, and competition for food (Almeida-Santos et al., 1998; Muniz-da-Silva & Almeida-Santos, 2013; Batista et al., 2021). The main feature of this behaviour is body contact between males to exert pressure on a rival to subjugate it (Carpenter, 1977). In some cases the combat may involve males biting each other's bodies and heads (Capula & Luiselli, 1997). Combat-related injuries have been observed in the colubrid *Leptophis ahaetulla*, the boid *Corallus hortulana* and xenopeltid *Xenopeltis unicolor* (Mattos et al., 2017; Goodyear & Gilbert, 2018; Santos et al., 2020). Here we report male combat in *O. aeneus* in the Brazilian Caatinga.

Around 08:00 h on 16 November 2021 (spring time), two male *O. aeneus* were sighted after falling from a tree (*Acacia* sp.) at Sítio Bom Sucesso (7° 54'23.4" S, 38° 10'40.2" W), a rural area of the municipality of Serra Talhada, Pernambuco state, north-eastern Brazil. On the ground, the males were intertwined and biting each other for approximately five minutes. Next, the males disengaged, climbed the tree, and started biting one another again. At this time, some blood was visible on the supralabial and infralabial scales of one of the males (Fig. 1A, B). The injury was possibly caused by the enlarged teeth on the posterior maxillae, since the males opened their mouths widely to grasp their rival's head. A third individual *O. aeneus* was then sighted at the top of the tree. This individual was probably a female, based on its large body size and subsequent reproductive behaviour reported below. The males disengaged soon after the observer approached the tree to record a video. One male moved to the left side of the tree, and the other moved to the upper right side, where the female was. The events reported above lasted around

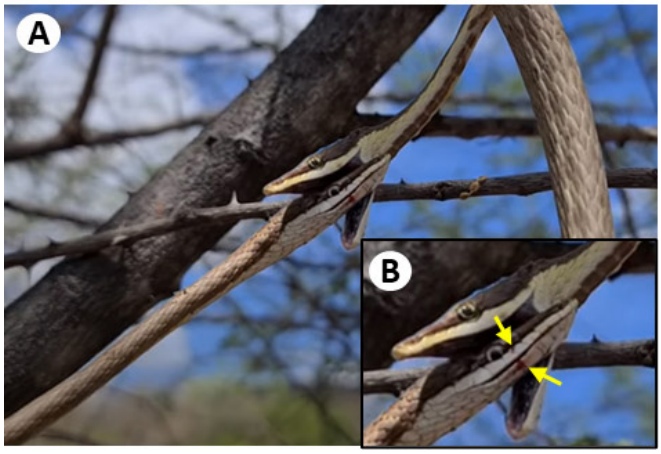

Figure 1. Male-male combat in the brown vine snake *Oxybelis aeneus* in Pernambuco, north-eastern Brazil - **A.** A male biting its opponent's head, **B.** Note the blood on the supralabial and infralabial scales of one of the males (arrows)

Figure 2. A courting male (blue arrow) and a female (red arrow) brown vine snake *Oxybelis aeneus* during a tail-search copulatory attempt (dashed circle) on a tree at night

90 minutes. At 19:20 h, one male and the female were seen together on the tree (Fig. 2). No copulation was observed, but some courtship-related behaviours were noted such as tactile-chase, tactile-alignment, and a tail-searching copulatory attempt (sensu Gillingham et al., 1977). The couple remained

together on the tree until at least 16:00 h the next day, when the observer stopped watching. The complete videotaped sequence is available in a database in the cloud "Banco de Vídeos GERES_LEEV" of the Laboratório de Ecologia e Evolução of the Instituto Butantan (001_LEEV_VD_HB).

Colubrid species that show male combat generally have a male biased sexual size dimorphism (SSD) index of lower than 0.27 (Shine, 1994). However, *O. aeneus* has a slightly female-biased SSD (0.07; calculated from mean SVL values provided by Goldberg, 1998; see also Mesquita et al., 2010). There is a similar SSD index (0.09) in the congener *O. fulgidus*, although no male combat has been reported in this species (Scartozzoni et al., 2009). Here, we recorded a male combat with biting in a female-biased SSD species. Bites in male combat have already been recorded in other New-World opisthoglyphous colubrids, such as *Sonora* and *Scolecophis* (Goode & Schuett, 1994; Wilson et al., 2002; Chernov et al., 2020; Senter, 2022). Vigorous biting during combat, such as that observed here for *O. aeneus*, has been previously reported in other colubrid such as *L. ahaetulla* (Mattos et al., 2017) and *Macroprotodon cucullatus* (Capula & Luiselli, 1997) which show enlarged teeth (bladed and grooved, respectively) on the posterior maxillae that aid in envenomation of prey or opponent (Achille, 2015; Sánchez et al., 2018).

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BOOK REVIEW

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The Secret Life of the Adder: The Vanishing Viper

Nicolas Milton
Pen and Sword Books, Yorkshire & Philadelphia,
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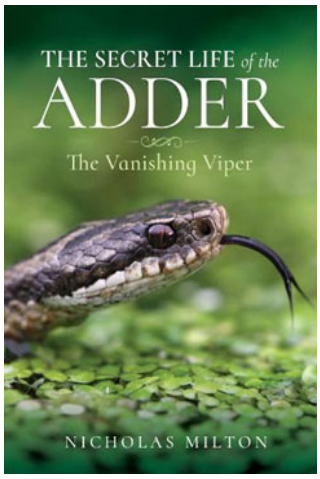

The British herpetological community is well aware that the northern viper or adder *Vipera berus* is of conservation concern. Its decline has been especially alarming since, in the past, the adder has been considered the most abundant of British snake species. A series of three 'Vanishing Viper' meetings, coordinated by the Amphibian and Reptile Groups of the UK (ARG UK) and ARC Trust, have addressed the issue with contributions from other European experts culminating in a detailed report on the threats and potential conservation solutions published in The Bulletin (Julian & Hodges, 2019). In the spirit of that report, it is exciting to welcome a new book on these vipers that devotes over a third of its text, in two chapters, to threats and conservation, preceded by chapters on the adder's history, decline and ecology. Attention is drawn to the fact that there are no nature reserves dedicated to the adder and that there are no SSSIs that designate adders as their principal concern. The book cites some projects that have already been designed and implemented to benefit adders, such as 'Adders are Amazing' and 'Back from the Brink'; although mention could also have been made of ARG UK's seminar series on 'Adder and Reptile Habitat Management for Landowners and Land Managers'. But, well-intentioned short-term projects only scratch the surface; much more is needed and the author makes ten suggestions to help direct conservation effort.

The 'Secret Life of the Adder' will be of particular interest to naturalists and wildlife conservationists. It follows the publication in 2011 of 'The Private Life of the Adder' by Rodger McPhail and is very well illustrated throughout, using many of McPhail's first rate photographs. There are pictures on most pages and there are also ten text boxes devoted to various asides and themes. The author is both an historian and a naturalist and the first chapter of the book, which is devoted to 'The Adder through History', benefits from this. It is very well written and makes interesting reading, even for a long standing adder enthusiast. It presents the very familiar story and photo of the eccentric new forest snake collector Brusher Mills from the nineteenth century, who did nothing for conservation, but could have benefitted from some mention of the late Tony Phelps who subsequently left Britain due to our failures in adder conservation (O'Shea et al., 2022). Also, I was a little surprised that the children's writer Enid Blyton is taken to task for writing about adders by "… repeating the misnomer that they are poisonous (as opposed to venomous)" when even up to the 1970s some books by herpetologists were still titled 'poisonous snakes'. Then follows the chapter on 'The Decline of the Adder', which opens with an interesting account of the first survey of British snakes organised by Gerald Leighton at the end of the 19th century. From this it is clear that even at that time adders had a tendency to be localised but with more sites occupied. Subsequently, the pre and post WW2 housing booms and drastic changes in agriculture had their negative wildlife impacts but the next adder survey wasn't initiated until 2005. This was ARG UK's 'Make the Adder Count', which to date is still ongoing, but delivered a set of conclusions in 2019. It told us that while large adder sites might remain relatively stable, the majority of sites (90 %) are small, typically with ten adult adders or fewer, and that they are at risk of extirpation.Consequently, adders may well disappear from much of the countryside in the next 15 to 20 years.

The 'Ecology of the Adder' chapter covers a basket of subjects ranging from aspects of anatomy, markings, venom, senses, movement, reproduction, feeding, and even the law. This is good background material but Ian Spellerberg's extraordinary 'posturing heliotherm' - nature's solar panel - barely emerges from the text and an opportunity has been missed to include recent advances in our understanding based on long-term studies. There are also misleading statements that will raise the eyebrows of adder researchers. One in particular is that besides vertebrate prey "Juveniles will also eat insects, spiders and earthworms." To the best of my knowledge there is no confirmed report of invertebrate prey being taken. Indeed, if juvenile adders do consume such prey then their food supply is more plentiful than researchers believe. However, in central and southern Europe a closely relayed species, *Vipera ursinii*, is well known to consume insects.

Then follows the chapter on 'The Threats to the Adder'. The first and foremost threat is stated to be climate change (warming) which presents several challenges to a cold-adapted species. Second comes habitat destruction, fragmentation, degradation, and mismanagement. The adder has been particularly affected by these owing to its complex habitat requirements and vulnerability to management extremes. Disturbance from the public is listed as a problem because the adder is in competition with a wide variety of recreational land uses. Although this is tempered with the

caution that there is very little quantitative data on the effects of disturbance and that the subject needs investigation. Game birds feature as a serious threat, particularly the very high stocking density of non-native species that consume pretty well any small creatures that will fit down their throats. There is new legislation to limit game bird releases within 500 m of special areas of conservation, but doubt is cast on the effectiveness of such a narrow buffer zone. The potential for adder inbreeding is discussed but it is acknowledged that this is probably not an issue and that British adder populations are currently genetically viable. The media are taken to task for their almost relentlessly biased accounts which shape negative attitudes in people. And naturally this leads to persecution by humans, something that is believed to be frequent but goes unreported. The point is made that since the 1981 Wildlife and Countryside Act was passed, no one has been prosecuted for killing an adder.

There follows the final chapter 'Conserving Adders' with the author's ten priority suggestions (or "….action plan") –

1. Protect in law all remaining adder sites
2. Create viable adder populations in every county/region
3. Teach 'Adders are Amazing' in schools
4. Recruit a new generation of adder champions
5. Report sensational and negative adder stories to the press regulator
6. Expand the 'Back from the Brink' projects to the whole of Britain
7. Ban dogs from all sites where adders occur
8. Make it illegal to release game birds within a mile of adder colonies
9. Build a nationwide network of adder corridors by rewilding, and
10. Designate adder nature reserves and fund a new adder conservation programme.

Each suggestion is discussed and all are likely to find support from the wildlife community. At least some of the suggestions vent the frustrations of many in the wildlife community who despair at the treatment of wildlife sites as social amenities where dogs roam free and where the behaviour of some photographers is focused on obtaining trophy photographs without respect for wildlife. The way ahead will certainly not be easy and some of the conservation measures suggested are politically sensitive as they conflict with pressure groups interested in the recreational use of land that is also adder habitat. Given these difficulties it makes sense to have a high profile adder champion. The book has a foreword by the naturalist Iolo Williams, who is a great adder enthusiast, but it is suggested that we need to go further and that perhaps Prince William might like to take on the cause of the adder in the way he supports endangered wildlife abroad; the royal estates may be a good focus for adder conservation efforts.

Fittingly, the two last pages of the book are devoted to a plea for adder conservation by project controllers from ARG UK and 'Back from the Brink'. This was written in response to the deliberate killing of an adder in a small Cotswold population, the few remaining individuals of which had been carefully studied and monitored by the local council rangers. The book is completed with a bibliography, a reference list, and an index. In the bibliography it is a pity there is no mention of Perti Viitanen's heroic study in Finland of adder behaviour in relation to seasonal movements and hibernation (Viitanen, 1967) as this a great inspiration (at least for me), and in the references the absence of the Vanishing Viper report (Julian & Hodges, 2019) seems a significant omission given that it draws together the herpetological community's thinking on adder conservation that underpins much of what is presented in this book.

A book such as this will mostly be read by those already sympathetic to the cause. But what is important is that it points the direction for those willing to devote time to securing the future of these beautiful creatures. During the final Vanishing Viper meeting, the veteran adder researcher Thomas Madsen mentioned that, in Sweden, people are very tolerant of adders and that the idea of killing them is considered to be outrageous; there is even a village that has built a 'snake wall' so that the villagers can live in proximity and harmony with adders. Our goal must be to try and reach the same kind of accommodation and ensure that the adder will remain a living icon of British wildlife. This book is a helpful step in that direction.

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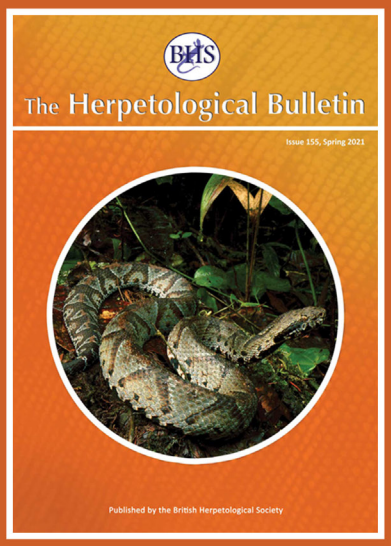
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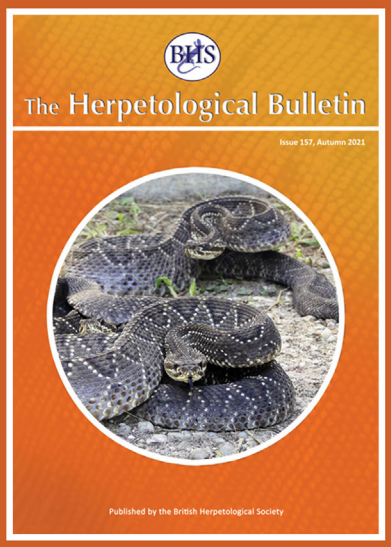
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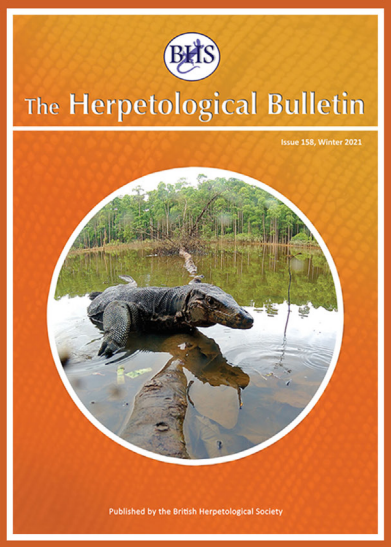
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