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Front Cover: Three adult female slow worms *Anguis fragilis* bathing in a shallow pool on an island off the south-western coast of Norway, photographed by Geir Inge Høines. See the article on p. 24.

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RESEARCH ARTICLE

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No amphibian chytrid fungus *Batrachochytrium dendrobatidis* detected in four introduced populations of the midwife toad *Alytes obstetricans* in eastern England

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ABSTRACT – The amphibian chytrid fungus *Batrachochytrium dendrobatidis* threatens amphibian species globally as the causative agent of chytridiomycosis, with the introduction of non-native species being one of the pathways that the pathogen can spread to naive populations. We have monitored and screened the common midwife toad *Alytes obstetricans* in four separate populations in eastern England, to investigate the potential threats to local amphibians. Forty-eight toads across all life stages were swabbed between May 2018 and August 2020, and screened for the presence of *B. dendrobatidis* DNA using qPCR. None of the samples tested were positive, indicating that it is unlikely that any of the *A. obstetricans* swabbed were infected with *B. dendrobatidis*. The populations surveyed represent only a small part of the species range in Britain, consequently a more widespread survey is recommended to increase confidence that British *A. obstetricans* are free of *B. dendrobatidis.*

INTRODUCTION

The amphibian chytrid fungus *Batrachochytrium dendrobatidis*, hereafter *Bd,* was discovered in the late 1990s. It is a non-hyphal zoosporic chytridiomycete fungus that causes amphibian chytridiomycosis, a disease that has been implicated in the decline of amphibian species globally (Berger et al., 1998; Skerratt et al., 2007). *Batrachochytrium dendrobatidis* is present on every continent except Antarctica and is known to affect more than 500 species globally, with the greatest impacts being in Mesoamerica, South America, and Oceania (Scheele et al., 2019). The spread of the fungus is thought to have been facilitated by the pet trade and the introduction of non-native species (Fisher & Garner, 2007).

In Europe, *Bd* is widespread in both geographic and host ranges, with few species displaying the symptoms of chytrid, and the impacts of disease being low for most species (Allain & Duffus, 2019). Several population declines of *Alytes* spp. recorded across continental Europe have indicated that this genus is highly susceptible to *Bd*, with subsequent lethal consequences (Bosch et al., 2001; Walker et al., 2010; Bosch et al., 2013; Doddington et al., 2013). Current data indicate that the *Alytes* spp. are the most susceptible taxonomic group to chytridiomycosis in the Palearctic (Bosch et al., 2001; Walker et al., 2010). Chytridiomycosis was responsible for the decline in the endangered Mallorcan midwife toad *Alytes muletensis*, following the release of infected captive-bred individuals (Walker et al., 2008). More recently, Moroccan midwife toads *Alytes maurus* have been found to be susceptible to chytridiomycosis (Thumsová et al., 2022). The rapid and significant geographic expansion of *Bd* across the entire range of *Alytes dickhilleni* in south-eastern Spain provides a relevant example of how chytridiomycosis severely affects midwife toads (Thumsová et al., 2021).

The salamander chytrid fungus *Batrachochytrium salamandrivorans*, hereafter *Bsal*, is a close relative to *Bd* which was first identified in 2013, after a dramatic decline of European fire salamanders *Salamandra salamandra* in the Netherlands (Martel et al., 2014; Spitzen-van der Sluijs et al., 2013). Since then, research has focused on how this additional pathogen may impact Europe's amphibian species. There is evidence suggesting that infection with *Bsal* in anurans is limited but species such as *A. obstetricans* may act as an intermediary host, infecting urodeles within the same ecological communities (Stegen et al., 2017). It is for this reason that previous screening of *A. obstetricans* in Great Britain targeted both *Bd* and *Bsal* (Allain & Goodman, 2017b; 2018). In the current study, individuals were not tested for *Bsal*, as previous analysis demonstrated that it was not present in *A. obstetricans* (Allain & Goodman, 2018) and in any case populations of *A. obstetricans* were introduced to their current locations before the emergence of *Bsal* in Europe. While *Bd* has previously been found in wild amphibians in Great Britain, *Bsal* is still yet to be detected (Allain & Duffus, 2019).

Alytes obstetricans is one of five recognised species of *Alytes* and is currently the only known species of this group to have established populations in Great Britain over the last century; mainly in southern Britain (Beebee & Griffiths, 2000). There are additional instances of introductions of this species outside of their natural range in Belgium, the Netherlands, and Germany (Speybroek et al., 2016). *Alytes obstetricans* are relatively small stocky toads compared to the native common toad *Bufo bufo*, with adults averaging

5.5 cm in length (Speybroek et al., 2016). Although variation in colour has been observed during surveys, the majority of individuals sampled have been a mottled stone colour, with visible warts and parotoid glands. A series of very small white warts usually 4–5 in number are also generally present along each flank. The native range for this species is western Europe, including the northern half of the Iberian Peninsula (Speybroek et al., 2016).

New populations of *A. obstetricans* are often being recorded in southern Britain, usually following the identification of the males' distinctive call (Allain & Goodman, 2019). This likely indicates an increase in detection rather than an expansion of their range within that same time frame. As a non-native species with an as yet unknown introduction pathway, disease surveillance is essential to ensure that any impacts on native species are minimised.

MATERIALS & METHODS

We surveyed midwife toad populations within urban and/or semi-urban environments at four locations in southern Britain (Fig. 1). Examples of our prime research habitats include, but are not limited to, private gardens and residential alleyways. To locate *A. obstetricans* in these environments during evenings between May and September, manual searches were combined with the use of call playbacks, which had previously been used to assist in the detection of the species (Allain & Goodman, 2017a). Upon capture, all toads were inspected visually for clinical signs of disease such as ulcerations and skin reddening.

To prevent cross-contamination, captured toads were held in individual zip-lock bags whilst each bag contained sufficient moisture and air to minimise stress. In the process of sampling, all appropriate biosecurity measures were taken including the use of nitrile gloves when handling the toads (Mendez et al., 2008), changing of gloves between each site, and the use of Vikron S when disinfecting field equipment (Young et al., 2007). Sterile cotton-tipped swabs (Medical Wire & Equipment, MW-100) were used to take skin surface samples from toads and tadpoles to check for chytrid fungus (Fig. 2). Each of the captured toads were sprayed with a small amount of water as part of the swabbing process in order to free their skin of any accumulated dirt or detritus, which may hinder the qPCR analysis (Kosch & Summers, 2013). The abdomen, thighs, groin, and feet of each individual were swabbed 15 times. After swabbing, data on sex, weight, and snout-to-vent length were recorded. Only in the case of the Cambridge population were tadpoles sampled, they were captured and subsequently released back into ponds using small aquarium nets. The tadpoles were sampled by placing a swab gently in contact with their mouthparts which was then spun for 10 seconds (Retallick et al., 2006). Once the samples had been taken, the swabs were refrigerated at 5 °C until they were sent to the Institute of Zoology at the Zoological Society of London for analysis, following the protocol described by Boyle et al. (2004). Briefly, swabs were tested for the presence of *Bd* DNA in duplicate using qPCR, with a positive result indicated by both duplicates showing amplification.

Figure 1. A map of Great Britain indicating the location of the four populations of common midwife toads *Alytes obstetricans* that were sampled in this study. Locations - 1 = Cambridge, 2 = St. Neots, 3 = Oundle, and 4 = Sutton.

Figure 2. A common midwife toad *Alytes obstetricans* being swabbed for amphibian chytrid fungus *Batrachochytrium dendrobatidis* during this study

RESULTS

A total of forty-eight *A. obstetricans* were swabbed from the four populations between 23 May 2018 and 27 August 2020. Five of these samples were from tadpoles, with the remainder coming from post-metamorphic toads. Of the toads captured, 7 were male, 3 were female, 3 were

Table 1. The qPCR testing for the presence of *Batrachochytrium dendrobatidis* DNA in common midwife toad *Alytes obstetricans* populations from four populations in eastern England, all tests were negative

Date	Location	Life-stage	Positive	Negative
23 May 2018	Cambridge, Cambridgeshire	Post-metamorphic	0	1
21 August 2018	St. Neots, Cambridgeshire	Post-metamorphic	0	1
27 July 2019	Cambridge, Cambridgeshire	Post-metamorphic	0	2
24 August 2019	Oundle, Northamptonshire	Post-metamorphic	0	16
3 September 2019	Sutton, Bedfordshire	Post-metamorphic	0	16
18 September 2019	Cambridge, Cambridgeshire	Post-metamorphic	0	3
18 September 2019	Cambridge, Cambridgeshire	Larval	0	5
27 August 2020	Cambridge, Cambridgeshire	Post-metamorphic	0	4

sub-adults, and 30 were of undetermined sex. None of these showed any clinical signs of chytridiomycosis. The average snout-to-vent lengths of the swabbed toads was 3.60 cm, and the average weight was 7.43 g. All samples tested qPCR negative for the presence of *Bd* DNA (see Table 1).

DISCUSSION

All samples were negative for *Bd* DNA but owing to the small sample size there remains some uncertainty about this result. This is especially true of the St. Neots population where only a single individual was swabbed due to problems associated with surveying; toads are not easily located as they often occur at low population densities and are found in private gardens, access to which depended on the co-operation of local residents.

Between 2018 and 2020 there was repeated testing of the Cambridge population (Table 1) but there was no evidence of chytridiomycosis during this period. The ongoing monitoring and disease screening of amphibian populations, including non-native species, is vital as an early warning system, which will enable swift mitigation to limit the exposure of British amphibian populations to *Bd*. This is especially true given the risk of *Bsal* spillover from captive collections to wild populations of amphibians within Great Britain (Fitzpatrick et al., 2018).

Whilst it is encouraging that there has been no evidence of *Bd* in any population of *A. obstetricans* in eastern Britain to date, the implications of such a discovery must also be considered. If and when *Bd* is detected in a population of *A. obstetricans*, it is unlikely that we would be certain whether they or the native populations of amphibians had been the source of infection. It is unlikely that the sampled populations of *A. obstetricans* mentioned here are vectors of *Bd* due to their high susceptibility to chytridiomycosis, and in any case their populations have persisted in southern Britain for over a century (Beebee & Griffiths, 2000). *Alytes obstetricans* typically inhabit gardens and breeding ponds with the common toad *Bufo bufo*, common frog *Rana temporaria*, and newts such as the smooth newt *Lissotriton vulgaris*. Of the amphibian species encountered on surveys, *A. obstetricans* is the most frequent, likely a consequence of our survey timing. Due to the greater susceptibility of *A. obstetricans* to *Bd*, the screening of *A. obstetricans* is an effective way to check whether or not the other amphibians within the same habitats were likely to be infected.

It is possible that exposure to less virulent strains of *Bd* could be beneficial in avoiding mass die-offs within populations of amphibians, as naive individuals are likely to be more susceptible (Greener et al., 2020). Other species such as the alpine newt *Ichthyosaura alpestris* are a recently established non-native amphibian within Great Britain, with a country-wide distribution (Allain & Lynn, 2021). Alpine newts may act as reservoirs for *Bd* (and other amphibian pathogens) that could potentially expose native amphibians to disease (Greener et al., 2020), highlighting the need to closely monitor amphibian populations where non-native species are known to be present.

This study has provided encouraging results, although it is only a snapshot based on populations in eastern England. Current known populations are considerably more widespread (Beebee & Griffiths, 2000). With adequate funding, all known populations could be screened regularly for *Bd* which would give more certainty of the status of the pathogen in Britain.

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RESEARCH ARTICLE

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Captive breeding and ex-situ conservation of the Caucasian pit viper *Gloydius caucasicus*

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ABSTRACT - The Caucasian pit viper *Gloydius caucasicus* is one of six snake species that is frequently reported as a cause of venomous snakebite in Iran. We present the results of successful captive breeding of 20 Caucasian pit vipers (10 males and 10 females) collected in August 2018 from the Lar National Park, northern Iran. Mating and copulatory behaviours were observed from mid-June to early July 2019. Five of the ten females gave birth with parturition occurring from 10 to 13 September, when 17 vipers were born in litter sizes ranging from 2 to 5. The present captive breeding programme has been successful and shows potential for both venom production and support for conservation by reducing the demand for wild caught specimens.

INTRODUCTION

In the Middle East, vipers are a significant cause of snakebite, as well as of importance to medical research and development, but they have been relatively little studied (Stümpel & Joger, 2009; Rima et al., 2018). One approach to securing a ready supply of vipers for the extraction of venom for therapeutic antibody production and pharmaceutical purposes is captive breeding; this also has the advantage of reducing the demand for the collection of vipers from the wild (Újvári et al., 2002).

The only pit viper species occurring in Iran is the Caucasian pit viper *Gloydius caucasicus* (Nikolsky, 1916) (Asadi et al., 2019). This is found in montane habitats at altitudes up to 3,000 m a.s.l. and belongs to the *Gloydius halys*/*Gloydius intermedius* species complex, a group of closely related vipers in the subfamily Crotalinae (Alencar et al., 2016). The Caucasian pit viper is a viviparous, diurnal snake distributed from the northwest of Afghanistan and southern Turkmenistan to Azerbaijan and northern parts of Iran (Rastegar et al., 2008; Khani et al., 2017; Asadi et al., 2019; Uetz et al., 2022). In Iran, the species is distributed along the Alborz Mountains, from the Hezar Masjed Mountains in Khorasan Razavi province to the western regions of Gilan province (Mozaffari et al., 2016).

The Caucasian pit viper is threatened by several anthropogenic impacts that include habitat loss due to agricultural development, urbanisation, livestock overgrazing, indiscriminate and irrational killing by local people, and most importantly, excessive collection and over exploitation by antivenom co-operatives in Iran (Rastegar-Pouyani et al., 2018; Asadi et al., 2019; Kaboli, pers. obs.). Overharvesting of this viper for antivenom production has dramatically reduced wild populations of *G. caucasicus*, especially in the Lar National Park. For instance, in 2020, out of 184 Caucasian pit vipers captured from the Lar National Park, few had reached full adult size. Each year, the co-operatives harvest many snakes directly from the wild yet remain firm in arguing that the extent of wild capture has been overstated and that, in any case, the snakes have been released back into the wild after venom extraction. This claim has gone unchallenged by the Department of Environment of Iran (DoE) for decades (Kaboli, pers. obs.) and in any case it would be expected that the survival rates of these snakes when released back into the wild would be drastically reduced (Roe et al., 2010; Harvey et al., 2014; Wolfe et al., 2018). Furthermore, such practices are not recommended as, according to the latest protocols, venom should be collected from infection-free captive bred snakes reared under controlled environmental conditions in order not to compromise the quality of the venom extracted. Manufacturers should fulfil a number of standards, including venom traceability, quality control, and animal welfare during snake milking, handling and maintenance in captivity (WHO, 2017).

To date, there is only one published research study on the captive husbandry and breeding of *G. caucasicus* that of Shakoori et al. (2015). The most fundamental requirement of a captive breeding programme is successful reproduction. However, the methods employed in that study appear to have resulted in low reproductive success and low offspring survival rates, which challenge the long-term sustainability and commercial viability of a breeding programme. Establishing a large-scale captive breeding programme for venom production or conservation breeding projects calls for a comprehensive assessment of captive breeding requirements as well as comparison of venom composition between wild and captive snakes (Leloup, 1984; Braz et al., 2012). With regard to venom composition, an investigation of the properties of venom obtained from our viper specimens at the time of capture, and subsequently, has confirmed that antivenom from the captive Caucasian pit vipers would be an effective treatment against bites from this species (Rasoulinasab et al., 2020). On that basis it has been recommended that capture of wild snakes for their venom be discontinued to reduce their risk of extinction (Rasoulinasab et al., 2020). In light of these findings, we present here data collected at the Laboratory of Herpetology of the University of Tehran that contribute towards the development of guidelines for the husbandry and captive breeding of Caucasian pit vipers.

CAPTIVE BREEDING METHODOLOGY

Collection of breeding stock from the wild

This project was authorised by the Iranian Department of Environment under license number 9061/97. In August 2018, a total of 20 Caucasian pit vipers, 10 females and 10 males, were collected from Lar National Park (central Alborz, northern Iran; Fig. 1) to be used as breeding stock for the captive breeding programme (Fig. 1). The park is located on the south-western slopes of the highest peak in Iran, Mount Damavand (5,671 m). The region is characterised by a cold, humid mountain climate marked by high precipitation, mostly in the form of snow that covers the landscape for approximately half of the year, during which time it is inaccessible to humans. During the day when temperatures rise, reptiles of this region are very active but at night when temperatures fall reptile activity decreases. Detailed records were made of geographical locality, habitat, season and date of capture. Venom was collected immediately from the snakes at the sites of capture to be stored as wild venom for future comparisons, reported by Rasoulinasab et al. (2020). The vipers were transferred to the captive breeding facility at the University of Tehran designed according to WHO guidelines. The facility comprises an administrative office, snake housing room, quarantine room, milking room, venom production and storage room, control laboratory, animal (rodent) housing room, and cleaning room (WHO, 2017).

Quarantine and disinfection

On arrival, the 20 snakes were kept for three months in the quarantine room which is located away from the maintenance and production rooms, to minimise transmission of parasites and diseases. During this period, the individuals were housed in separate boxes with good ventilation, warm and cool sides, cover objects (e.g. broken flower pots or small hollow logs), and a water dish in the cooler part of the box. Ventilation of the quarantine room itself was supplied using fans and air conditioning. On arrival, biometric measurements for all snakes including weight and other morphological characteristics were recorded. They were then all examined by an experienced veterinarian familiar with reptiles for any ectoparasites, wounds or fractures. Ectoparasites (e.g. lice, mites, and ticks), endoparasites (e.g. nematodes, protozoans, cestodes and pentastomids), and infections, if present, were treated using antiparasitic and anti-inflammatory drugs, including diluted ivermectin spray for the ectoparasites. The snakes were inspected every 15 days by the veterinarian for any unwanted symptoms. To prevent pathogen transmission, personnel footwear was treated using a disinfectant before entrance.

Design and maintenance of the routine enclosure

Each snake was housed separately in a semi-transparent plastic container (120 cm long × 60 cm wide × 50 cm high) with perforations in the sides. Clean sawdust was used as a substrate which was replaced monthly. A water dish, two hides (one in the warm and another in the cool end of the box), and a piece of stone were provided in the container. Each container was assigned a unique number on a label stating the animal's sex, feeding times, venom collection and cleaning schedules. The enclosures, the hides and the production room were cleaned and disinfected twice a week.

Temperature and humidity

The temperature regime was arranged to heat to 30–32 °C one-third of the posterior end of the box during the day using six 32-watt heating strips and cooling to 20–24 °C along one-third of the anterior end of the box at night. The average relative humidity was maintained between 30–45 % by placing a water dish in the box. When the ambient humidity dropped to 30 %, the boxes were sprayed with warm water at least twice per week, increasing humidity up to 55 %. Water should be sterilised with ultraviolet light (WHO, 2017); however, we used regular tap water.

Sexing and marking

The snakes were initially sexed using tail/bodylength ratio and later confirmed by applying gentle pressure on the tail base to evert the hemipenes.

Feeding

In the wild, Caucasian pit vipers feed on a variety of lizards, rodents and insects (Mallow et al., 2003). In captivity they

Figure 1. The Caucasian pit viper *Gloydius caucasicus* - **A.** Habitat of Lar National Park in central Alborz, northern Iran, **B.** Conducting field searches during the early hours of the day using snake tongs, **C.** The Caucasian pit vipers captured from Lar National Park in August 2018

were offered laboratory mice (10–15 g). In accordance with ethical principles and to prevent the snake from sustaining any potential rodent bite injuries and to reduce venom usage, laboratory mice *Mus musculus albinus* were euthanised with carbon dioxide (CO_2) prior to feeding. For euthanasia, a cage (50 cm long x 25 cm wide x 25 cm high) with a CO_2 flow rate of 5.6 L/min was used. We maintained CO_2 flow for 2–5 minutes to induce anesthesia and death and continued for at least one minute after observing death signs including lack of respiration and faded eye colour.

After euthanasia, the mice were stored at -20 °C for a week to eliminate any possible pathogens or parasites. After completion of an initial adaptation period in the enclosures, the vipers were fed once a week with a diet of newborn and adult mice. We used small prey at the beginning of feeding and after several acceptances of small prey graduated to larger prey. After a few hours, any uneaten or regurgitated food and faeces were removed. Details of food and feeding frequency for each individual snake were recorded on an information sheet attached to each cage and stored electronically. The captive vipers were weighed monthly to estimate their body condition. Freshwater was provided at all times and was changed every three days but if it was contaminated with faeces, it was changed immediately.

Hibernation

Prior to lowering temperatures, all food was withheld from the snakes for six weeks during which time lighting and heating were gradually reduced. The temperature was lowered by 1 °C every week and light was gradually reduced. This started in early October 2018 and lasted until the end of November until the amount of daily light reached zero and temperature had fallen to 8–10 °C. In order to reach such a temperature in the laboratory, cooling was provided by a freezer that gave temperature control through the operation of a small fan in the top of the freezer that was in operation for 5 minutes every hour to exchange the air between the inside the freezer and the room. Humidity was controlled for hibernating snakes at around 60–80 % as a decrease in humidity could lead to water loss and eventually death. To control humidity, a water dish and a clean, wet frozen cloth were placed in the box. During hibernation, water was available to snakes at all times. Also, regular visits were made to check the conditions and change the water. In late February, light and temperature were gradually increased and the snakes emerged from hibernation in the third week of March 2019. The same hibernation procedure was followed in 2020.

OBSERVATIONS ON REPRODUCTION

Pairing the snakes

To avoid outbreeding, we paired only males and females from the Lar National Park (Chanhome et al., 2001; Mitchell, 2004; WHO, 2017). In the spring of 2019, the ten breeding pairs of Caucasian pit vipers were placed together in the boxes for courtship and copulation, although the pairings were not simultaneous. To stimulate mating activity, we placed two male snakes with a female following her postovulatory slough (Siegel & Ford, 1987). Male-to-male combat behaviour was observed prior to mating. After a couple of minutes, the weaker male escaped to the corner of the box. We immediately removed the defeated snake from the box. If a female showed no interest, the remaining male was removed from her box after 3–4 days. To witness copulation, the vipers were checked on an hourly basis. After mating, the male vipers were kept with the females for about two weeks and then returned to their own boxes to reduce any stress during the period of embryonic development. Gravid female snakes are known to consume less food and to have a reduced appetite during pregnancy (Gregory et al., 1999) and so during this period we gave smaller prey to the gravid snakes to avoid any possible harm to the embryos (Osborne, 1982). The diet of the gravid vipers consisted of 2–3 baby white mice weighing less than 10 g per week.

During the last week of pregnancy, gravid vipers ceased feeding and spent their time in close contact with the heat source. In the final stages of pregnancy, we put a wet sponge in the cage to provide adequate humidity for the female viper to give birth; it also helped neonates to emerge from the amniotic sac more easily. We only used sawdust as the substrate rather than other fine particles (e.g. sand, soil etc.) because the particles could stick to the amniotic sac and make it difficult for neonate emergence and may enter the eyes and mouths of the newborn snakes (Gregory et al., 1999).

Reproduction and neonatal care

Mating and copulatory behaviours were observed from 18 June to 5 July. Copulations lasted for up to three hours and of the ten females five were impregnated successfully (Fig. 2A). From 10 to 13 September, 17 Caucasian pit vipers were born (Fig. 2B), only one of which was stillborn. They were kept separately in boxes with perforated sides (30 cm long × 10 cm wide × 10 cm high) with a water dish, a hide, and a sheet of paper as substrate. After parturition, the female moved to the darker and cooler side of the box. Upon emergence from the amniotic sac, the neonates took shelter in the warmest area of the box. The mean (±SD) litter size was 3.40 ± 1.14 (range 2–5), similar to Shakoori et al. (2015) who reported a litter size ranging from 3–5. Normally, on the first day after birth, the young slough but few had sloughed even ten days after birth. Following the first slough, the neonates refused to feed readily and were therefore stimulated to eat or gently force-fed every ten days with newborn mice weighing 2 g. We removed bones from the newborn mice and only fed the soft tissue to the neonates. We also supplemented their food with calcium and vitamins A, C, and D3. It took the neonates approximately three to four weeks to accept food (pre-killed newborn mice) readily. The neonates were not put into hibernation and we continued to feed them during the autumn and winter of 2019.

As parturition occurred in early September, the gestation period for the Caucasian pit viper can be estimated to be 70–90 days. A similar gestation period (2.5 months) for the Caucasian pit viper was reported by Shakoori et al. (2015). Of the 18 females used in their study, 14 were gravid at the time of capture but their reproductive output was poor as

Figure 2. Captive Caucasian pit vipers *Gloydius caucasicus* - **A.** A gravid female, **B.** A female (bottom left) that gave birth to three young, two of which are visible

Table 1. Length and weight measurements for the adult male and female Caucasian pit vipers in the breeding study

Sex	Number	Total length range and mean ± SD (cm)		Tail length range and mean ± SD (cm)		Weight range and mean ± SD (g)	
Male	10	40.50 – 53.70	45.67 ± 1.39	4.50 – 7.00	5.90 ± 0.21	27.31 – 61.44	39.34 ± 3.41
Female	10	41.50 – 47.70	45.42 ± 0.73	4.50 – 5.30	4.86 ± 0.14		
	5 (gravid)					51.85 – 67.84	58.41 ± 2.64
	5 (not gravid)					36.40 – 50.35	42.14 ± 3.21

Table 2. Length and weight measurements for neonate Caucasian pit vipers

Postnatal age	Total length Range and mean ± SD (mm)		Tail length range and mean ± SD (mm)		Weight range and mean ± SD (g)	
Newborn	150.00 – 219.00	189.29 ± 14.57	18.00 – 26.00	22.00 ± 1.70	2.57 – 4.34	3.49 ± 0.49
1 month	181.00 – 224.00	198.17 ± 11.41	20.00 – 28.00	23.41 ± 2.67	3.19 – 4.62	3.83 ± 0.41
2 months	191.00 – 228.00	205.70 ± 10.17	20.00 – 29.00	25.00 ± 2.50	3.91 – 5.66	4.52 ± 0.44

they state that "…. only three young were able to survive for three months", which suggests the need for revised captive breeding protocols. The remaining four females were subjected to hibernation in the hope that they would breed in the following year, but as yet no information has been provided on whether this was successful.

Growth and development of neonates

Total length (from the tip of the snout to the end of the tail), tail length and weight were measured for adult male and female snakes in the breeding study (Table 1). To avoid stressing gravid females and harming developing embryos, the last weighing was done on 29 August (two weeks before parturition). We used an independent t-test to compare differences between adult male and female measurements. Adult females were significantly heavier than male vipers at the time of mate seeking and mating behaviours (t = -3.55, *p* = 0.003). There was no significant difference between male and female vipers in total length (t = 0.14, *p* = 0.890), although males had significantly longer tails (t = 3.27, *p* = 0.005). Shakoori et al. (2015) report that the mean weight for gravid females was 68.92 ± 10.41 g and snout-vent length (SVL) 48.39 ± 2.69 cm, indicating that their female snakes were larger than ours, which were on average 58.41 g and SVL 40.56 cm.

Neonates were measured for total length, tail length and weight at birth, one month after birth (mid-October) and two months after birth (20 November) (Table 2). Shakoori et al. (2015) reported a mean birth weight as 2.81 ± 0.39 g and SVL 148.2 ± 8.4 mm, values that on average were lower than ours which were on average 3.49 g and 167.29 mm SVL.

DISCUSSION

The demand for venom from the Caucasian pit viper for medical purposes and the decline of wild populations of this species make a captive breeding programme essential. A venom comparison of our wild-caught adults, long-term captive adult and captive-born neonates has demonstrated that the venom obtained from captive-bred Caucasian pit vipers is an appropriate source for the production of antivenom in Iran (Rasoulinasab et al., 2020). We believe that the continuation of captive breeding programmes should be a high priority for the Iranian Department of the Environment, to ensure an adequate supply of pit vipers in light of the serious population declines and habitat loss over recent years (Ahmadi et al., 2019; Asadi et al., 2019). Furthermore, this will support the conservation of wild populations by reducing the demand for wild caught specimens.

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RESEARCH ARTICLE

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Natural history of an anuran community in a coastal zone of north-eastern Brazil

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ABSTRACT – We investigated the species composition and reproduction of an anuran community in a Restinga habitat in Ceará State of north-eastern Brazil. Twenty species of four families were observed and most of these reproduced in temporary ponds, whereas *Boana raniceps* and *Scinax x-signatus* were observed in a larger number of reproductive sites and micro-habitats. Vocalisation was seasonal, with the majority of the species showing reproductive activity only during the rainy season. Only three species also vocalised during the dry season. Increases in the number of vocalising species were associated with increases in rainfall and humidity but appeared to fall with rising temperatures. For most species, there was spatial overlap of vocalisation sites and strong temporal overlapping. The anuran community in the area was composed of a mix of species that can also be found in the Caatinga and Cerrado domains but not those of the Atlantic Forest.

INTRODUCTION

There have been several previous studies of amphibian communities in Restingas (coastal, moist broadleaf forest habitats) of eastern Brazil (Xavier et al., 2015) but most of those have focused on anuran composition on the south-east coast, mainly associated with Atlantic Forest (e.g. Rocha et al., 2008; Oliveira & Rocha, 2014; Xavier et al., 2015; Oliveira et al., 2020). There have been fewer studies on the northern coast, in the states of Ceará, Piauí and Maranhão, mostly in the Delta do Parnaíba (Loebmann & Mai, 2008; Loebmann et al., 2010; Andrade et al., 2014; 2016; Araújo et al., 2018; 2020) and only one on the coast of Ceará (Borges-Leite et al., 2014).

The Restinga habitats in Brazil, especially in the north-east region, suffer from a high degree of degradation, due to hotels and beach houses built for tourism, which has led to deforestation, sand dune degradation and pollution of water bodies (Lima et al., 2000, Silva et al., 2011; Gondim-Silva et al., 2016). These impacts may lead to the disappearance or even extirpation of amphibian species. Our research has focused on anuran species composition, habitat use and calling activity in a Restinga habitat in Ceará state, north-eastern Brazil to better understand the natural history of the anurans in this region.

MATERIALS & METHODS

Study area

The study area was located in the Ecological Station of Pecém (3° 35´33” S, 38° 47´24” W, 18 m a.s.l.), a state Conservation Unit in the Municipality of Caucaia, Ceará state, 1.5 km from the Atlantic Ocean and 40 km from Fortaleza, the state capital. The area is located on Cenozoic sedimentary deposits of the Barreiras Formation (Souza, 1988), having Quartzarenic Neosol (Embrapa, 1999) as the predominant soil type. The climate is Hot Tropical semi-arid, with a pre-rainy season from November to January and a rainy season from February to May, with the highest rainfall in February to April (Funceme, 2006). The vegetation of the area is composed of coastal forest on sandy soil, with a predominance of herbaceous and shrub vegetation, which are associated with sand dune forests (Figueiredo, 1997; Castro et al., 2012).

Study sites

Observations on anuran vocalisation were made at the following sites:

Site I: A permanent pond (accumulating water the entire year), dimensions 1,195 m x 200 m, to a depth of 2.5 m with a sandy bottom and muddy areas. The vegetation included an abundance of cyperaceous *Kyllinga* sp and *Eleocharis interstincta* at the margins and the pond was set within an arbustive-arboreal vegetation with a predominance of Anacardiaceae and Malpighiaceae. This was the only water body with fish - *Cichla ocellaris* and *Hoplias malabaricus*.

Site II: An ephemeral pond (accumulating water for a maximum of three months yearly), dimensions 10 m x 6 m to a depth of 0.5 m. It had muddy and sandy portions at the margins, was 100 m from site I and was surrounded with the same vegetation as site I.

Site III: A semi-permanent pond (accumulating water for more than three months but less than a year), dimensions 500 m x 20 m to a depth of 1.5 m. It was located between sand dunes in the forest interior, 20 m from site I. There was a predominance of *Eleocharis interstincta* both within the pond and on its margins.

Site IV: A shaded forested area, without water accumulation, with sandy soil and accumulated leaf litter. The vegetation was dominated by *Anacardium occidentale*, *Byrsonima crassifolia*, *Chrysobalanus icaco*, and *Ipomoea asarifolia*.

Methodology

Data were collected monthly from April 2005 to May 2006 using a complete species inventory methodology (sensu Rocha et al., 2004). Observations were made between 17:00 h and 23:00 h for four days/month in the wet season and one day/month in the dry season, giving a total of 26 days of observation and 156 man-hours of effort.

Each site was monitored every hour from 17:00 h and 23:00 h, and for each species observed, we recorded the microhabitat used, calling site, and the vocalisation period. Specimens of the plants associated with the anuran calling sites were collected and deposited in the Herbário Prisco Bezerra at the Federal University of Ceará, Brazil.

Voucher specimens of the anurans were collected, euthanised with Tiopental anaesthetic, fixed with 10 % formalin and preserved in 70 % alcohol. The specimens were deposited in three Brazilian collections (for details see Appendix 1 in the Supplementary Material). For the species taxonomy we followed Frost (2023).

Mean monthly data on temperature, humidity and rainfall were obtained from the Fundação Cearense de Meteorologia (FUNCEME) station located in the municipality of São Gonçalo do Amarante, about 30 km from the study area.

RESULTS

Composition and calling site

We recorded a total of 20 anuran species, distributed in four families: Leptodactylidae (10 spp), Hylidae (7 spp), Bufonidae (2 sp) and Microhylidae (1 sp) (Table 1).

The ephemeral and semi-permanent ponds had higher and similar diversity of calling species than the permanent pond and the forested area (Table 2). Some species had characteristic calling sites. For example, *Leptodactylus pustulatus* was only found vocalising from within the water bodies where it was well camouflaged among the debris and dead vegetation of *Eleocharis* sp. *Scinax fuscomarginatus* was found calling at 40–60 cm above the water's surface and *Dendropsophus minusculus* at 10–30 cm above the water's surface.

Leptodactylus troglodytes and *L. mystaceus* were occasionally observed calling from the swampy water body margins but most of the time they were calling in the leaf litter of the forest, in site IV, usually hidden under roots, dead trunks or in small holes. *Boana raniceps* and *Scinax x-signatus* called while partially submerged in the water within the ponds as well as from arbustive-arboreal vegetation away from the water. *Physalaemus albifrons*, *P. cuvieri*, *Leptodactylus macrosternum* and *Pleurodema diplolister* vocalised only from the water's surface, usually hidden among the aquatic vegetation. *Elachistocleis* cf. *piauiensis* was found calling in a vertical position leaning against the aquatic vegetation, partially submersed in the water. *Rhinella granulosa* was only found foraging in the site IV, we did not record calling and/or reproductive activity for the species in the area.

Table 1. Anurans species recorded at different sites at ESEC Pecém, Ceará, Brazil and those vocalising at the various study sites, from April 2005 to May of 2006. All species have been classified as of Least Concern (IUCN, 2022).

Family	**Species**	Site I	Site II	Site III	Site IV
Bufonidae	*Rhinella granulosa* (Spix, 1824)				X
	Rhinella diptycha (Cope, 1862)	X	X		
Hylidae	*Dendropsophus minusculus* (Rivero, 1971)	X	X	X	
	Dendropsophus soaresi (Caramaschi & Jim, 1983)		X	X	
	Boana raniceps (Cope, 1862)	X	X	X	
	Scinax fuscomarginatus (Lutz, 1925)	X	X	X	
	Scinax nebulosus (Spix, 1824)		X		
	Scinax x-signatus (Spix, 1824)	X	X	X	
	Pithecopus gonzagai Andrade, Haga, Ferreira, Recco-Pimentel, Toledo & Bruschi, 2020			X	X
Leptodactylidae	*Leptodactylus fuscus* (Schneider, 1799)		X		
	Leptodactylus vastus A. Lutz, 1930		X	X	
	Leptodactylus mystaceus (Spix, 1824)				X
	Leptodactylus macrosternum Miranda-Ribeiro, 1926				X
	Leptodactylus pustulatus (Peters, 1870)		X	X	X
	Leptodactylus troglodytes Lutz, 1926	X			X
	Physalaemus albifrons (Spix, 1824)		X	X	X
	Physalaemus cuvieri Fitzinger, 1826		X	X	
	Pseudopaludicola mystacalis (Cope, 1887)	X	X	X	X
	Pleurodema diplolister (Peters, 1870)		X	X	
Microhylidae	*Elachistocleis* cf. *piauiensis* Caramaschi & Jim, 1983	X	X	X	X
	Total number of species vocalising	**8**	**15**	**13**	**9**

Calling season

At the beginning of the rainy season, January and February, the first species to start calling were *Rhinella diptycha*, *Leptodactylus fuscus*, *L. troglodytes*, *Dendropsophus minusculus* and *Pseudopaludicola mystacalis*. Two months

Table 2. Reproductive seasonality based on male anuran vocalisation at ESEC Pecém, Ceará, Brazil, from April 2005 to May 2006

SPECIES	A	M	J	J	A	S	O	N	D	J	F	M	A	M
Rhinella diptycha										■	■			
Dendropsophus minusculus	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Dendropsophus soaresi		■											■	
Boana raniceps	■	■	■	■									■	■
Scinax fuscomarginatus	■	■	■										■	■
Scinax nebulosus			■											
Scinax x-signatus		■	■									■	■	■
Pithecopus gonzagai	■	■												■
Leptodactylus fuscus	■									■	■	■	■	
Leptodactylus vastus												■	■	
Leptodactylus mystaceus	■	■										■	■	
Leptodactylus macrosternum													■	
Leptodactylus pustulatus		■	■	■	■	■	■						■	■
Leptodactylus troglodytes	■	■								■	■	■	■	■
Physalaemus albifrons		■	■									■	■	
Physalaemus cuvieri	■	■	■									■	■	■
Pseudopaludicola mystacalis	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Pleurodema diplolister		■										■	■	■
Elachistocleis cf. *piauiensis*		■	■									■	■	■
TOTAL	9	14	10	4	3	3	3	2	2	5	5	11	16	11

later, after the intensity of rainfall had increased, we observed a greater number of calling species in the area with the arrival of the *Physalaemus* spp and most of the hylids (Table 2).

Most of the species began to call at dusk, around 17:30 h, with increasing calling activity at the beginning of the night, most intensively from 19:00 to 21:00 h. The only species observed calling during the day time was *Pseudopaludicola mystacalis*, which continued calling into the night.

Most of the species showed an explosive or intermediate breeding behaviour, concentrated over a few weeks or months only during the rainy season (Table 2). Only *D. minusculus*, *P. mystacalis* and *L. pustulatus* were observed calling in the dry season. *Dendropsophus minusculus* and *P. mystacalis* called all year long while *L. pustulatus* vocalised for six continuous months from May to October (Table 2).

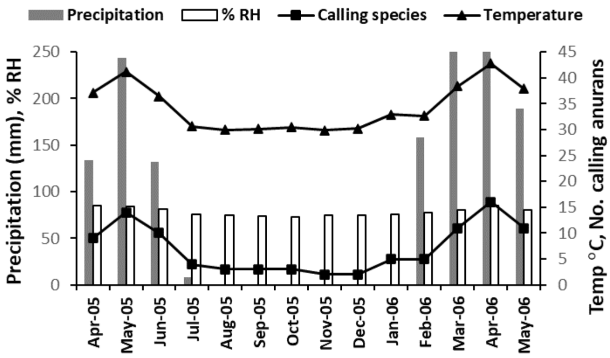

Figure 1. Number of anuran species calling plotted against the prevailing climatic conditions - total monthly rainfall (mm), % relative humidity and mean monthly temperature at ESEC Pecém, Ceará, Brazil, from April 2005 to May 2006. Environmental data from FUNCEME (2006)

DISCUSSION

The anuran community we have described comprised 20 species that are typical of the open environments of the Caatinga and Cerrado domains (Valdujo et al., 2012) and could also have included a further three species *Proceratophrys cristiceps*, *Adenomera hylaedactyla* and *Dermatonotus muelleri* that are known to occur at the Ecological Station of Pecém. The community is similar to that of São Gonçalo do Amarante, another coastal area in Ceará State closely located to our study area, where 23 species were recorded (Borges-Leite et al., 2014). The more southerly coastal areas include species associated with Atlantic forest, which as a result have greater anuran diversity (Araújo et al., 2020; Oliveira et al., 2020). Most of the species observed in the Ecological Station of Pecém anuran community use temporary ponds for reproduction and these species are explosive breeders (sensu Wells, 1977). To exploit temporary ponds they are rapid colonisers and have fast growth rates (Wilbur & Collins, 1973; Wilbur, 1997). In temporary ponds the availability of suspended nutrients for tadpoles, such as phytoplankton, is greater than in older or permanent ponds, which can influence the suitability of ponds for reproduction (Wilbur, 1995). Furthermore, the presence of carnivorous fish in the permanent pond may be a deterrent (Heyer et al., 1975; Azevedo-Ramos & Magnusson, 1999).

A high overlap in the spatial distribution of calling sites was verified for most of the species, including congeners such as *Physalaemus albifrons* with *P. cuvieri*, and *Leptodactylus mystaceus* with *L. troglodytes*. This has been reported previously for other leptodactylid species (e.g. Pombal Jr, 1997; Rossa-Feres & Jim, 2001), mostly because of the exclusive use of the horizontal substrates of soil or water, instead of vertical substrates as used by the hylids (e.g. Cardoso et al., 1989; Bertoluci & Rodrigues, 2002; Protázio et al., 2015).

Most species showed either explosive or intermediate reproductive patterns, vocalising only during the humid and rainy period of the year, typical of the seasonal tropics (Duellman & Trueb, 1986). Only three species vocalised during both the hotter and drier period of the year; these species show prolonged or continuous patterns of reproduction: *Dendropsophus minusculus* and *Pseudopaludicola mystacalis* vocalised throughout the year, while *Leptodactylus pustulatus* vocalised for six consecutive months in 2005, three of them during the dry season. Haddad & Cardoso (1987) found continuous reproduction through the year for *Pseudopaludicola mystacalis*. However, it should be noted that vocalisation can also play different social roles, not related to reproduction (Toledo et al., 2015).

At the beginning of the rainy season there is an increase in the rate of precipitation which is associated with decreasing air temperature and increasing relative humidity (Fig. 1). In anuran communities with greater seasonal climatic changes, as in the Caatinga, Chaco and Cerrado habitats, weather is strongly correlated with the calling activity and breeding period (Prado & Pombal Jr, 2005; Kopp & Eterovick, 2006).

A strong temporal overlap was observed in the reproductive period among the species, which suggests that reproduction at different times of the year may not represent an important mechanism of reproductive isolation or habitat partitioning between species of this community. The same pattern was also observed for other anuran communities in open area domains, such as Cerrado and Caatinga (e.g. Arzabe, 1999; Toledo et al., 2003; Prado & Pombal Jr, 2005; Cascon & Langguth, 2016).

Our study contributes to the understanding of the diversity and natural history of anurans on the coast of Ceará state. Even though all of the amphibian species recorded at this site are classified as of least concern (IUCN, 2022), the negative impacts of deforestation, forest fires and discarded waste inside this conservation unit present a significant conservation threat.

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RESEARCH ARTICLE

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Husbandry and first record of captive breeding of the Asian giant river toad *Phrynoidis asper*

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ABSTRACT – The Asian giant river toad *Phrynoidis asper* is a large species of bufonid from south-east Asia that is apparently obtained by the international exotic-pet trade from wild populations. Captive breeding of this species seems not to have been documented. The donation to Chester Zoo in October 2021 of an adult group of five males and one female of this toad species provided an opportunity to study captive breeding. The specimens were maintained separated by sex until signs of reproductive condition were apparent and then they were placed together in a large breeding enclosure. This had three distinct environmental zones simulating a riverbank. Six days after mixing the sexes a large spawn mass was laid. On hatching, the tadpoles were transferred to a rearing aquarium and then, after reaching Gosner stage 42, the developing toads were transferred to terrestrial vivaria to complete development. Captive breeding of this species could replace collection from the wild.

INTRODUCTION

The genus *Phrynoidis* (Bufonidae) currently comprises two described species, *Phrynoidis asper* and *Phrynoidis juxtasper* (Frost, 2023) that are widely distributed in south-east Asia (Frost, 2023). *Phrynoidis asper* (Gravenhorst, 1829), commonly known as the spiny river toad or Asian giant river toad, is known to occur in Vietnam, southern Myanmar, western and peninsular Thailand, Malaya, Sumatra, Borneo, and Java (Frost, 2023), where it may be found from sea level up to 1500 m (Inger, 1966).

Phrynoidis asper is a large species, with females attaining snout vent lengths (SVL) of 95–215 mm and males 70–100 mm (Inger & Stuebing, 2005) although despite the species' large size in the wild it shows a diet preference for ants and termites (Hui et al., 2014). Throughout its range it is closely associated with rivers and streams and reproduction is believed to be non-seasonal. Males can be observed calling from water bodies year round (Inger & Bacon, 1968), amplexus is axillary, and a single female may deposit up to 12,000 eggs in a single spawning (Inger & Bacon, 1968). Dorsally the colouration is generally brown with green or red flecks, ventrally the colouration is yellowish cream. There are large parotoid glands the size of which varies depending on geographic location (Inger, 1966) and the skin is covered in conical tubercules usually tipped with melanin (Inger, 1966). Skin glands release toxins that predominantly contain bufotelin and have had significant effect on mice in laboratory tests (Daly et al., 2004).

Although populations of *P. asper* have been noted to be declining, the species is assessed as of Least Concern by the IUCN (Chanson et al., 2021). The species is also increasingly present as wild collected individuals within the international exotic pet trade with no evidence of captive breeding (Choquette et al., 2020). This report details captive breeding of *P. asper* at Chester Zoo (Great Britain) during 2022 and may represent the first successful breeding of this species in captivity.

MATERIALS & METHODS

An adult group of one female and five male *P. asper* were donated to Chester Zoo's Herpetological collection by a private keeper in October 2021. This group is believed to have been collected from the wild for the pet trade at an unknown locality and had been in captivity for two to three years previously. The sexes were initially maintained separately under dry conditions to reduce activity during acclimation, and to begin reproductive cycling, before transfer to a large breeding enclosure as detailed below.

The equipment used to measure the environmental parameters described herein were as follows; Surface temperatures - a Mini RayTemp infrared thermometer; Air temperatures and humidity - a temperature and hygrometer digital probe (Electronic Temperature Instruments Ltd); UVB zones - a Model 6.5R Reptile UV Index Meter (Solar Meter®); and water chemistry parameters - an API Freshwater test kit and colour chart (Aquarium Pharmaceuticals Inc.).

Dry Period Enclosure

From October 2021 until March 2022 two identical enclosures were used to maintain sexes separately. These consisted of large preformed open top containers measuring (2.4 m long x 1.4 m wide x 1.4 m high) with a substrate of loose soil and gravel, leaf litter, live plants *Dieffenbachia seguine* and *Spathiphylum* sp. for low cover, large cork bark pieces and a preformed plastic hide box to provide an additional refuge. A large shallow water bowl was always present and was refreshed daily. The specimens were provided with heating and lighting from a 24 w Light wave canopy (Growth Technology Ltd) containing two 6 % T5 Arcadia (Monkfield Nutrition Ltd) UVB emitting lamps combined with a UVB emitting basking spot of a 100w Arcadia D3 basking lamp. This created a localised area of heat and light with a UVB gradient of 0–3.5 and temperature of 28–30 °C across an area of approximately 60 cm^2, the photoperiod was 12:12. The ambient temperature was between 23–28 °C during daytime and 18–20 °C at night. The enclosure was lightly misted with water once each evening, the open top enclosure allowed the environment to become dry after a short period following spraying. Food was offered three times per week and consisted of adult black field crickets *Teleogryllus commodus*, adult brown cricket *Acheta domesticus*, locusts *Schistocerca gregaria* and dubia cockroaches *Blaptica dubia*. All food items were gut loaded with Repashy Superload (Repashy Specialty Pet Products ©) 24 hours prior to feeding and dusted with Arcadia Earth Pro A vitamin and mineral supplement before being offered to the toads.

Breeding Enclosure

This enclosure simulated a stream and large open water area to induce mating behaviour and oviposition (Fig. 1). It was constructed from a large commercially available paddling pool (4 m long x 2 m wide x 1 m high) and divided into three distinct environmental zones; a land area (1 m x 2 m), an intermediate shallow water area (1 m x 2 m) and a fully aquatic area (2 m x 2 m).

The land area of the enclosure was constructed using plastic pallets to create a raised false flooring. A lower level was added using the same false floor method to create an intermediate shallow water area in a ‘step’ effect before the drop into the pool. A tarp was used to create a barrier between the false flooring and the substrate, which was a mixture of soil, gravel and leaf litter on the land area, and solely gravel on the intermediate area. These areas were furnished with logs ranging in length from 1–2 m, creating a landscaped environment. A basking zone area of increased heat, light and UVB exposure measuring 45 x 80 cm was provided by an Arcadia Thermal Zoo Pro unit which was fitted with a 50 w Arcadia deep heat projector, 100 w Arcadia incandescent spot lamp, 24 w Arcadia 6 % UVB T5 and a 24w Arcadia Jungle dawn LED. The temperature of the basking zone was 28–32 °C and the UVI range was 1.0–1.8. The ambient daytime temperature range was 23–27 °C and the nighttime temperature was 20–22 °C.

Within the open pool the water depth was approximately 30 cm, emergent large boulders were placed to provide areas above the water line from which males could call. The aquatic plant *Elodea densa* was provided for cover, and as potential egg deposition sites. The water temperature was 18–20 °C. Two circulation pumps were used, the first circulated water from the pool to within a curved, hollow log on the land area which channelled the water back down into the pool, simulating a stream (Syncra Silent 4.0, Sicce S.r.l). The second (Green Line Pond Pump 8000, Velda B. V.) was linked to an overhead rain system constructed from plastic pipework with irrigation sprayer nozzles (LBS Worldwide Ltd) placed throughout, which simulated rainfall when the pump was in use. The rainfall covered the entirety of the aquatic section and approximately two thirds of the land area ensuring that specimens had continuous access to some drier areas. The water from simulated rainfall was circulated from the main pool within the enclosure through the rain system, with water being topped up in the pool when required. Netting was used above the enclosure to prevent escape whilst maintaining high air flow.

Figure 1. The captive breeding enclosure for *Phrynoidis asper*

Five male specimens were introduced to the breeding enclosure on 21 March 2022. The stream remained flowing permanently and the rain system was not used during this time, the land area was sprayed up to twice daily with a pump action spray gun to provide humidity. From 16 April the rain system was turned on 15:00–16:30 h to initiate an environmental change. The female was introduced on 18 April at which point the rain system was turned on for 24 hours. At the time the sexes were together a CamPark T150 trap camera (CamPark Electronics ©) was used to record behaviour and activity of the group both night and day.

Care of eggs, larvae and metamorphosed toads

Following egg deposition, the spawn remained in-situ within the breeding pool for the duration of development and hatching, an aquarium air stone was placed by the egg mass to provide additional oxygenation. Upon hatching, tadpoles were transferred into a rearing aquarium (90 cm long x 30 cm wide x 30 cm high) filled to 60 L at a stocking

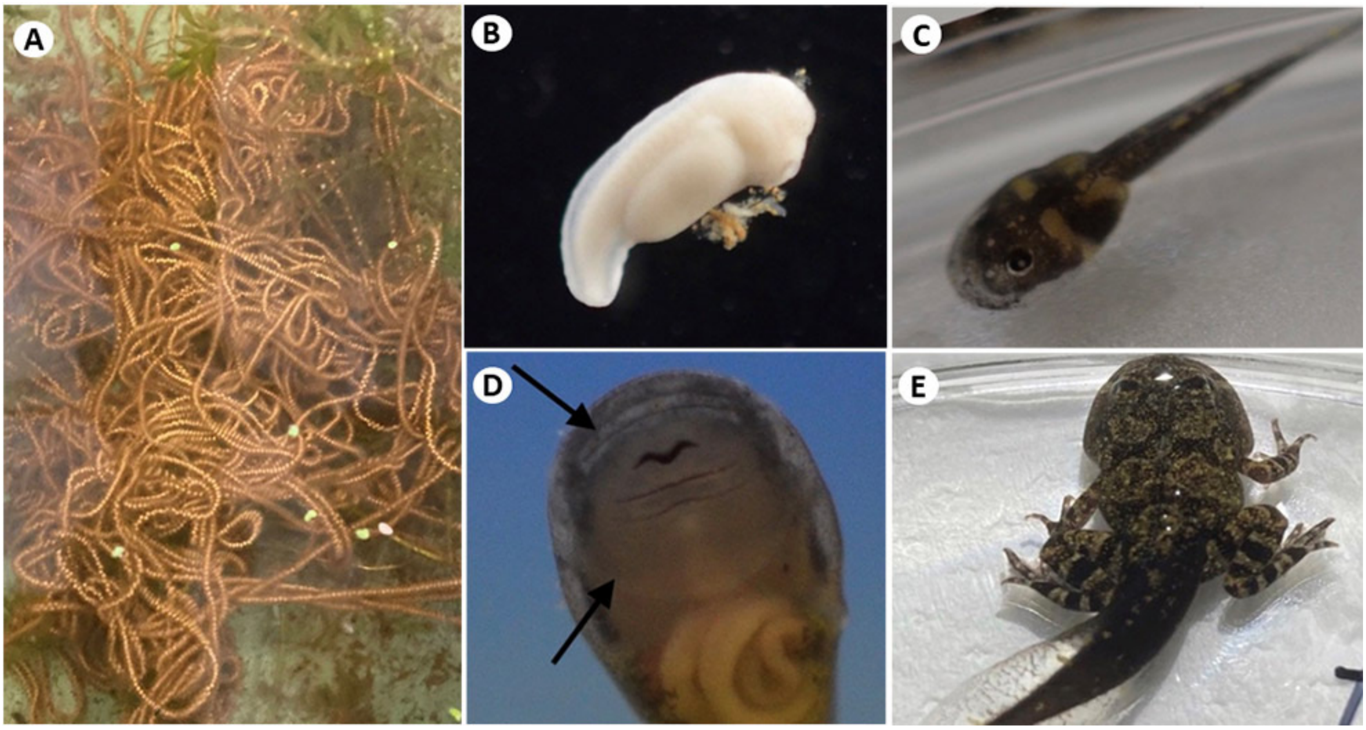

Figure 2. Some developmental stages of the captive bred *Phrynoidis asper* - **A.** Spawn mass, **B.** Prematurely hatched tadpole at Gosner stage 19, **C.** Tadpole in dorsal view showing pattern and pigmentation, **D.** Ventral view of tadpole showing the specialised oral disc (arrows), **E.** Developing toad at Gosner stage 42

density of one tadpole per litre. Water was maintained at a temperature of 18.5–21.5 °C, a pH of 7.6–7.8 and general hardness was 143.2 ppm. All water used for aquarium water changes and maintenance was Heavy Metal Axe (HMA) filtered tap water. Filtration was provided by two airline aquarium sponge filters and one Fluval mechanical filter (Rolf C. Hagen Inc.), providing oxygenation and directional water movement across the aquarium, simulating a high flow-rate stream. Lighting was provided by a 15 w 10,000 k Daylight Photon Energy LED (Wave Point® Technology) on a 12:12 photoperiod. Aquarium furnishings consisted of large smooth stones, angled slates providing shelter and a surface for the tadpoles to feed upon and cling to, and cuttings of plastic pipe. *Elodea densa* was provided for cover and the surface of the water was approximately 25 % covered by duckweed (*Lemnoideae* sp.) providing a photo gradient. No substrate was used. Waste was spot cleaned daily, and 25 % water changes took place every 3–5 days.

Food was provided daily and consisted of an Arcadia Amphibi gold pellet which was crumbled into the aquarium and sunk to the floor, generic tropical fish flake, and spirulina powder mixed with water to make a paste which was then dried onto the slates provided as above; Repashy Soilent Green, which was also prepared using the same method as the spirulina slates, and a mixture of Repashy Soilent Green, Repashy Red Rum and Arcadia Amphibi gold pellet ground into a powder with pestle and mortar at a 2:1:1 ratio and then also dried onto slates. All food slates were replaced every 24–48 hours.

At Gosner stage 42 (Gosner, 1960) (Fig. 2E) the developing toadlets were removed from the tadpole rearing aquarium and housed at a density of 20 individuals in an Exo Terra glass vivarium (Rolf C. Hagen Inc.) (45 cm long x 30 cm wide x 30 cm high) with a shallow water body at the front aerated using an aquarium pump and air stone. The land area was provided using an EpiWeb (Dusk Tropic ©) sheet covered with a thin layer of tropical soil seeded with springtails *Siera* sp. for food, and covered with dried leaf litter. Stacks of cork bark were used to provide a humidity gradient and as refuges. Lighting was provided by an Arcadia ProT5 6 % UVB and an Arcadia Jungle Dawn LED. Enclosures were heated using room air conditioning that gave an air temperature gradient of 20–28 °C and a UVB gradient of 0.0–2.0. Specimens were fed daily with a mixture of *Drosophila melanogaster*, *Drosophila hydei*, and 1st instar *A. domesticus* dusted with Repashy Calcium Plus. Approximately two months after metamorphosis the substrate was covered with rehydrated sheet moss and additional cork bark refuges were installed.

After four months specimens were transferred to a larger Exo Terra glass vivarium (60 cm long x 45 cm wide x 30 cm high) with the aerated water bodies replaced by large shallow water bowls. Enclosure furnishings were the same as above although specimens were provided with an Arcadia 35 w halogen basking spot, producing a localised surface temperature of 30 °C. Specimens were observed in groups of up to eight individuals under pieces of cork bark beneath and around the basking spot. The same food was provided as above although cricket instar size offered were larger, between 1st (5 mm) and 3rd (8–10 mm) instar.

RESULTS

During the initial dry period, weight and visual body scoring was used to determine health status; all the specimens

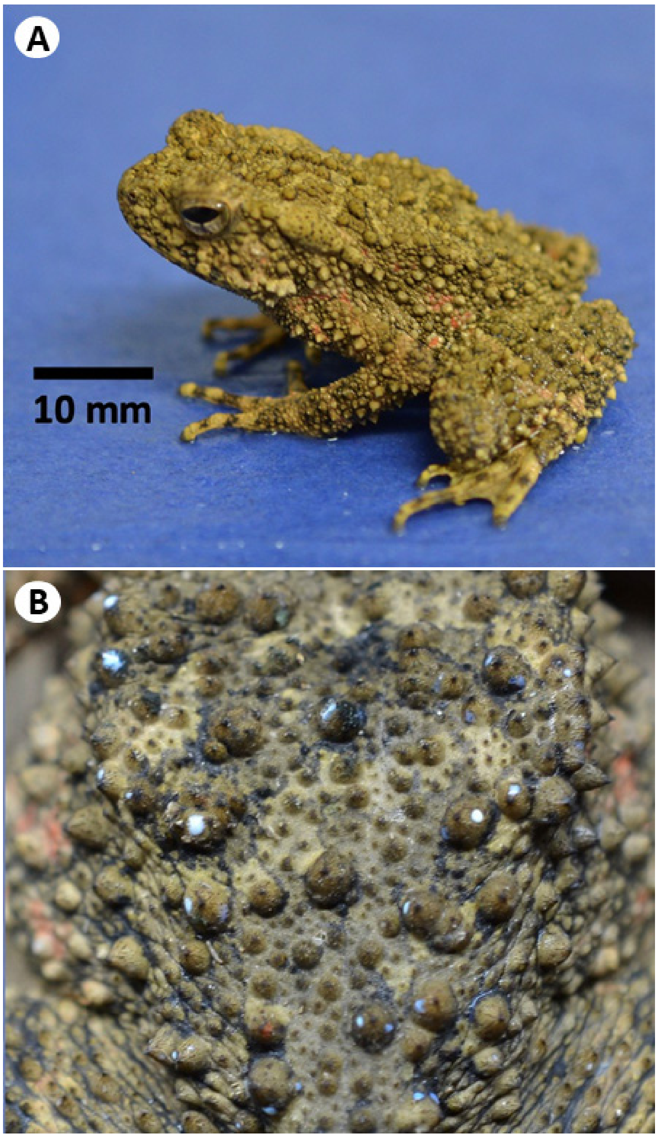

Figure 3. **A.** Six-month-old captive bred *Phrynoidis asper*, **B.** Toxic white secretions exuding from tubercules on the skin of a captive bred individual

appeared healthy. The mean ± sd weight of five males was 234.8 g ± 66 g. The female began this period with a weight of 608 g and following the dry period regime weighed 600 g. The consistent weight of the female together with the development of nuptial thumb pads in males was used as an indicator of readiness for a breeding attempt. Upon the initial introduction of the male specimens to the breeding enclosure they appeared to be very active, with specimens noted by keepers to be in different positions of the enclosure each day. Following the introduction of the female to this enclosure on 18 April, coinciding with initiation of rainfall, a high level of nocturnal activity was observed, with males vocalising predominantly from the water's edge but also from emergent rocks within the pool area. This behaviour persisted for an additional five nights.

On the morning of 21 April males were recorded to be very active from 01:00 h, amplexus with the female was initiated early morning at 04:45 h, when the female paired with a male was actively moving around the pool. Subsequently, conspecific males attempted to displace the successful male and over time up to three males were observed in amplexus with the female. From 09:00 h, two males were in amplexus with the female and at approximately 11:00 h spawn was observed in the pool (Fig. 2A); the spawn appeared to have been fertilised by these two males. Following oviposition the two males separated from the female and she was returned to the dry enclosure. At the time of removal from the breeding enclosure the female was weighed and was observed to have lost 56 g as a result of oviposition.

The spawn mass consisted of one continuous string of several thousand individual cream to white eggs measuring approximately 2 mm in diameter. At a water temperature of 18–20 °C the first stages of development were observed within 72 hours. Five days following oviposition at Gosner stage 19 (Gosner, 1960) when the egg mass was disturbed during monitoring, developing larvae were observed to hatch prematurely (Fig. 2B), falling from the egg mass to the floor of the pool, where normal development continued. Eight days following oviposition, those larvae that remained within the egg mass had completed normal stages of development and begun hatching naturally; prematurely hatched larvae had reached the equivalent stage. Hatched larvae used short bursts of movement to disperse from the spawning site but they were then observed remaining motionless attached to the walls and objects within the enclosure such as plants, pipes and rocks. The tadpoles, which still had a pale pigmentation, appeared not to be feeding at this time and were probably relying on the large yolk reserves that could be seen through the ventral body wall. The hatched egg mass was then removed to prevent polluting the water body.

After a further 48 hours the tadpoles had developed dark pigmentation (Fig. 2C) and begun to feed. Their total length was about 5 mm and they possessed a specialised large oral disc (Fig. 2D) that was used to cling to surfaces constantly. Free swimming behaviour was not observed, instead movement was made by short bursts along surfaces whilst clinging by the mouthparts. Individuals fed readily from the food slates provided and upon algae forming on the glass of the aquarium. During the later stages of larval development tadpoles showed a preference for higher protein content foods such as Repashy Soilent Green and slates containing Arcadia Amphibi gold pellets, food slates containing algae alone were visited less frequently at stages close to metamorphosis. The first individual reached Gosner stage 42 (Gosner, 1960) and metamorphosis following 120 days aquatic development with a total length about 20 mm. Upon being moved to the aquatic area of the rearing enclosure, metamorphosing toads were observed on land within 24 hours, completed metamorphosis and feeding in an additional 48 hours, and following absorption of the tail, their total length was about 10 mm. Under the described conditions, at eight months following metamorphosis 38 captive born *P. asper* had a mean ± sd body weight of 12.63 g ± 3.51 and SVL of 46.31 mm ± 3.76 (Fig. 3A).

DISCUSSION

Despite being a non-seasonal breeder in the wild (Inger & Bacon, 1968), keeping *P. asper* initially in drier conditions appeared to aid in gaining and maintaining the body condition needed for reproduction. Prior to our study, there were no descriptions of captive breeding for *P. asper*, consequently we devised a breeding enclosure with three zones to simulate as far as possible the toad's ecological niche. Once the toads were introduced into this breeding enclosure this led swiftly to reproduction. However, it should be noted that the introduction of the female to the breeding enclosure coincided with a natural low-pressure weather system. This could have helped induce reproductive behaviour in the males as it is known that captive amphibians may use external changes in barometric pressure as a reproductive cue (Poole & Grow, 2012). Further research at times with low barometric pressure should help clarify this issue.

To improve the success of future captive breeding, we recommend further investigation into the best method of maintaining the spawn, in particular improvements that would avoid premature hatching of the larvae. Premature hatching in amphibians is known to be a defence mechanism (Warkentin, 2011) and although in our study the prematurely hatched larvae continued development they eventually suffered a higher mortality rate than those that hatched at the normal time. A further recommendation is to use matured water bodies with established algae growth upon walls and furnishings. This would allow newly hatched tadpoles to begin feeding when ready and reduce the need to manually rehouse tadpoles during this delicate stage, which risks causing damage to them. It is worth noting that we found that a temporary fault with the aquarium air stone led to some tadpole deaths. This may indicate a particular need for water oxygenation, which would be expected in a species that breeds in streams and rivers.

In the experience of the authors, *P. asper* adapt well to captivity provided that their space requirements are met. Their large size means that they are an engaging species for display in zoological collections, although keepers must exercise caution when handling specimens due to the toxic secretions from the skin (Daly et al., 2004), which are even released by the captive born juveniles, when stressed during handling (Fig. 3B).

The international trade of wild collected amphibian species has been shown to be linked to the spread of pathogens such as the amphibian chytrid fungus *Batrachochytrium dendrobatidis* and ranavirus (Fisher et al., 2007; Picco & Collins, 2008), which presents a risk to zoological collections as well as to wild amphibian populations worldwide (Greenberg & Palen, 2019). Wild collected amphibians may also have high parasite burdens and fail to acclimate to captivity without appropriate specialist care (Write & Whitaker, 2001). A responsibility of modern zoological collections is to establish repeatable methods of captive breeding to reduce these risks by providing sustainable populations without the need for wild collection. Knowledge gained and shared from captive breeding may also, in the future, prove beneficial for the ex-situ conservation of endangered species with similar requirements. Due to the large number of eggs produced by *P. asper*, it would require only relatively few but regular breeding episodes to replace the collection of this species from the wild.

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SHORT NOTE

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Interactions between the endemic gecko *Phelsuma inexpectata* and the introduced *Phelsuma laticauda*: understanding the drivers of invasion on Reunion Island

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INTRODUCTION

The introduction of non-native species of reptiles raise many concerns regarding the potential impact they may exert on indigenous species. The day geckos (*Phelsuma* spp) are territorial lizards. Several species have been repeatedly introduced to many areas outside their natural range (Fieldsend et al., 2021). One species, the gold dust day gecko *Phelsuma laticauda* is frequently reported as an invasive species (Dubos et al., 2014) and considered an aggressive competitor (Lund, 2015). It was first reported on Reunion Island in 1975 (Moutou, 1995) and is today present in habitats alongside the endemic *Phelsuma inexpectata*, a species listed as Critically Endangered (CR) on the IUCN Red List (Sanchez, 2021). Here we report observations on interspecific interactions between the two species, including filmed material (BHS video, 2023), from a botanical garden where the native species *P. inexpectata* was first observed in 2012 and the non-native *P. laticauda* was first observed in 2015 (Personal obs., J.M. Luspot).

MATERIALS & METHODS

The study site was located in the botanical garden Café Grillé (21.30° S, 55.42° E) in the south of Reunion Island of the Western Indian Ocean. On 23 November 2022 we monitored basking sites and floral food resources of an occupied patch for one hour. These resources typically attract a large number of individuals of both *P. laticauda* and *P. inexpectata* (Hoarau et al., 2021). Two observers (JMP & JC) were positioned in front of an inflorescence of the introduced palm *Dypsis lutescens* and two observers (GD & XP) in front of the native *Pandanus utilis*. Basking sites were located at different heights on branches and trunks of the trees, while floral food resources were located about 10 m above ground. Geckos were filmed using a Sony RX10 (1-inch sensor /24-200 mm f/2.8 lens) camera. In addition we counted geckos (JC, XP & ND) along the edges of *Pandanus* thickets. We performed four 5-minute counts between 08:30 h and 14:30 h. On 8 February 2023, we repeated the observation of basking sites and floral food resources, but not the observations on thicket edges, using the protocol described above.

We counted interactions between the two gecko species on two types of substrates: floral food resources (e.g. fruits, nectar and pollen of *Pandanus utilis* and *Dypsis inflorescences*) and sunspots (basking sites) on the branches and trunks of *Pandanus utilis*. Interactions were classified as either aggressive or non-aggressive. We considered an aggressive behaviour as an attempt by one gecko to either bite or intimidate another, leading to its retreat. A non-aggressive (passive) interaction was recorded when both species were present on the same substrate (e.g. food or sunspot) with no observed aggressive interaction. We also recorded for both species the number of ritual displays, i.e. tail movements (raising or flicking) that are behaviours displayed by *Phelsuma* spp. when they are, for example, hunting (Wehsener & Noss, 2022) or territorial signalling (Caceres et al., 2010; Wehsener, 2019). We also recorded the number of retreats following an interspecific interaction.

RESULTS

The count across sampling occasions for *P. inexpectata* was 16 individuals and 188 for *P. laticauda*. We observed 18 interactions between the two species (Fig. 2). Non-aggressive interactions were the most frequent (67 %; n = 12). At sunspots, we observed five non-aggressive interactions and two aggressive interactions (one for each species). On floral food resources we counted five interactions that were non-aggressive with three aggressive interactions of *P. laticauda* against *P. inexpectata* and one of *P. inexpectata* against *P. laticauda* (BHS video, 2023). *Phelsuma laticauda* signalled their presence (tail movements) six times but *P. inexpectata*

Figure 1. Group of *Phelsuma laticauda* and *Phelsuma inexpectata* on the same screwpine *Pandanus utilis*

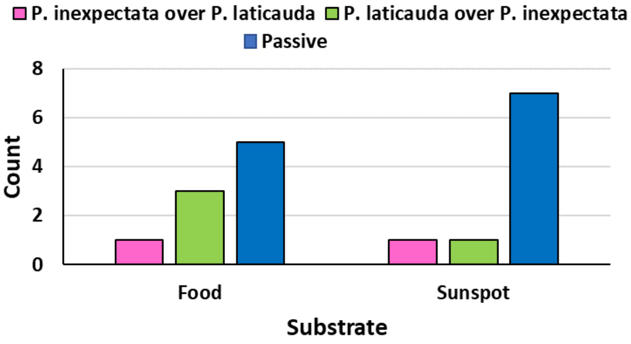

Figure 2. Number of interspecific interactions between *Phelsuma inexpectata* and *Phelsuma laticauda* and the outcome of these interactions on food resources and sunspots (basking sites)

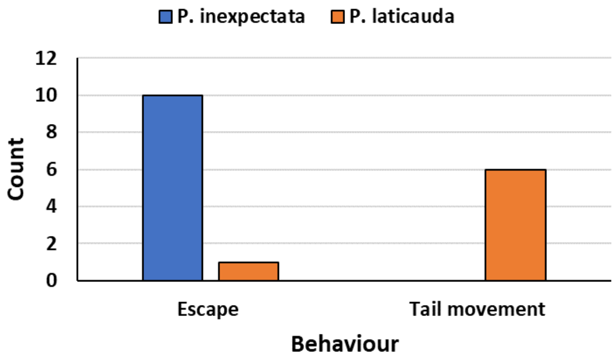

Figure 3. Number of ritualistic displays and escape behaviours during interspecific interactions between *Phelsuma inexpectata* and *Phelsuma laticauda*

was not observed to display this behaviour (Fig. 3). On ten occasions *P. inexpectata* avoided contact with *P. laticauda* by circumnavigating, whilst *P. laticauda* retreated only once from *P. inexpectata* (Fig. 3).

DISCUSSION

Our study was brief, comprising observations from two days only, hence, represent limited data. Furthermore, gecko behaviour may show seasonal variation (Choeur et al., 2023) so that any conclusions about the interactions between the two species are at present tentative until more data are available. This should include information on population size and how populations are distributed in different habitats. Nevertheless, we provide some evidence of direct competition for floral food resources and basking sites between the invasive *P. laticauda* and the endemic *P. inexpectata*. While both species are capable of physically repelling each other (BHS video, 2023) most of the observed interactions were non-aggressive. However, *P. laticauda* showed frequent use of ritual displays towards the endemic species. *Phelsuma inexpectata* showed these displays intra-specifically suggesting that the signals from *P. laticauda* were possibly recognised and likely causally related to *P. inexpectata* retreat behaviours.

In lizards, inter-specific interactions are complex with subtle differences in behaviour and habitat occupancy that allow coexistence, such as differences in activity peaks (Luiselli & Capizzi, 1999; Porcel et al., 2021), use of supports (Williams et al., 2020; Porcel et al., 2021), food resources (Simbula et al., 2019), and sometimes distinct diurnal and nocturnal roosting sites (Zughaiyir, 2016; Zduneck, 2022; Meek & Luiselli, 2022). All species of the genus *Phelsuma* are frequent visitors of floral food resources, these are often very localised, thus attracting several individuals to the same location. This increases the likelihood of contacts and hence conflicts. It should be noted that our observations were made at a site with abundant floral food resources that might be expected to increase tolerance between the two species. However, when resources are limited, aggressive interactions may increase. The local association Nature Océan Indien addresses this issue in the framework of the 'Refuges pour le Gecko vert de Manapany' project using citizen conservation. The association works with local homeowners and provides endemic plants and guidance for the restoration and protection of urban habitat within the *P. inexpectata* distribution.

Despite the fact that *P. inexpectata* was first detected in the botanical garden, three years before *P. laticauda*, the latter is in now the most abundant species. This suggests that it has higher population dynamics; a factor that may contribute to its status as an invasive species. In addition, *P. inexpectata* has colonised only specific sections of the botanical garden whereas *P. laticauda* is now found widely across the whole estate, suggesting the use of a wider range of habitats. The displacement of other native gecko populations by non-native lizards to more specialised habitats has been documented, a good example being *Phyllodactylus pulcher* by *Hemidactylus mabouia* in Barbados (Williams et al., 2020).

We recommend demographic studies and the monitoring of habitat use for both species to better understand the population dynamics of *P. laticauda*. Potential avenues to explore are the observed more frequent use of native plants by *P. inexpectata* than *P. laticauda* (Porcel et al., 2021) and comparisons of the geckos thermal ecologies.

This study found some support for the notion that aggressive behaviour of *P. laticauda* is a driver of its invasiveness. However, we emphasise that wider habitat use, potentially higher population dynamics and the predicted positive response of *P. laticauda* to climate change (Dubos et al, 2022a; 2022b) may be even more powerful drivers of invasion in the future.

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SHORT COMMUNICATION

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Mass bathing in the slow worm *Anguis fragilis*

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There are few published accounts of slow worms *Anguis fragilis* swimming or bathing, and where there are reports they describe only single individuals in water. On 7 July 1967, one adult *A. fragilis* in Germany was described by Dathe (1971) swimming in a channel 10 m wide and 1 m deep. The water temperature was about 18 °C. Petzold (1971) writes that slow worms do not voluntary move into water, but if threatened they may swim well, with the head above water. Poivre (1980) reports that the slow worms bathe voluntarily and swim very well, but drown if they do not get to dry land fast enough; he doubts that they swim voluntarily. Gollman & Gollman (2008) reported an unusual diving behaviour of one juvenile slow worm in a puddle near Vienna, Austria, on a sunny day, 27 April 2008. They suggested that *A. fragilis* may dive into richly structured water bodies for both escape and foraging. Eggert (2016) observed a male slow worm swimming in a large pond in the evening of 10 May 2012, and he could not see any reason to believe that it was forced into the water. Badziukiewicz (2021) writes that slow worms can swim perfectly, however this is not normally mentioned in reptile field guide books (for example: Fog et al., 1997; Arnold & Ovenden, 2002; Speybroeck et al., 2016), though it is mentioned that the species is often found in damp or moist areas. However, on the internet there are a few photos and videos on YouTube that show slow worms bathing and swimming, and there is even a photo that documents a foraging slow worm swimming in water in Britain at the end of June 2019 (RSPB Wildlife, 2021). From these various reports of swimming slow worms, we may conclude that *A. fragilis* may forage in water.

However, these earlier observations show only one individual at a time, and the purpose might be foraging. Here we report mass bathing by up to 12 female slow worms *A. fragilis* together, and their purpose did not seem to be foraging. We also report repeated observations of mass bathing slow worms on three different dates at six sites. All the observations are made in the same area, at the centre of the island Karmøy, north of Stavanger city, on the south-western coast of Norway (Fig. 1). Karmøy is a relatively flat island of 177 km^2, the highest point is 132 m a.s.l., the open landscape consisting of rocks, swamps, heather, lakes and pastures. Climate is mild and moist, with most rain in October and the least in May, however the spring and summer of 2022 was warm and dry. On 18 August 2022 the second author went for a walk at Revsåna where he discovered two small, shallow puddles close together, with a total of 12 slow worms in the water (59° 12’58.1978” N, 5° 14’57.6926” E, Fig. 2). This was first time the author observed such an aggregation of slow worms, and the first time to find slow worms in water (YouTube, 2022). These small, shallow puddles were about 10 cm deep with an area of 2 m^2. They were laying still in the water with the head above water, or swimming slowly around before laying still again. The author did not observe

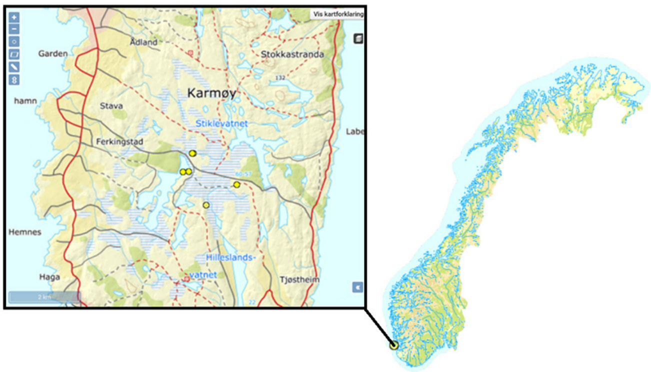

Figure 1. Map of Norway and Karmøy island on the south-western coast. The six locations with bathing slow worms *Anguis fragilis* are indicated with yellow dots. Two dots nearly overlap. The map is made in Artsobservasjoner.no.

Figure 2. Mass bathing by six female slow worms *Anguis fragilis* in a shallow puddle at Karmøy, Norway, 18 August 2022. There were 12 slow worms in two nearby puddles on that day.

Figure 3. Three female slow worms *Anguis fragilis* bathing together in a shallow puddle at Karmøy, Norway, 20 August 2022

Figure 4. Three female slow worms *Anguis fragilis* in a shallow puddle at Karmøy, Norway, 26 August 2022

any behaviour that could seem like foraging or diving, nor did the slow worms appear to be escaping from a predator. They were relaxed and enjoying a bath.

Two days later, on 20 August the second author went for another walk 1 km further south, on the southern shore of the lake Sandvatn (59° 12’40.6775’’ N, 5° 14’51.6647’’ E) where he found several bathing slow worms. A new location along Burmavegen the same day was also found, where several slow worms were bathing inside three small puddles (59° 12’28.5653’’ N, 5° 16’20.1437’’ E, Fig. 3 & YouTube, 2022). Their behaviour was similar, they were laying still in the water with heads up, seemingly enjoying their bath. This made the second author curious to see if the slow worms were still present in the first puddles at Revsåna observed two days earlier, and a further visit to that site showed seven slow worms in the two puddles, still relaxing in the water with heads up.

On 26 August the second author went for a walk to a new area at Karmøy, where he discovered a small puddle with three slow worms bathing (59° 12’09.2999’’ N, 5° 15’23.6778’’ E, Fig. 4). He also returned to Sandvatn and found a new puddle where four slow worms were bathing (59° 12’40.5327’’ N, 5° 14’41.0577’’ E).

In total, six localities were found with shallow puddles with several bathing slow worms in each, on 18, 20 and 26 August 2022. Apart from these summer time observations, there was a single slow worm observed in a puddle much earlier in the year, on the 19 March 2022 (59° 12’8.2577’’ N, 5° 14’59.7690’’ E).

Why were the slow worms bathing en mass in August? It appeared that all the slow worms were adult females with dark sides, a thin dark stripe on their backs and small heads (Smith, 1990). Also, their thickened bodies imply that they were gravid. In Norway, slow worms are known to give birth in August–September (Fog et al., 1997; pers.obs.) which means that the bathing females at Karmøy during the period of observation of 18 to 26 August were probably soon to give birth. Although slow worms are active at a range of body temperatures from 10 °C to nearly 40 °C (Fog et al., 1997) the optimal body temperature for slow worms, at least in France, is 28 °C (Poivre, 1980). However, it is not uncommon for gravid female reptiles to maintain higher body temperatures (Capula & Luiselli, 1993; Poivre, 1980; Fog et al., 1997), otherwise prolonged gestation periods would result in gravid females hibernating and giving birth the following year (Fog et al., 1997). In an alpine population of *A. fragilis* the mean body temperature of gravid slow worms was 27.0 °C compared to 25.2 °C in non-gravid females (Capula & Luiselli, 1993). August 2022 at Karmøy was mostly warm and dry except a few cold nights, and the vegetation was dry as there had been little rain from May to August. The temperature in the shallow puddles was not measured, but it is realistic to expect the water to have been nice and warm since it was only 10 cm deep and the surrounding open terrain was not shaded by trees. If the water in the puddles was warmer than the surroundings then the simplest explanation for this behaviour is that the gravid slow worms were bathing to raise their body temperature so increasing the rate of embryo development and shortening their pregnancy. This behaviour appeared to make the females very visible to predators on land and in the air, which is contrary to the usually very secretive behaviour of slow worms. The advantages of maintaining a higher body temperature would appear to outweigh the increased risk of predation (Capula & Luiselli, 1993). It will be interesting to see if mass bathing by slow worms at Karmøy will be observed in future years, if so then it may be possible to make some associations with prevailing climatic conditions.

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SHORT COMMUNICATION

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Palmate newts *Lissotriton helveticus* infected with *Amphibiocystidium* sp. in France

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Infectious diseases are listed among the main causes of global species extinctions (Smith et al., 2006), especially in amphibians (Catenazzi, 2015) which are very sensitive to chytridiomycosis (Fisher & Garner, 2020), Ranavirus (Gray et al., 2009) as well as other viral or fungal pathogens and trematode flukes (Hoverman et al., 2012). The parasite *Amphibiocystidium* (Pascolini et al., 2003), a fungal-like protist on the animal-fungal boundary (González-Hernández et al., 2010) has been found in both frogs (Pascolini et al., 2003; Fagotti et al., 2019) and newts (Raffel et al., 2008; Courtois et al., 2013) in Western Europe and North America. The lesions that result from this infection mainly occur on the skin, which facilitates its detection (Courtois et al. 2013, González-Hernández et al., 2010), but internal organs, particularly the liver, may also be lethally affected (González-Hernández et al., 2010; Raffel et al., 2008). In France, this parasitosis has been reported in populations of palmate newt *Lissotriton helveticus* inhabiting areas with low human influence, in the Pyrenees (Ariège) (Courtois et al., 2013) and in Larzac (Aveyron) (González-Hernández et al., 2010). In this study, we report the case of these large skin lesions, characteristic of infection by *Amphibiocystidium* sp., in the palmate newt *Lissotriton helveticus* in a small forest pond at low altitudes in western France.

On 15 April 2022, around 21:30 h, we were pond netting in search of amphibians suffering from *Amphibiocystidium* sp. infection in two small forest ponds located about 150 m apart in Deux-Sèvres department, France (-0° 25'31.44" W, 46° 8'48.84" N, 70 m a.s.l.). We captured a total of 106 *L. helveticus* from both ponds (Table 1). In pond 1, of 69 specimens we detected eight infected females (12 %) and one infected male (1.4 %). Individuals presented lesions that resemble those of *Amphibiocystidium* (see Courtois et al., 2013), but the presence of the parasite was not formally tested for using DNA analysis. The lesions observed ranged from single or few cutaneous lesions to multiple coalescing skin ulcers (Fig. 1). In pond 2, of 37 *L. helveticus* none were infected. In the two ponds combined, we observed that 8.5 % of individuals were infected of these 7.5 % were female and 1 % male.

The prevalence of *Amphibiocystidium* sp. in newts can vary between populations or ponds. Courtois et al. (2013) investigated the prevalence of *Amphibiocystidium* sp. in nine populations of palmate newt and found that five populations had infected individuals (21 individuals out of 356 were

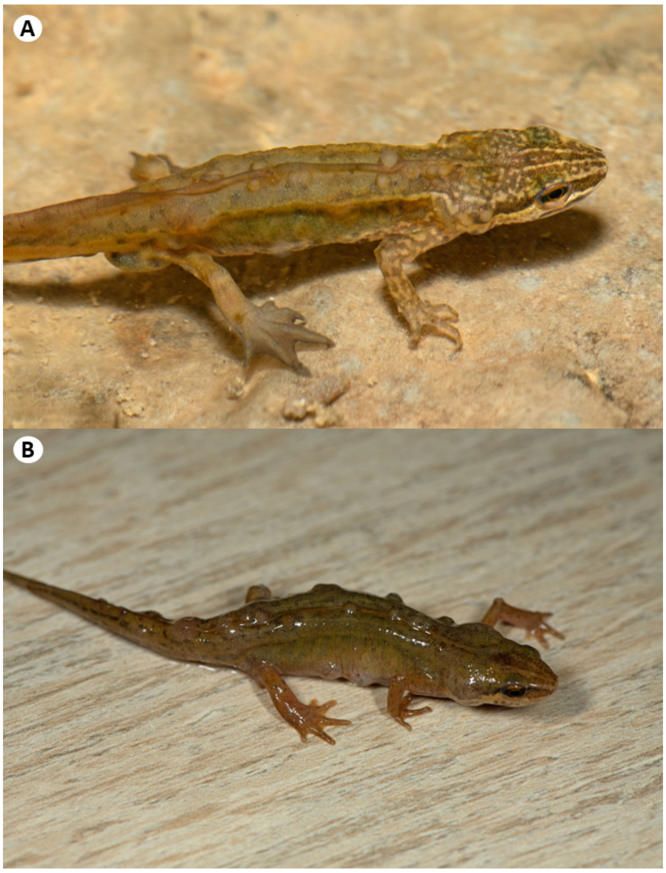

Figure 1. **A.** Male and **B.** female *Lissotriton helveticus* suspected to be infected with *Amphibiocystidium* sp. found in a pond in the Deux-Sèvres Department (Western France)

Table 1. Numbers of uninfected *Lissotriton helveticus* and those presumably infected with *Amphibiocystidium* sp, in two ponds in the Deux-Sèvres epartment (Western France)

	Male		Female		
	Uninfected	Infected	Uninfected	Infected	Totals
Pond 1	11	1	49	8	69
Pond 2	16	0	21	0	37
Totals	27	1	70	8	106

infected). In these populations, prevalence ranged from 2.5 to 25 % (mean ± sd 12.1 % ± 9.5 %). In our study, the difference in prevalence between two closely located ponds, 13 % in pond 1 and 0 % in pond 2, is puzzling and deserves specific investigations in order to understand the individual and environmental drivers of *Amphibiocystidium* infection.

The skin lesions of *Amphibiocystidium* infection may interfere with gas and water exchange through the skin (González-Hernández et al., 2010), increase the risk of secondary infections by other diseases and microorganisms, and the costs of immune responses may also be a trade-off with growth, maintenance, and reproduction (Raffel et al., 2008). Infection may reduce fitness and survival imperiling amphibian populations. Consequently, there is an urgent need to document both the potential impact of this pathogen on amphibian species as well as its geographical distribution.

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SHORT COMMUNICATION

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Scarfing - a novel agonistic behaviour between a copulating and a competing male northern viper *Vipera berus*

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In the Spring of 2021, I was asked to help film the northern viper *Vipera berus* for a new wildlife series, Wild Isles. This highlights the natural history and habitats of the British Isles and was released in March 2023 by the British Broadcasting Corporation (BBC). Over the first two weeks of April, we captured on film the spring emergence, basking, sloughing, courtship, wrestling and breeding of vipers, but what made the trip unique was a behaviour between adult males that I have never observed before; 'scarfing' - not an official technical term, but rather the best description given by those who observed it.

Filming took place in northern England at a wild location chosen for its vastness, a glaciated upland valley of grassland, heather, bilberry and bracken and, most importantly, supporting a scattered, robust population of vipers. To capture numerous snake interactions and breeding behaviours meant travelling to a wild landscape location, perhaps a sad reflection on how fragmented a once widespread British snake species has become, certainly in the counties of lowland central and southern England, where many populations of vipers are now in small populations on sites under growing anthropogenic pressures (Gardner et al., 2019). In such places the chances of capturing widespread natural interactions is difficult and fraught with disturbance issues.

From the first day of arrival on site, 1 April, male sloughing, courtship and male combat were in full swing, earlier than reported at many other sites in England that year. This was happening despite overnight freezing temperatures, sleet, snow and rain. During the day, heather moorland on nearby hillsides was being burnt as part of moorland management but also to improve the habitat for game birds, particularly the red grouse *Lagopus lagopus* and common pheasant *Phasianus colchicus*. This burning was being undertaken whilst snakes over this landscape were emerging and attempting to breed.

The viper group on which we focused occupied a steep, rock strewn river valley, a less favourable area for moorland burning. Despite this it was still noted that some of the adult snakes had what appeared to be old burn scars and healed possible peck wounds from birds. Over 50 individual vipers were seen throughout the valley over two weeks filming, suggesting a robust, widely scattered population, possibly even benefitting from widescale control of local predators. Shooting estate moors have a reputation for low tolerance towards game bird predators, these include birds, especially corvids, mammals, such as mustelids and foxes, all of which are also viper predators. However, any benefit to vipers is likely offset by both widescale annual moorland burning and pheasant releases which can result in damage to the populations of both vipers and other reptile species (Graitson et al., 2022), in this case viviparous lizards *Zootoca vivipara* and slowworms *Anguis fragilis*; viviparous lizard are considered an important prey species for juvenile vipers.

Figure 1. A copulating male with neck encircled by the tail of a male interloper. This 'scarfing' could go on from a few seconds up to 4 minutes. The scarfing male would lose its grip and then re-assert its hold again. The scarfing male did not have its hemipenes everted during these interactions.

Scarfing

For the first time, I witnessed courtship groups of male and female vipers where an interloper male intruded on a mating pair and wrapped his tail tightly, almost constrictor like, around the neck and head area of a copulating male (Figs. 1 & 2). The hold was positioned just behind the head and occasionally a little further down the body, but the head and neck area were the main focus. This unusual behaviour was given the name 'scarfing' due to its focus on the neck area. A second male, identified by its markings and scars, was observed scarfing in a separate courtship group (Fig. 3). Scarfing continued for just a few seconds up to 4 minutes until the copulating male could pull his head out from the hold after which the interloper again encircled the neck and head area; this happened over the 30–40 minutes that the snakes were mating.

Figure 2. The same pair of male snakes as in Figure 1 with interloper laid across the back of the copulating male and encircling tightly the other male's head and neck

Figure 3. Scarfing between a further pair of males at a different area of the site from that shown in Figures 1 & 2

Adult male vipers engage in vigorous combat bouts during the mating season, with larger males driving smaller individuals away from receptive females (Andrén et al., 1986; Madsen et al., 1988). A dominant and possibly larger snake usually pushes off a competitor, engaging in multiple combats, and then mates with the female. This is different from scarfing, which did not involve males in active combat and occurred when one of the males (the scarfed male) was already mating with a female.

So is scarfing a deliberate attempt to disrupt or dislodge the copulating male or simply an aspect of frenetic, confused courtship behaviour? At first I thought that the interloper male had confused the head of the copulating male with the female's tail and was trying to locate the female cloaca. However, the behaviour was repeated many times and would appear to involve deliberate encircling and gripping with the tail rather than attempts to frantically locate the cloaca with his tail. Hemipenes were never seen everted by the interloper males so it seems unlikely that the interloper males had confused the sexes. The behaviour appeared too deliberate and prolonged to be frantic confusion, but rather an attempt to restrain the neck area of the male in coitus, perhaps to irritate, disrupt, even force the connected male snake to disengage from the female.

It is a puzzle that scarfing has remained undescribed for so long despite the fact that *V. berus* is one of the most intensively studied snake species. The behaviour may perhaps be rare and/or confined to certain populations. Its late description brings to mind the observation of death feigning (thanatosis) in northern vipers which was first described in only 2013 (Hodges, 2013). In that case, it was suggested that the rarity was due to it being a behaviour of last resort, perhaps that is also true of scarfing. Let's hope there are still more rare viper behaviours for the enthusiast to record.

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SHORT COMMUNICATION

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Treatment of dystocia in a captive false map turtle *Graptemys pseudogeographica*

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Dystocia, often referred to as egg binding, is common in reptiles (Mader et al., 1994; Sykes, 2010) and birds (Abou-Zahr et al., 2019). It describes the situation where eggs are either retained within the body or stuck within the cloaca (pelvic canal). Egg binding can be caused by attempts to pass abnormally large eggs, behavioural stress caused by lack of adequate areas to nest, nutritional deficits, or disease. It is a serious health risk and can result in death if the eggs are not removed.

In the Biodiversity Laboratory of Saint Joseph's University (SJU), we maintain large colonies of turtles for behavioural research and conservation. Recently we encountered a young female *Graptemys pseudogeographica* with an egg lodged in her cloaca (Fig. 1). Attempts to manually dislodge the egg failed, so the animal was transported to our attending veterinarian at the Radnor Veterinary Hospital.

At the hospital the map turtle was weighed (450 g) and X-rays were taken, which revealed several eggs within the animal (Fig. 2). A number of these eggs were misshapen, with some being smaller than normal, and two being larger than normal. To remedy the situation, the egg was extracted from the cloaca by the following procedure (percloacal ovocentesis). An 18-gauge catheter (without stylet) was placed onto a 3 cc syringe containing lubrication gel, and the catheter inserted around the egg, through the cloaca. Once the egg was lubricated with the gel, the catheter was removed and a 20 cc hypodermic needle was used to puncture the egg and extract the yolk. The egg shell was then squeezed out to clear the cloaca. Following the procedure, the turtle was prescribed a 0.1 mL injection of the antibiotic Fortaz every 72 hours and 0.45 mL of calcium gluconate every 12 hours for 14 days. The calcium gluconate was provided to replace female calcium, which is used in the egg-laying muscle contractions. Following the procedure, the turtle successfully passed the remaining eggs.

Figure 1. View of *Graptemys pseudogeographica* female with an egg lodged in her cloaca

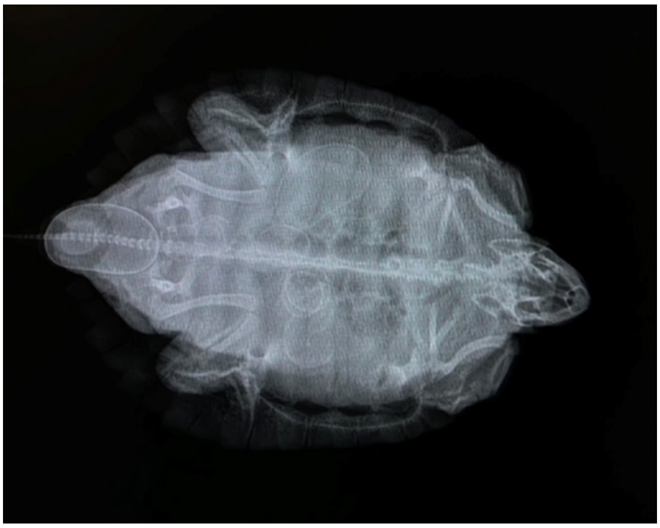

Figure 2. X-ray of *Graptemys pseudogeographica* female, showing large egg lodged in cloaca, along with another large egg near left rear leg, and 3-5 smaller eggs in central body region

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SHORT COMMUNICATION

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Supernumerary rattle growth in a Mexican lance-headed rattlesnake *Crotalus polystictus*

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Cases of teratological conditions and other developmental abnormalities are important to document in reptiles and amphibians because they can bring to light new emergent pathogens, diseases or conditions that can have ecological and conservation implications (Barr et al., 2020; Kaiser, 1997; Schmidt, 1997; Bishop et al., 1991; 1998; Bell et al., 2006) or highlight understudied physiological, developmental or healing processes that are in need of more focused research (Barr et al., 2019; Alibardi & Meyer-Rochow, 2021). In rattlesnakes (*Crotalus* and *Sistrurus*), a well-studied group of New World pit vipers (Crotalinae), a wide variety of developmental aberrations have been recorded (for example Klauber, 1956; Pendlebury, 1976; Wallach, 2007; Sant'Anna et al., 2013; Murphy, 2018), but with regard to the specialised crotaline rattle, abnormalities have been rare; they include a case of accelerated growth in the structure which resulted in the accumulation of an abnormal number of rattle segments over a brief period (Walker et al., 2008), and the loss of the rattle style and matrix (Rice et al., 2016). Here, we report on an unusual aberration in an adult Mexican lance-headed rattlesnake *Crotalus polystictus* that resulted in the growth of secondary rattle tissue.

As part of an Association of Zoos and Aquariums-managed Species Survival Plan (SSP) for the species, *C. polystictus* has been maintained by Audubon Zoo since 2002. In August 2019, a 13-year-old captive-bred male (AZA regional studbook #192) that had been maintained at Audubon Zoo since 2009, underwent a veterinary physical examination due to concerns over an uncharacteristic decrease in its body mass (30.6 %) over the previous winter cooling period, and apparent difficulties with regaining its lost weight despite feeding regularly. The examination was performed by staff veterinarians while the animal was conscious and restrained in a tube, and its demeanor was bright, alert, and responsive. Full body orthogonal radiographs and coelomic ultrasonography showed no abnormalities. Blood was collected from the caudal coccygeal vein and submitted for a complete blood count, biochemistry panel, and protein electrophoresis; no parameters were significantly abnormal. Upon close visual inspection, a small (ca. 1 mm), firm, subcutaneous swelling was noticed on the right lateral aspect of the tail at the base of the rattle, which, based on its size and general appearance, was presumed to be a caseous abscess. The mass was aspirated using a 22-gauge hypodermic needle, and a small amount of yellowish liquid was collected and submitted for cytological examination. No microbial organisms were observed; occasional white blood cells were noted. The mass was left untreated to be closely monitored for changes in size and potential impacts on the animal's activity and behaviour.

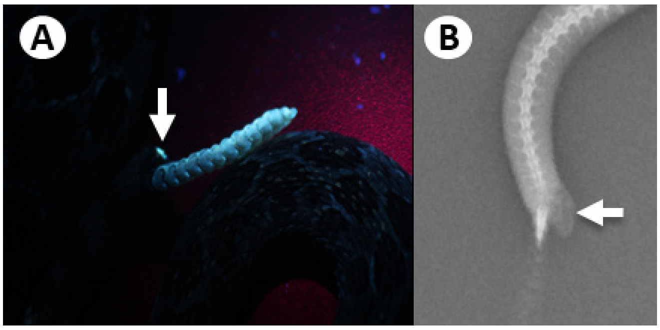

Figure 1. *Crotalus polystictus* rattle and secondary rattle - **A.** tissue growth (white arrow) fluorescing under 365 nm UV light, and **B.** Dorsoventral radiograph showing the soft tissue opacity (white arrow) of the secondary rattle tissue growth

A follow-up examination in September 2020 revealed that the mass had increased in size to ca. 2 mm in diameter and had begun to protrude from the skin. With the specimen restrained in a tube, the exposed emergent tissue was closely examined and revealed by palpation not to be an abscess, but rather the same keratinised tissue as the rattle. This was later reaffirmed during a study on pit viper biofluorescence (Paul & Mendyk, 2021) in which the emergent tissue fluoresced the same colour and intensity as the primary rattle when illuminated with a 365 nm ultraviolet LED torch in a darkened room (Fig. 1A). Since the snake had begun to regain its body weight, was eating well and was otherwise behaving normally, it was decided not to intervene surgically and to continue monitoring the mass.

In December 2021, the specimen was re-examined, measured (69.2 cm, SVL 74.9 cm, TL 212 g), and radiographed again. Dorsoventral radiographs showed a soft tissue opacity growth lateral to the rattle on the right side (Fig. 1B), and there was no evidence of infection or other disease process to the underlying bone. The continued outward growth of the mass appeared to be displacing the primary rattle, causing it

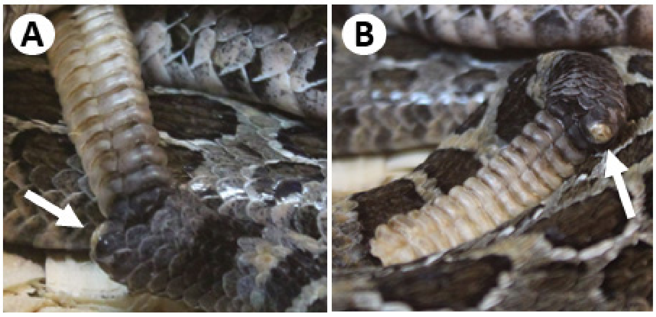

Figure 2. *Crotalus polystictus* rattle and secondary rattle tissue growth - **A.** Displacement of the primary rattle by the secondary rattle tissue growth (white arrow), and **B.** Detail of supernumerary rattle growth (white arrow)

to angle upwards at an inclination of ca. 50 degrees (Fig. 2A), with the externalised portion then protruding ca. 3 mm (Fig. 2B) and the entire mass measuring ca. 4 mm in diameter. Just a few months later in April 2022, it was noticed that the mass had been ejected from the body, possibly during ecdysis, and could not be located, leaving an empty walled-off cavity that eventually closed up on its own after a few weeks. No immediate signs of regrowth have since been observed. Notably, defensive usage of the rattle was not observed during the development and subsequent loss of the supernumerary growth; this has continued long after recovery and no further usage of the rattle has been observed to date.

This account appears to represent the first documented case of supernumerary rattle growth in a rattlesnake. Considering that no similar cases have been reported in the literature for either *Crotalus* or *Sistrurus* when probably tens of thousands of specimens have been collected as museum vouchers, studied in the field, and maintained in captivity over the last two centuries (e.g. Klauber, 1956; Murphy & Armstrong, 1978; Murphy, 2017), the condition is presumed to be extremely rare. Since biopsy of the affected tissues at the base of the rattle for histological analysis was not considered essential for the health and welfare of the individual, and because the ejected mass could not be located for further analysis, the etiology of the condition remains unclear.

In lizards, the growth of bifid and supernumerary tails is typically the result of failures in tail regeneration related to trauma or disturbance to the spinal cord (Bellairs & Bryant, 1985; Alibardi, 2010), but in rattlesnakes, the rattle is produced through an entirely different mechanism that is independent of the spinal cord (Meik & Schuett, 2016). Moreover, since there was no evidence of prior trauma to the tail of the *C. polystictus*, an injury-related etiology seems unlikely. The possibility of a teratoma or other neoplastic disorder could not be ruled out.

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NATURAL HISTORY NOTE

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Attempted predation of a cave olm *Proteus anguinus* by a dice water snake *Natrix tessellata*, in Bosnia and Herzegovina

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The blind European cave salamander or olm *Proteus anguinus* is a large and slender neotenous salamander, only found naturally in the western Balkans (Speybroeck et al., 2016). Their maximum length varies according to which river basin they inhabit but is typically 25–55 cm. They are characterised by their pinkish-white colouration, paddle-like tail, and reduced number of digits on all four limbs. The species inhabits both flowing and stagnant subterranean waterbodies in karstic limestone formations throughout their range, as long as these are chemically unpolluted waters, with a stable temperature range of 8–15 °C. *Proteus anguinus* are almost always observed in caves, for which they have a number of adaptations to allow them to survive such a challenging environment (Hervant et al., 2001; Issartel et al., 2009; Balázs et al., 2020). Due to their specialised adaptations to subterranean life, *P. anguinus* are particularly vulnerable to changes to the karst ecosystems they inhabit, such as contamination from anthropogenic sources (Kolar, 2019). The European cave salamander also has no known predators within its natural underground habitats.

The dice snake *Natrix tessellata* is a semi-aquatic species that may grow to over a metre in length and is found throughout most of central and south-eastern Europe (Speybroeck et al., 2016). *Natrix tessellata* is typically olive-green or brown in colour with four rows of square-like black blotches along the body, although not all individuals are marked in this way. The species feeds primarily on fish and amphibians (Hutinec & Mebert, 2011; Weiperth et al., 2014), and inhabits both freshwater and saltwater environments such as lakes and shorelines (Speybroeck et al., 2016).

On 4 July 2021, a *P. anguinus* was observed near the town of Trebinje (Bosnia and Herzegovina) swimming in a small stream after inadvertently straying out of a nearby cave system (Fig. 1). While seeking shelter under nearby boulders, the *P. anguinus* was dislodged by a juvenile *N. tessellata*, which latched onto the tail (BHS video, 2023). The *N. tessellata* was far too small to be able to consume the *P. anguinus*, and may have mistaken the tail for a smaller prey item. Unfortunately, the *P. anguinus* later died as a likely consequence of being flushed into sub-optimal conditions in the strong flow of the adjacent Trebišnjica River. The individual was some 36 cm in length and so almost certainly a fully mature adult *P. anguinus*. The *N. tessellata* is gauged to be approximately 54 cm long. These body-length measurements were estimated against those of the easily recognisable rocks in the video recording, and were accurately measured at a later date. A few minutes after the start of the confrontation, both animals were washed out into the strong flow of the surface river, and not observed again.

Figure 1. The dice snake *Natrix tessellata* can be clearly seen wrapping its jaws over the underside mid-tail area of the stray olm *Proteus anguinus*

In Bosnia and Herzegovina, this iconic amphibian is endemic to certain parts of only five river basins, where they live in many cave ecosystems of the Dinaric Karst. During periods of exceptionally high rainfall, *P. anguinus* can be washed out of their underground aquatic habitats into the adjacent surface waterways such as the observation we report here, although such observations have rarely been reported in the local media. The species has exceptionally good survival strategies including that of naturally swimming upstream (positive rheotaxis) (Durand & Parzefall, 1987;

Lewarne & Balázs, 2019). Occasionally, during low-flow underground conditions, individuals may also inadvertently stray out of their safe cave environment into the adjacent surface waterways. For whatever reason they appear on the surface, *P. anguinus* cannot survive long in the aquatic environment away from the cave systems, due to exposure to threats such as encounters with potential predators.

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Slow worms and greater white-toothed shrews sharing refuges

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The slow worm *Anguis fragilis* is a legless anguid lizard native to Britain. There is also a colony in the Burren area of the west of Ireland, in counties Clare and Galway, where it is presumed to have been introduced. I have studied this colony since 2015 and to this end have employed the use of artificial cover objects (ACOs) of various materials including roofing felt, hardboard, and corrugated iron. The refuges had an area of about 0.05 m^2 and were positioned close to various thick vegetation and bushes that provided good cover for slow worms.

The presence of the greater white-toothed shrew *Croidura russula* was first discovered in Ireland in 2007 in pellets of birds of prey (Tosh et al., 2008) in Counties Tipperary and Limerick. It is thought to have originated from France and is believed to be spreading at a rate of 5 km per annum.

It was on 16 June 2022, while completing my regular survey of ACOs, that I discovered the two species sharing the same refuge, in this instance a piece of corrugated iron placed on a roadside verge in the townland of Dereenatloghtan in County Clare. Upon lifting the refuge, a pair of white-toothed shrews were observed along with a male slow worm. One of the shrews immediately departed leaving just a single shrew and the slow worm (Fig. 1). While the slow worm was not disturbed by my frequent visits it appears the greater white-toothed shrews were, as they were not observed there again. These shrews have increasingly been noted under a number of refuges at different sites, but this was the only occasion when a photograph was possible of the two species together.

Shrews are insectivores whose diet is not confined to insects and it has been noted that young slow worms are at risk of predation by shrews (Simms, 1970; Beebee & Griffiths, 2000). In the case of white-toothed shrews, a study in Algeria showed that of 115 items in the stomach contents of 15 shrews, a total of three (1.9 %) were lizards/geckos which accounted for 14.9 % of prey biomass (Brahmi et al., 2012). I have not observed slow worm predation by the white-toothed shrew but this very probably happens. The same is likely to be true for Ireland's only native reptile, the viviparous lizard *Zootoca vivipara*, which may be even more vulnerable as it lacks the protective osteoderms that are present as a 'chain-mail' protection in the skin of slow worms (Beebee & Griffiths, 2000).

Figure 1. Male slow worm and one of a pair of greater white-toothed shrews under a corrugated iron refuge

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NATURAL HISTORY NOTE

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Dor beetle *Anoplotrupes stercorosus* found within the flank of a live northern viper *Vipera berus*

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On 13 August 2021, during radio telemetry studies of northern vipers or adders *Vipera berus*, in the Forest of Dean (England), a male viper about 440 mm long was found with a large swelling half way down its flank (Fig. 1). On capture and closer inspection the swelling was found to be a dead and decomposing adult woodland dor beetle *Anoplotrupes stercorosus*. This species is a fairly large (12–19 mm), earth-boring, metallic blue-black dung beetle. Weithmann et al. (2020) have pointed out that this is an opportunistic, copro-necrophagous species that is the most common dung beetle found on vertebrate cadavers. It is attracted to the volatile organic compounds generated by decomposition.

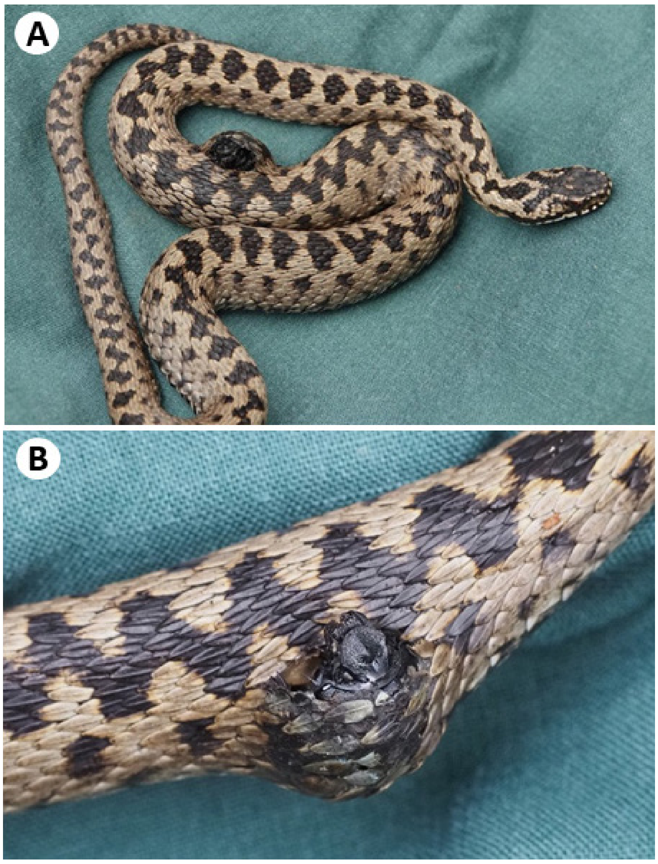

Figure 1. Male northern viper with a dor beetle embedded in its flank - **A.** The viper is alive, but appeared underweight, and **B.** Close up showing the head of the dead dor beetle protruding from the viper's flank

Figure 2. The dor beetle removed from the viper leaving a 'pocket'. The beetle had begun decomposing and the wound area contained blowfly eggs.

The snake was taken to the Vale Wildlife Hospital and Rehabilitation Centre (Tewkesbury, England) where they removed the decomposing beetle, along with blowfly eggs, and the wound was cleaned with saline solution (Fig. 2). The body of the beetle had formed a pocket just under the skin with the muscle covering the ribs, appearing undamaged, and with no damage to internal organs. After treatment, I released the snake at the capture location where it was seen basking in the same area on 14 September, but not seen in the following spring of 2022, or since.

I know of no other reported case of this kind of attack within the distribution of *V. berus*. It is intriguing to speculate how it may have happened. The snake was unlikely to have predated the beetle as there was no evidence of the beetle burrowing out of the snake's digestive tract and this scenario would surely have killed or seriously compromised the snake. Instead, it seems likely that the dung beetle burrowed down to where the snake was hidden, perhaps during a period when the snake was cool, less active and unable to move away. Indeed, the dor beetles were actively moving across the forest floor and footpaths, even in November (when the snake would be in hibernation). The snake may have had a wound on the flank possibly attracting the beetle which then became trapped between the skin and body wall.

This beetle is prolific throughout the Forest of Dean, when walking through the woodland and heath habitat areas these

Figure 3. Dor beetles on boar dung. Dor beetle are attracted to boar dung and they are frequently seen on the forest floor and pathways across the Forest of Dean.

beetles are regularly observed (Fig. 3). Possibly the growth in feral wild boar numbers may have had a positive impact on the beetle population. My observation raises two questions related to viper conservation in such habitats. The first question relates to the apparently long period that the snake survived in this condition, which is suggested, since the beetle would have taken a while to succumb and then decompose and also blowflies are less active during the winter months, so their eggs were likely laid during warmer periods. So, would the beetle attack have eventually led to a deterioration in the health and condition of the viper, compromising its ability to forage? The second question is whether dor beetle attack is confined to animals with wounds? If not then they may present a more significant hazard than we realise.

Predations of snakes by insects and specifically by Coleoptera is extremely rare in Britain. In August 2021, a dead neonate *V. berus* about 11 cm was photographed protruding from what was believed to be a green tiger beetle *Cicindela campestris* larval burrow; the viper's head and upper body appeared to have been consumed whilst either having been dragged down the hole or attacked whilst the snake investigated the burrow (T. Farrer, personal communication).

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I am grateful to Giles King-Salter for confirming the beetle species.

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NATURAL HISTORY NOTE

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A water snake uses the tail and body to scan for fish prey in tide pools

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Some snake species use the tail as a lure to attract potential prey within striking distance. Caudal luring is mostly recorded for the Boidae, Colubridae, Dipsadidae, Elapidae, and Viperidae (Murphy et al., 1978; Sazima & Puorto, 1993; Sazima, 2006; Hagmant et al., 2008; Andrade et al., 2010). An extreme example of elaborate caudal luring displayed to attract birds is that of the viperid *Pseudocerastes urarachnoides*, whose tail tip bears an extraordinary resemblance to a spider (Fathinia et al., 2015).

One or two snake species use the tail to flush potential prey from their shelters and cause them to move. The movement attracts the attention of the snake, which attempts to catch the fleeing prey. This poking behaviour is recorded for the dipsadid *Hydrodynastes gigas* (Strüssmann & Sazima, 1990) and is suggested for the dipsadid *Echinanthera undulata* (Gomes & Marques, 2012).

We describe here another hunting tactic - scanning - with the use of tail and body to flush fish prey from crevices in tide pools, displayed by the dipsadid water snake *Erythrolamprus miliaris*. Tail poking differs from scanning by the use of tail only (Strüssmann & Sazima, 1990). This water snake has a wide distribution in South America, recorded from Brazil, Ecuador, Bolivia, Paraguay, Argentina, and Uruguay (Giraudo, 2006). It dwells in different habitats including lakes, rivers, swamps, mangroves, and occasionally tide pools and beaches (Sazima & Haddad, 1992; Marques & Souza, 1993; Duarte et al., 2014, Rocha-Lima et al., 2018; Van der Burg, 2020). *Erythrolamprus miliaris* is an opportunistic and generalist feeder, preying on fish, amphibians, reptiles, and small mammals (Van der Burg, 2020; Eisfeld et al., 2021), and even scavenges on roadkill frogs smashed to pieces (Gomes et al., 2017).

Figure 1. The water snake *Erythrolamprus miliaris* scans a tide pool for fish prey - **A.** The snake in the pool during its searching, **B.** Scanning flushed a frillfin goby *Bathygobius soporator* (red asterisk) from its shelter - note the snake's tail position while moving it in the crevice, **C.** Further scanning caused the goby to move towards the snake, which quickly caught the prey underwater, **D.** The snake moves to higher ground on a rock and swallows the prey tail first

We recorded an *E. miliaris* individual on 30 October 2022, at 12:25 h at the Pontinha beach (25° 33'47.2" S, 48° 19'06.5" W, WGS84, 9 m a.s.l.), in the Ilha do Mel, Paranaguá, Paraná, Brazil. It searched for prey in a tide pool and displayed a hunting tactic we are calling 'tail and body scanning'. The sequence of events can be viewed on the video we made of its behaviour (BHS video, 2023). The snake had its head out of the water (Fig. 1A) when it began to make waving movements with the tail and part of the body to scan the tide pool. This scanning flushed out a frillfin goby *Bathygobius soporator*, which was previously undetected by the snake (Fig. 1B). The scanning repeatedly disturbed the fish, which swam from one spot to another of the tide pool looking for a safe place. At one moment of the scanning, the goby swam towards a higher point of the pool, being quickly detected by the snake and was caught (Fig. 1C). The snake held the prey and carried it to a rock out of the water (Fig. 1D), where it was swallowed tail first. From our first sighting of the snake in the tide pool, the above-described event lasted about 1 min 15 s.

This is the first report of tail and body scanning behaviour for *E. miliaris* and, to our knowledge, the first one by any snake. Our record adds to the already known variable feeding tactics of this water snake, which forages in diverse microhabitats and preys on a wide array of vertebrates (Sazima & Haddad, 1992; Marques & Souza, 1993; Duarte et al., 2014, Rocha-Lima et al., 2018; Van der Burg, 2020; Eisfeld et al., 2021).

In a study on the aquatic snakes *Nerodia* spp., Gillingham & Rush (1974) comment that water snakes display non-stereotyped (variable) hunting tactics according to habitat. The same may apply to *E. miliaris*, which forages in a variety of aquatic habitats (Sazima & Haddad, 1992; Marques & Souza, 1993; Duarte et al., 2014). It is possible that the frillfin goby recorded as prey of *E. miliaris* by Marques & Souza (1993) was hunted in the tide pool using the same scanning tactic reported here, as it seems very effective for such a microhabitat. As *E. miliaris* displays some surprising foraging behaviours (e.g. Duarte et al., 2014; Gomes et al., 2017; this study), we expect that further natural history observations will disclose additional behaviours not yet described for this snake.

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NATURAL HISTORY NOTE

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Winter predation of the viviparous lizard *Zootoca vivipara* by the Eurasian kestrel *Falco tinnunculus* in Britain

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The viviparous lizard *Zootoca vivipara* is one of the mostly widely distributed reptile species and one of the most northerly, having been recorded within the Arctic Circle as far as 71° N. As protection from sub-zero temperatures during the winter months these lizards brumate in a hibernaculum under debris (Costanzol et al., 1995) or in a carefully prepared cell in the soil (Hodges & Seabrook, 2022). Lizard activity is dependent on weather conditions and in particular the amount of solar radiation available (Van Damme et al., 1987).

The Eurasian kestrel *Falco tinnunculus* is a predator of small mammals, reptiles, other birds and invertebrates (Village, 1990) and is a significant predator of *Z. vivipara* with which it shares many habitats. One of the most comprehensive studies on this predator-prey relationship found that the likelihood of a viviparous lizard being delivered to a kestrel nest by the parent birds increased towards midday and independently increased with increasing ambient temperature (Steen et al., 2011). When lizards were delivered to the nest, the average temperature was 20.2 °C but other types of prey were delivered at an average temperature of 15.7 °C. Delivery of *Z. vivipara* to the kestrel nests could be a functional response to the increasing availability of lizards with increased temperature, as well as solar height (Steen et al., 2011). Those lizards basking or actively foraging will be at risk of predation (Lima & Dill, 1990; Caro, 2005) while at other times they stay well hidden within the vegetation. For the lizards, this means there may be a trade-off reaching their optimal body temperatures for maximum physiological performance (for foraging, mating etc.) and the likelihood of being captured by kestrels (Steen et al., 2011).

In the middle of winter on 17 December 2020, I observed a female kestrel predating two viviparous lizards on the Solent coast in the Havant area of Hampshire (England) at 13:38 h, one of these is shown in Figure 1. The weather conditions at the time were unusually mild with a temperature of 11 °C, relative humidity of 85 % and wind speed of 9.3 mph (Timeanddate website, 2020). The observed temperature (11 °C) falls within the lower end of the temperature range at which lizards have been recorded to be delivered to nests by kestrels 7.1–31.4 °C (Steen et al., 2011). Due to the lack of orange ventral colouration, no distinct hemipenal bulge at the tail base and adult size, the lizard in this observation appears to be female. This is interesting to note, as males may be expected to emerge from hibernation earlier than females in readiness for reproduction (Van Damme et al., 1987). However, due to the disfigurement of the body, it is difficult to determine the lizard's gender with certainty.

Figure 1. A kestrel eating a viviparous lizard on 11 December 2020, Havant, Hampshire, England

This may be the first record of predation by kestrels on viviparous lizards in December in Britain. Certainly, warmer British winters in recent times may be rendering *Z. vivipara* more vulnerable to winter predation (Davies et al., 2021).

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NATURAL HISTORY NOTE

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King cobra *Ophiophagus hannah* exhibiting breaching behaviour while swimming in response to a predation threat from a white-bellied sea eagle *Haliaeetus leucogaster*

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The king cobra *Ophiophagus hannah* is a predominantly terrestrial species (Rao et al., 2013) but it will take to water and is a capable swimmer. In Singapore, the species has been observed swimming in reservoirs and quarry lakes (Lim et al., 2011; Tan et al., 2020) and attacking conspecifics while swimming (Ananthanarayanan & Harrick, 2019). However, swimming in open water bodies exposes them to threats from both above and below the water surface.

On 22 October 2018, 09:21 h, a king cobra (c. 1.5 m in length) was observed interacting with a sub-adult white-bellied sea eagle *Haliaeetus leucogaster* at Upper Peirce Reservoir, Singapore (1° 22’14.3” N, 103° 47’47.0” E). The eagle was first observed flying close to the water surface, seemingly to hunt for fish (Fig. 1A). The observers were approximately 350 m away. A closer observation, however, revealed that it was in fact approaching a king cobra (Fig. 1B). The snake raised its head towards the approaching eagle (Fig. 1C). The eagle retreated, but circled back again, making two more dives at the snake. Each approach of the eagle elicited the same response, with the snake breaching an estimated maximum of 1 m above the water surface at its highest (Fig. 1D). The event lasted for about a minute, after which the eagle flew away.

It seems likely that the white-bellied sea eagle was attempting to predate the king cobra (Corbett & Hertog, 2011), which, in response, breached to deter the eagle; this upright posture is similar to the king cobra’s defensive display in a terrestrial environment (Jones, 2017). The olive sea snake *Aipysurus laevis* has also been photographed breaching in Australia (Thomas, 2021). The behaviours demonstrated by these snakes suggests that the ability to breach in aquatic environments may be important either in deterring predators and/or in enabling a better view of their surroundings. Such sightings present novel information about the king cobra’s possible responses to threats in aquatic environments and add to the current knowledge of its behaviours.

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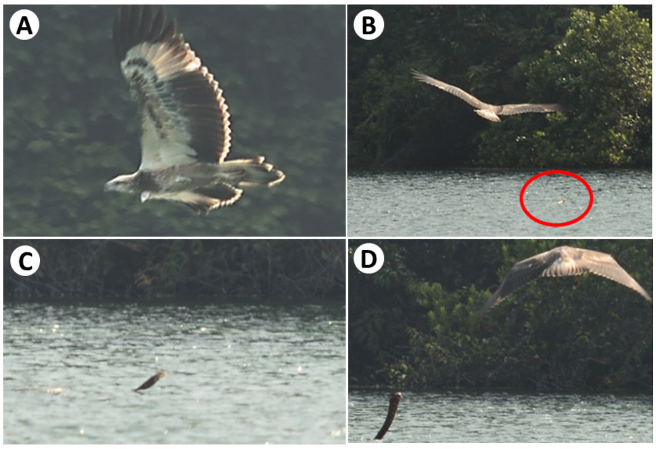

Figure 1. Stages of interaction between the king cobra (circled in red for Fig. 1B) and white-bellied sea eagle

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NATURAL HISTORY NOTE

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Consumption of putrescent carrion by a free-ranging western Montpellier snake *Malpolon monspessulanus*

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Snakes can be endangered by live prey that struggles and retaliates, but scavenging on dead prey can minimise such risks (Bonnet et al., 2010; Kornilev et al., 2023). A wide range of snake species accept dead prey, both in captivity and under natural conditions (Shivik & Clark, 1997; DeVault & Krochmal, 2002; Glaudas & Alexander, 2017; Deso et al., 2022; Oliveira et al., 2023) and mass prey mortality can even trigger intensive scavenging episodes (Ayres, 2012). A potential disadvantage of scavenging is that the bacterial proliferation in decaying carcasses produces toxins that are avoided by most predators, except those with specific adaptations (Ortiz & Smith, 1994). There are few reports of snakes successfully feeding on substantially putrified carcasses but examples are a *Micrurus frontalis* that began but eventually failed to swallow a rotten pitviper (Marques et al., 2017), *Agkistrodon piscivorous* eating dry fish carcasses abandoned by birds (Lillywhite & McCleary, 2008), and a large female *Natrix helvetica* captured in the field (western France, WGS84 46.011/0.590, May 1998) that due to handling stress regurgitated a 40 g vole covered with ~0.5 cm long maggots, the vole was likely eaten one day after its death (XB, pers.obs).

Free-ranging western Montpellier snakes *Malpolon m. monspessulanus* have been observed scavenging and accepting chicken thighs, but all these food items were fresh (Ventura, 2012; Weitzmann & Pretus, 2018; Deso et al., 2022). Here, we provide evidence of consumption of rotting carrion during a field experiment in south-eastern France (WGS84 44.033/4.878). On 17 July 2022, we placed bait in locations where snakes have been frequently observed and these were monitored with a camera trap (Num'Axes Trail Camera PIE 1023; Deso et al., 2022). One bait, a 50 g unfrozen rat placed at 09:30 h and exposed to the sun, was detected by an adult male snake 29 hours later (BHS video, 2023) during which time the hot weather in the study area (air temperature max. 38 °C, min. 23 °C) would have resulted in advanced decomposition. The snake tongue-flicked the rat and then consumed it. The snake was observed later digesting the prey with no ill effects. This observation indicates the western Montpellier snake will scavenge putrescent carrion.

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Predation of a grass snake *Natrix natrix* by a Peloponnesian freshwater crab *Potamon pelops*

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The grass snake *Natrix natrix* (L., 1758) has a broad Palearctic distribution that extends from central Germany in the west to southern central Russia in the east, and from central Scandinavia in the north to the Balkans and Middle East in the south (Speybroeck et al., 2016; Kindler et al., 2017; Schultze et al., 2020). It is known to be predated by a wide range of vertebrates, including various species of birds, mammals, fish, amphibians and reptiles (e.g. Kabisch, 1999; 2020; Cugnasse, 2001; Strugariu et al., 2014). It should however be noted that many reports mentioning predation refer to observations of snakes that are currently considered to be the barred grass snake *Natrix helvetica* (Lacépède, 1798), which has only recently been elevated to full species status (Kindler et al., 2017). In addition, some recent reports don't take this taxonomic split into account. Records of *N. natrix* sensu lato being predated by invertebrates are rare but do include the predation of juveniles by *Carabus* ground beetles (Kabisch, 2020).

In this report we share an observation of a juvenile *N. natrix* being predated by a Peloponnesian freshwater crab *Potamon pelops*, Jesse, Schubart & Klaus, 2010, on the south of the Peloponnese peninsular, Greece. On 10 July 2020, at 12:16 h, about 750 m south of Charavgi (36.98473° N, 21.85503° E, WGS 84) we were undertaking a herpetological survey from a trail adjacent to the stream that includes the Polylimnio waterfalls when we spotted a juvenile *N. natrix* (i.e. likely born in the previous season) in shallow water of the riparian zone of the stream. Upon further inspection, it turned out that the snake was being predated by a specimen of *P. pelops* (BHS video, 2023), which was partially hidden under a piece of dead wood. The crab was holding the snake with its pincers and eating it tail first (Fig. 1). The snake was fully conscious and trying to escape, but wasn't capable of releasing itself from the crab's grip. At the moment the observation took place, the crab had completely devoured the snake's tail, the cloaca and part of the lower abdomen, indicating that it had been feeding for a while and that the snake had no chance of survival. We observed and documented the situation for twenty minutes, decided not to intervene and then left the scene.

To the best of our knowledge, this is the first documented case of predation of a European snake by a crustacean. To understand the role of a snake species in an ecological system, it's important to know the factors that play a role in its survival rate. With this contribution, we add to the knowledge regarding Greek herpetofauna and particularly to the ecology of *N. natrix*. Since *N. natrix* and other species of *Natrix* share their range with several other species of *Potamon*, it is likely that more interactions between members of these two genera are yet to be recorded.

Figure 1. Predation of a juvenile grass snake *Natrix natrix* by a Peloponnesian freshwater crab *Potamon pelops*

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BOOK REVIEW
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Snakes of the World: A Guide to Every Family

Mark O'Shea
Princeton University Press, Princeton, USA and Oxford
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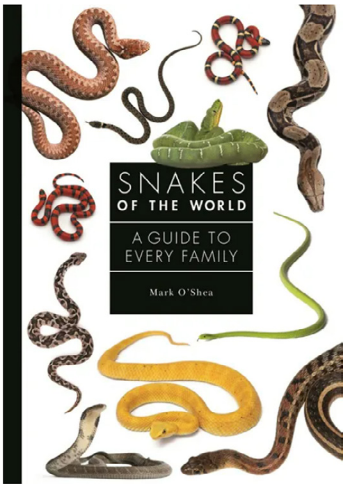

There are over 4,000 species of snakes in the world, present on every continent except Antarctica, and found in most habitat types. This remarkable success has not, alas, been matched in their appreciation by humans with snakes typically feared and disliked. This relationship prompted in part by the fact that some are highly venomous, results in large numbers of human fatalities and permanent injuries every year. But much of this fear is due to a lack of understanding, as most snakes are harmless, shy and rarely encountered. Education about their behaviour and the wider availability of cheap antivenoms would be important developments allowing these creatures to co-exist with humans, and continue to contribute to the ecosystems in which they exist.

Thus the publication of 'Snakes of the World', describing the wide range of species of snakes is timely and significant. An important development following the invention of digital cameras and modern publishing methods is that the quality of presentation in books has made huge advances. This book, published by Princeton University Press and produced by the Bright Press, is an excellent example of this. The volume contains many high quality photographic images of a wide range of snakes species and is beautifully presented, making it attractive and accessible to not just snake converts (mea culpa), but to the wider and more general audience who might be interested to learn more about these wonderful creatures.

'Snakes of the World' is well laid out, with a useful introduction containing sections on evolution and origins, taxonomy, anatomy, sense organs, locomotion, reproduction and conservation. The main section of the book is a comprehensive description of the world's species following the taxonomic ordering of the two infraorders Scolecophidia and Alethinophidia, with these containing various clades, superfamliles and families. The current taxonomic structure is nicely explained allowing the subsequent ordering and presentation of the various snake taxa. For each taxonomic unit a number of species are described with particular characteristics explained, along with more general information on distribution (including maps), habitat, size, reproduction and diet. And for many species, excellent photographic images are presented to illustrate the snake's appearance, often those specifically described in the text.

As there are many snake species, this descriptive section of 'Snakes of the World' is extensive. Hence I suspect it will not be read cover to cover, but will instead be dipped into by most as a resource, though this will reveal much fascinating information, especially important for the some of the less well known species, of which there many. 'Snakes of the World' finishes with a glossary, resource list and index. The glossary was useful but, as the resource list only describes various field guides, I felt it limited and superficial. As an academic I like to know the source of information presented in the text, and in almost all cases I was unable to do this; but I accept that perhaps this was beyond the intended audience and remit of the book.

My inference is that 'Snakes of the World' was intended instead to be a bridge between more scholarly volumes, with more detail and cited sources, and a 'coffee table' type book, containing limited text and information, but with many images. If this was the case I feel that that it has been largely successful. I learnt much from the text, and appreciated seeing many beautiful images of snake species I had not seen before. That this was all brought together in one relatively small volume is a credit to the author and publishers, and so I strongly recommend 'Snakes of the World'. Finally, I very much hope that this book increases understanding and appreciation of these wonderful creatures.

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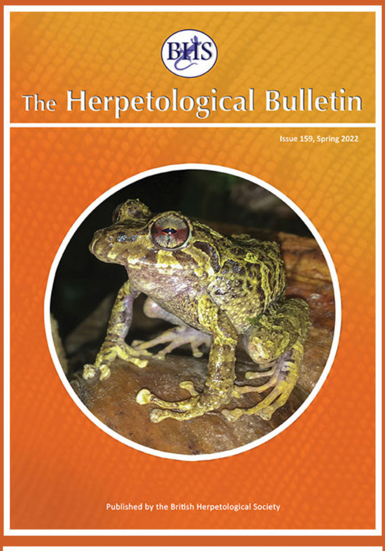

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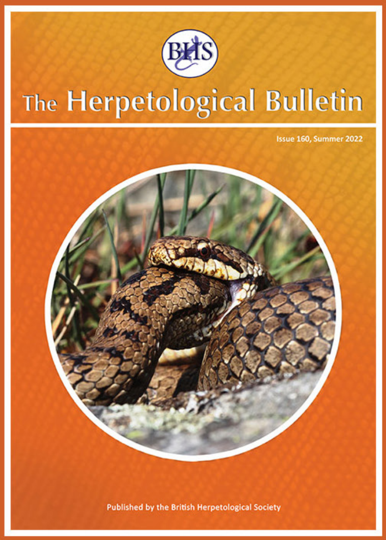

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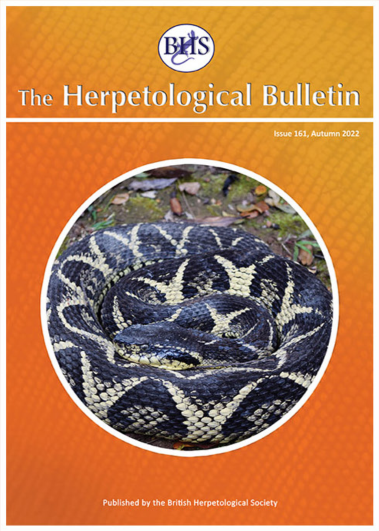

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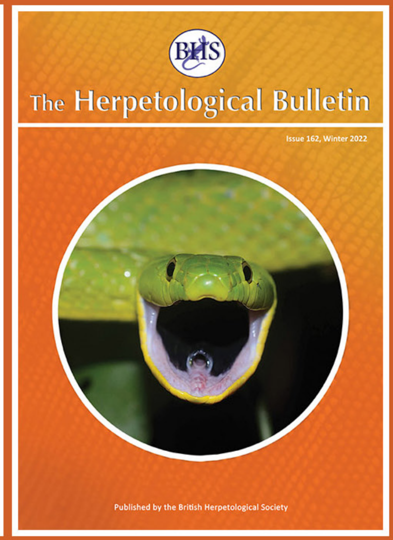

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