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Front Cover: A pair of northern pool frogs, *Pelophylax lessonae*, reintroduced to England, in amplexus. See article on page 7. Photograph by John Baker.

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Modulation of foraging strategy in response to distinct prey items and their scents in the aquatic frog *Xenopus longipes* (Anura: Pipidae)

CHRISTOPHER J. MICHAELS^{1*}, SANDEEP DAS², YU-MEI CHANG³ & BENJAMIN TAPLEY¹

¹Zoological Society of London, Regent's Park, London, NW1 4RY, UK ²Forest Ecology & Biodiversity Conservation Division, Kerala Forest Research Institute, Peechi, Kerala, India 680653 ³The Royal Veterinary College, Royal College Street, London, NW1 0TU, UK *Corresponding author Email: christopher.michaels@zsl.org

ABSTRACT - Aquatic predators must forage for prey in a complex three-dimensional environment where the availability of different prey types with different spatial niches may vary. Aquatic predators have evolved a number of ways in which they may respond to this variation, including phenotypic adaptation and behavioural modulation. We investigated whether clawed frogs (*Xenopus longipes*) can modulate their foraging behaviour in response to benthic (bloodworms) and pelagic (glassworms) prey species to which they had already been exposed, and whether any response would be elicited by chemosensory prey cues alone. Frogs responded to the presence of prey items by foraging more than in a control treatment (no cues at all) and were able to respond appropriately to prey type, foraging more in the water column for glassworms and on the aquarium floor for bloodworms. This effect was maintained in a second set of trials where frogs were exposed only to the chemosensory cues of the same prey items. These data show that *X. longipes* can modulate its foraging strategy to match the type of prey available and that this behaviour is at least in part informed by chemosensory cues.

INTRODUCTION

It has been established that aquatic predators live in complex environments where the availability of different prey types may vary in both space and time (Ahlgren et al., 1997; Bogan & Lyle, 2007; Downes, et al., 1993; Hart, 1985; Outridge, 1998). Prey abundance in a particular spatial zone may be dependent on season, and the behaviour and ecology of a particular prey species (Beckett et al., 1992). Previous research has shown that, correspondingly, the composition of the diet of such predators can vary seasonally with relative abundance of different species (Fasola & Canova, 1992).

The fitness of a predator may depend partly on the foraging success of individuals in order to invest in reproduction (Blanckenhorn, 1991; Morse & Stephens, 1996), and so predators must be able to cope with spatial heterogeneity of prey types in order to maximise evolutionary success. In some species, morphological phenotypic polymorphisms may allow individuals to specialise more on particular feeding niches and thus improve foraging success (Ehlinger & Wilson, 1998). Alternatively, individual animals may modulate foraging strategies to optimize them for given prey types as and when they are available (Deban, 1997; Ehlinger, 1989; Heiss et al., 2015; Montgomery & Hamilton, 1997; Persson & Greenberg, 1990).

Amphibians have relatively conserved body forms compared with fish and so are more likely to fall into the latter category than the former. They are often an important component of aquatic vertebrate predator assemblages, especially in temporary and fishless waters (Wells, 2007). Amphibians use a variety of sensory organs to detect prey, including eyes and chemosensory organs (e.g. Miles et al., 2004; Placyk & Graves, 2002) and, in aquatic amphibians, mechano- and electro-receptors (e.g. Himstedt et al., 1982; Martin et al., 2013; Reiss & Eisthen, 2008). Species-specific biases exist in their relative importance in prey detection. Some species use multiple senses (e.g. Avila & Frye, 1978; Manenti et al., 2013; Placyk & Graves, 2002; Uiblein et al., 1992), while others may rely almost entirely on single senses (Martin et al., 2013; Miles et al., 2004).

It is well established that once prey has been located, amphibians may modulate their prey seizing (Anderson, 1993; Deban, 1997; Maglia & Pyles, 1995; Monroy & Nishikawa, 2011; Valdez & Nishikawa, 1997) and processing (Avila & Frye, 1978) tactics in response to different prey types, as well as to different feeding environments (Heiss et al., 2013; Manenti et al., 2013). Moreover, search tactics may be modified flexibly to deal with different distributions of the same prey type (Nomura & Rossa-Ferres, 2011). However, little is known about flexibility of the strategies used by amphibians to search their environment for prey prior to encountering prey items. For example, it is not known whether amphibians are capable of exhibiting flexible (i.e. variable according to treatment), if stereotypic (i.e. little variation within treatments), responses (Wainwright et al., 2008) to cues produced by different prey types in order to improve chances of foraging success. In other aquatic predators (mainly fish), such behaviour is well documented (Ehlinger, 1989; Montgomery & Hamilton, 1997; Persson & Greenberg, 1990) and given the similarity of niche, aquatic amphibians are likely to exhibit similar capabilities.

The Lake Oku clawed frog (Xenopus longipes) is a

Critically Endangered (IUCN SSC Amphibian Specialist Group, 2017) pipid frog endemic to the highland crater lake of Mount Oku in Cameroon. Xenopus longipes is a generalist predator and feeds largely on aquatic invertebrates (Tapley et al., 2016) and will readily feed on prey items at any position in the water column (C. Michaels pers. obs.), although it occupies the benthic zone and mats of submerged vegetation when not foraging. It is equipped with upward-pointing eyes, dermal mechanoreceptors, typical amphibian chemosensory organs and specialised chemosensory subocular tentacles (Loumont & Koebel, 1991), which could all be employed in foraging. We investigated whether these frogs are able to modulate their foraging behaviour in response to cues from different prey type, and whether chemical cues may be important in detecting prey.

MATERIALS AND METHODS

Study Animals and Husbandry

In this study, we used adult founder (F0) and captive bred F1 X. longipes held as part of the Living Collection at ZSL London Zoo, UK. Details of the source of founder animals and the husbandry of the frogs is reported by Michaels et al. (2015); briefly, frogs were maintained in biologically filtered aquaria containing soft, neutral pH water (Total Dissolved Solids <30mg/L, pH c. 7.2), which were held at 16-20 °C. Before this study, animals were fed on a combination of pelleted food (Xenopus Express, USA), live bloodworm (Chironomidae), live glassworm (Chaoborus), live earthworm (Eisenia hortensis) and water fleas (Daphnia), with an equal proportion of blood- and glassworms. Frogs remained part of the Living Collection at ZSL London Zoo after the study was completed. The experimental protocol was reviewed by the Zoological Society of London (Zoological Project Database number: ZDZ58) and was designated as not requiring a Home Office license under the UK Animals (Scientific Procedures) Act 1986, as methods did not diverge significantly from normal husbandry.

Experimental Array

Six groups of six frogs, outside the reproductive period, each group comprising one F1 female, one F1 male, three F0 females and one F0 male, were housed in glass aquaria measuring 45x45x45cm filled with water to a depth of 35cm. This combination of sexes and filial generations was used simply to ensure even distribution of these categories between tanks. Aquaria were part of a filtered system linked to a sump and large external canister filter filled with filter foam and ceramic biological filter media (Eheim, Germany). The return plumbing for each aquarium was equipped with a tap allowing isolation of each enclosure from the system, which prevented the circulation of prey scents between enclosures. Each enclosure was equipped with identical hides (one PVC plastic pipe, one 25x10cm piece of plastic trellis and one granite pebble c. 10cm in diameter). There was no bottom substrate.

Experimental Protocol

Groups of frogs were exposed to two trials between the 13 and 22 April, 2016 (Trial 1 and Trial 2). In Trial 1, each group of frogs was presented with three prey treatments (PreyTypeCue) over three consecutive days, the order of which was systematically varied between tanks so that no group of frogs received the treatments in the same order. The three treatments (PreyTypeCue), presented as 3ml of live prey items, were: Chaoborus Glassworms, a pelagic species; Bloodworms (Chironomidae), a benthic species; Control, where tank water was disturbed with a clean, plastic 3ml spoon. Immediately after the stimulus was added to a tank, the animals were scan sampled for 15 minutes with counts made instantaneously every three minutes. This 15 minute period was long enough to capture foraging behaviour. At each three-minute interval, frogs were allocated to combinations of the following two category pairs: 'foraging' (repeated flicking of the front limbs, which aims to push any food items into the mouth and is the primary feeding behaviour in this species) or 'non-foraging' (any other behaviour), and 'on the substrate' (any part of the body in contact with the substrate of the aquarium) or 'off the substrate' (no part of the body in contact with the substrate). These paired categories (e.g. foraging on the substrate) were exhaustive and mutually exclusive such that all frogs were in one of the four paired states. A count of the number of frogs engaged in each category was made using a hard-copy check sheet. All observations were made by a single observer (SD).

In Trial 2, the same experiment was repeated one week later, but rather than presenting prey items, each treatment consisted of the addition of 40ml of Reverse Osmosis (RO) water in which live prey items had been soaked for 24 hours to provide chemosensory cues only; the control treatment consisted of plain 40ml RO water delivered using a clean pipette. Powder-free vinyl gloves were worn for all interactions with the aquaria and these were changed between enclosures to avoid contamination with chemical cues.

Statistical Analyses

For each tank in each treatment, the mean number of frogs foraging in the water column and on the aquarium floor over the six observation intervals was calculated, resulting in a single number for each behaviour-position category per tank per treatment. Given the small sample size (6 tanks) and the repeated measures within the same tank, Friedman's test (Q statistics) and Dunn's post-hoc comparison (Z statistics) were employed to assess the foraging patterns between different prey items or between different chemosensory cues. A Wilcoxon paired signed rank test (W statistics) was used to compare numbers of frogs exhibiting appropriate behavioural responses to Bloodworms and Glassworms between Trials 1 and 2. All analyses were conducted in SPSS 23.0 for Windows.

RESULTS

Trial 1

There was a significant effect of PreyTypeCue on the number of individuals foraging on the aquarium floor $(Q^2=12, P=0.002)$. Using Dunn's post-hoc comparisons (see Fig. 1A), Bloodworms significantly increased the number of frogs that were foraging and on the substrate against Control (Z=-2, P=0.001). The differences in numbers of frogs that were foraging and on the substrate were not significant between Bloodworms and Glassworms (Z=-1, P=0.083) or between Glassworms and Control (Z=-1, P=0.083)1, P=0.083). There was also a difference in the number of frogs that were foraging and off the substrate across treatments (Q^2 =10.182, P=0.006) where Glassworms had a greater number of frogs foraging and off the substrate than Bloodworms (Z=1.667; P=0.004) and Control (Z=1.333, P=0.021). No significant difference between Bloodworms and Control (Z=0.333, P=0.564) was detected.

Trial 2

There was a significant effect of PreyTypeCue on the number of individuals foraging and on the substrate $(Q^2=10.182, P=0.006)$. Using Dunn's post-hoc comparisons (see Fig. 1B), Bloodworms significantly increased the number of frogs foraging and on the substrate compared to Glassworms (Z=-1.333, P=0.021) and Control (Z=-1.667, P=0.004). There was no difference in this respect between Glassworms and Control (Z=-0.333, P=0.564). There was also a significant effect of PreyTypeCue on the number of frogs that were foraging and in the water column (Q^2 =7.913, P=0.019); Glassworms had a greater number than Control (Z=1.583, P=0.006) and Bloodworms (Z=1.592, P=0.012), but there was no significant difference between Bloodworms and Control (Z=0.667, P=0.248).

Appropriate Response Comparison

The number of frogs foraging and on the substrate did not differ between Trial 1 and two under the Bloodworms treatment (W=8, P>0.05). The number of frogs foraging and off the substrate was significantly lower in Trial 2 than in Trial 1 (W=0, P<0.05).

DISCUSSION

In Trial 1, the results establish that frogs did indeed respond to the presence of blood- and glass-worms with distinct foraging behaviours. Further, our results show that chemical cues alone (Trial 2) elicit the same preyspecific foraging responses. The small amount of foraging behaviour seen in control frogs was probably a result of a conditioned response to the approach of a human and of a low level of baseline foraging exhibited by the frogs.

Flexible foraging behaviour allows animals to exploit resources efficiently by using prey search behaviour appropriate to the prey detected (Wainwright et al., 2008) and is an alternative to the evolution of prey-specific morphologies (Ehlinger & Wilson, 1998). Such a flexible hunting strategy also suggests that these frogs specifically target prey items in their environment rather than simply



Figure. 1. Mean numbers of frogs foraging on the aquarium floor and foraging in the water column when exposed to glassworms, bloodworms and control treatments in Trial 1 (prey items present; Panel A) and Trial 2 (chemosensory cues only; Panel B). Error bars represent the standard error of the mean. Asterisked brackets reflect significant differences (P<0.05) between the bars at the tips of the brackets identified with Dunn's post-hoc tests; for exact p values, see text. An absence of brackets linking bars implies no significant difference.

ingesting food items when encountered randomly. This specific aspect of plastic foraging behaviour is not well studied in amphibians. Amphibians are known to modulate prey handling behaviour in response to different prey types once encountered (Anderson, 1993; Avila & Frye, 1978; Deban, 1997; Maglia & Pyles, 1995; Monroy & Nishikawa, 2011; Valdez & Nishikawa, 1997), to foraging in different environments (Heiss et al., 2013; Manenti et al., 2013) and to different prey distributions of the same prey type (Nomura & Rossa-Ferres, 2011), but little evidence exists concerning prey searching strategy in this context. It is worth noting that while the frogs in this study displayed

flexible foraging strategies to align their own behaviour with that of the prey item, the method of actually seizing the prey items did not vary noticeably. Xenopus laevis is known to use very different means of prey seizure (Avila & Frye, 1978), but this was in response to very different prey forms. In the present study, Bloodworms and Glassworms are probably too similar (both being similarly sized, elongate 'worms') to require different handling behaviours. Chemosensory organs are a dominant sense for prey detection in a number of amphibians (Avila & Frye, 1978; Manenti et al., 2013; Martin et al., 2013; Placykl & Graves, 2002; Telfer & Laberge, 2013). In aquatic species, especially those living in habitats with poor visibility (which does not apply to Lake Oku, which is a relatively low turbidity system - T. Doherty-Bone, pers. Comm.), chemosensory cues may be particularly important (Manenti et al., 2013; Martin et al., 2013; Uiblein et al., 1992). In species where single senses have become highly dominant, other organs are typically reduced (Martin et al., 2013). Although Xenopus frogs are able to find prey using olfaction alone (e.g. Avila & Frye, 1978), other sense organs are highly developed including eyes, mechano- and electroreceptors and these are also used to find prey (Elepfandt, 1985; Himstedt et al., 1982). Our data suggest the chemosensory stimuli are an important component of prey detection in X. longipes. The number of frogs that were both foraging and in the area in tank occupied by the prey items (or, in Trial 2, where the prey item associated with a chemical would be) did not differ between trials in the Bloodworms treatment. Contrastingly, this was significantly lower in Trial 2, where only olfactory cues were presented, than in Trial 1, where prey items were also present. This suggests that frogs may rely more on chemosensory stimuli to locate Bloodworms than Glassworms, where other senses may be important. Xenopus longipes forages for food both during the day and the night in the field (B. Tapley, pers.obs.) and it is also likely that the sensory stimuli used for hunting varies with light levels; in this study, frogs were only fed during hours of light and so any such variation could not be detected. Further work to establish which senses are used for each prey type, any hierarchy in their importance and variation influenced by environmental parameters such as darkness and turbidity, was beyond the scope of this study. The role of learning in the origin of these behavioural strategies is not elucidated by this study, as all frogs had been historically exposed to the prey items used. Given that the prey species used in this study are not sympatric with X. longipes in the field, it is unlikely that the prey-specific behavioural response displayed by frogs when presented with both prey and with chemosensory stimuli alone can be explained by innate reflexes. Rather, it is probable that frogs learned to associate particular prey scents with corresponding prey types and locations. Xenopus laevis can learn to associate given prey types with particular vibrational signatures (Elepfandt, 1985, 1986; Elepfandt et al., 2000, 2016) and moreover other amphibians show similar associative learning capabilities (Suboski, 1992; Ewert et al., 2001) including using prey chemosensory cues (Dole et al., 1981). The use of naive captive bred frogs may provide insight into the relative importance of innate and

learned responses in driving foraging strategy selection. Similarly, as frogs were held in groups for logistical reasons, there may have been a facilitating influence of conspecifics on frog behaviour; however, use of 'tank' as the experimental unit was intended to address this. Further work comparing the behaviour of lone and group-housed frogs in naive and experienced states would allow this to be investigated.

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A head-starting trial for the reintroduction of the pool frog *Pelophylax lessonae* to England

JOHN M.R. BAKER

c/o Amphibian and Reptile Conservation, 655A Christchurch Road, Boscombe, Bournemouth, BH1 4AP, UK Author Email: johninhalesworth@aol.com

ABSTRACT - Head-starting tadpoles was trialled to assist the programme for the reintroduction of the northern clade of the pool frog *Pelophylax lessonae* to England. Ten spawn clumps (estimated 1228 eggs) were removed from the reintroduction site and hatched under captive conditions. Survival rate of the eggs varied greatly between clumps, from 11% to 100%, but overall survival to the free-swimming tadpole stage was approximately 50%. Survival rates thereafter were high; 97% for tadpoles reared indoors and 81% for tadpoles grown for part of the larval period outdoors in artificial ponds. Releasing head-started tadpoles substantially increased metamorph productivity at the reintroduction site. The head-starting methodology described is labour intensive but provides an approach that could be used in secondary reintroductions of this species. It therefore merits further development for its potential contribution to the reintroduction of the northern pool frog to England.

INTRODUCTION

fter extinction in England the northern clade of the Apool frog *Pelophylax lessonae* (the northern pool frog) has been subject to a carefully planned reintroduction (Buckley & Foster, 2005). Over a four-year period (2005 to 2008) frogs were translocated from Sweden to a specially prepared site in Norfolk. Individual frogs have fared well and a population has become established (Foster et al., 2018). Nevertheless, the population has remained small (approximately 50 adults) which puts it at risk of extinction from stochastic events. The specific habitat requirements of the northern pool frog mean that it is unlikely to spread to new sites naturally. Hence the highly desirable establishment of further populations is reliant on translocation (Buckley & Foster, 2005), but while the first population remains small there are insufficient post-metamorphic animals to provide the necessary donor stock. Further importation of frogs from Sweden would be legally complicated and the pool frog is a rare species in that country anyway.

Head-starting is a management technique that rears early life stages (eggs, larvae, juveniles) in captivity before releasing them into native habitats (Smith & Sutherland, 2014). It boosts population productivity by protecting these life stages from the high rates of mortality normally experienced in the wild. Head-starting tadpoles has been recommended as a cost-effective method of establishing new populations of crawfish frogs *Lithobates areolatus* with minimal cost to the donor population(s) (Stiles et al., 2016) and within the British Isles it has been used as a successful technique in the recovery of the agile frog *Rana dalmatina* on Jersey (Ward & Griffiths, 2015).

In 2012 a limited head-starting trial was undertaken for the northern pool frog reintroduction programme. One hundred and thirty-eight eggs and hatchlings were taken into captivity from the established population and 113 of these were released back into the donor population as well-grown tadpoles. The current trial further investigated the potential of taking spawn from the wild, rearing tadpoles in captivity and subsequently releasing them to supplement the numbers of metamorphs produced naturally. Both trials aimed to release tadpoles immediately prior to metamorphosis to capitalise on the rapid larval growth stage but to avoid the relative difficulties of rearing large numbers of juvenile frogs. Both head-starting trials were carried out under licence from Natural England.

METHODS AND RESULTS

Collection of spawn

The behaviour of adult frogs at the reintroduction site was monitored to anticipate spawning. Behaviour indicative of imminent spawning included amplexus or the movement of female frogs towards male choruses. Spawn clumps were deposited on top of mats of vegetation, floating at the pond surface, or adhering to stems of broad-leaved pondweed Potamogeton natans, just below the surface. All of the clumps were found some distance from the pond shoreline and were collected by wading into the pond or from an inflatable dinghy, taking care not to disturb remaining spawn in the process. Each female produces two to five small spawn clumps in a single spawning (Sjögren, 1991). Effort was made to find spawn in different locations within a pond, or from different ponds, to maximise the number of donor females. Ten clumps of spawn were collected from two ponds (six clumps on 8 May, two on 29 May and two on 2 June).

The number of eggs in nine of the clutches was counted from photographs. One of these spawn clumps contained 246 eggs, more than twice the mean number of eggs in the others (mean=109, n=8), suggesting that in the former case two clumps were deposited simultaneously. Using the mean value of 109 to substitute for the number of eggs in the clutch where eggs were not counted, the estimated total number of eggs taken was 1228. These were equivalent to the reproductive output of one or two females, based on clutch sizes of six females which ranged from 587 to approximately 2,000, dependent on body size (Sjögren, 1991).

Head-starting facility

The spawn was hatched and the subsequent tadpoles reared in a private home approximately 50 km from the reintroduction site. No amphibians, or other animals, were kept at this residence, greatly reducing biosecurity risks. All equipment used in maintaining spawn and tadpoles was dedicated to the rearing protocols (i.e. not used for any other purpose) as a biosecurity measure.

Care of spawn

Spawn clumps were held separately in small plastic containers (used margarine tubs [Fig. 1] and food storage boxes) and maintained at room temperature. Immediately after hatching the tadpoles moved little and did not require feeding. As they became mobile they began to feed on algae growing on the remaining spawn and on fragments of adhering vegetation. At this point the tadpoles were transferred to larger containers by pipette. Some eggs failed to develop and survival rate varied between clumps from 11% to 100%. Some hatchlings were malformed, mostly oedematous. None of these survived long after hatching. Six hundred and nine healthy tadpoles were produced from an estimated 1228 eggs, giving a survival rate from egg to free-swimming tadpole of approximately 50%.



Figure 1. A clump of pool frog spawn collected from the field and hatched out in a used margarine container.

Care of tadpoles

Tadpoles were housed in plastic food storage containers, increasing in size from two to six litres, and then transferred to ten-litre plastic containers (domestic washing-up bowls) as they grew. The containers were partially filled with a mixture of water taken from ponds at the reintroduction site and tap water. Tadpole stocking densities ranged from approximately 30 per litre initially, reducing as they grew to approximately three per litre. Water hornwort *Ceratophyllum demersum*, also taken from the reintroduction site, was added to each container to provide refuge and surfaces for periphyton growth, upon which tadpoles could feed. Boiled spinach, as used by Orizaola et al. (2010), was provided, initially daily, then increasing to three or four times a day, to ensure ad libitum feeding (Fig. 2). Tadpoles did not consume a variety of pelleted food that was offered (fish and rabbit pellets) nor algae wafers, although during the early



Figure 2. Boiled spinach was the only easily available food identified which northern pool frog tadpoles fed upon readily

stages a little goldfish flake food (Aquarian) was consumed.

For most of the time the rearing containers were kept indoors, moving them daily to benefit from sunlight from south-, east- and west-facing windows. On most days the rearing containers were also temporarily moved outside on to the flat roof of a two-storey building (Fig. 3). This exposed them to direct sunlight in a location relatively safe from potential predators, with no resident amphibians and, hence, minimal risk of pathogen transfer. Nevertheless, rearing containers left in full sun had to be monitored closely to ensure that overheating did not occur.

During the early stages containers were cleaned by transferring tadpoles, using a pipette or small hand net (a



Figure 3. Rearing containers were moved onto a flat roof to benefit from exposure to sunlight on most days. Mesh lids protected against predation by birds.

plastic tea strainer), to a container of fresh water (prepared as above) at intervals of three to five days. As the tadpoles grew larger and were transferred to ten-litre containers, cleaning was carried out by siphoning detritus from the bottom. This was carried out once or twice a day when the tadpoles were large and growing fast. The survival of tadpoles maintained under these conditions was 97%. At least three of the mortalities were related to trauma sustained during cleaning.

Completion of tadpole development outdoors

One hundred tadpoles were transferred (50 on 22 June and 50 on 24 June) to two artificial ponds constructed outdoors, 15 km from the reintroduction site. These ponds were 76-litre plasterers' baths sunk into the ground and filled with approximately 55 litres of tap water inoculated with pond water from the reintroduction site and with tadpole densities of approximately one per litre. The ponds were protected from birds by a fruit cage, but additionally, they had closely fitting mesh lids to exclude other amphibians that may have been present in the area, grass snakes and large, predatory aquatic invertebrates. Tadpoles released into these ponds had a high rate of survival (81%). Nevertheless, growth and development were slower than that of tadpoles grown indoors. On 1 August the first individuals from the artificial ponds were released at the reintroduction site and by this date all but 11 of the tadpoles reared indoors had reached a similar developmental stage.

Release of tadpoles

Development rate of individuals varied and tadpoles reached the pre-metamorphic stages asynchronously. Eighteen releases were carried out between 20 June and 27 August 2013. The first two releases (20 and 24 June) took place earlier than was ideal, due to lack of rearing space, and involved relatively small, less developed tadpoles. Tadpoles released after this, in July and August, had all developed to at least the point of having small hind limbs. Tadpoles were transported back to the reintroduction site in ten-litre plastic food canisters with a large hole cut from the centre of the lid to allow ventilation. These transport containers were partially filled with a mixture of tap water and water from the rearing containers and packed with water hornwort. Tadpoles were released shortly after arrival at the reintroduction site rather than allowing further time for acclimation in the transport containers. Five hundred and seventy-five well-grown tadpoles or pre-metamorphic froglets were released at four ponds within the reintroduction site.

Monitoring released animals

Tadpoles were released into ponds where pool frogs from the parental population had not spawned. This allowed comparison between head-started and free-ranging productivity. Metamorphosing northern pool frogs bask on floating vegetation in their natal ponds and remain in and around these ponds for several days prior to dispersing. Metamorph emergence was monitored using a standardised count procedure developed during the course of the reintroduction. A count was the number of metamorphs seen on a circuit of each pond. Monitoring was carried out on



Figure 4. Counts of free-ranging and head-started metamorphs in 2013

nine occasions from 23 July to 3 September. Metamorphs were identified as individuals judged to have recently transformed. These included froglets during the stage of tail absorption and those assumed to have just completed tail absorption. The ponds surveyed for metamorphs included two where spawning occurred earlier in the year, four ponds at which head-started tadpoles had been released and four nearby neighbouring ponds to which metamorphs dispersed. The total number of metamorphs observed during each survey visit was recorded as a site count.

Metamorph emergence

Head-started individuals began to metamorphose earlier than free-ranging tadpoles and were found at all four of the release ponds. For eight of the nine monitoring visits the release ponds yielded higher metamorph counts than were obtained from the two free-ranging (spawning) ponds (Fig. 4). Metamorphs at the release ponds contributed from 44% to 100% of the site counts, which ranged from 13 to 95.

DISCUSSION

Husbandry

Collection of spawn and rearing tadpoles mostly indoors at high densities was relatively successful. Although survival of spawn to the free-swimming tadpole stage was only approximately 50%, this seemed to reflect the viability of the spawn itself rather than any effects of capture and captive husbandry.

Once they reached the free-swimming stage subsequent survival rates of tadpoles were high and they fared well under the captive rearing conditions described. Factors that may have contributed to the success of rearing tadpoles were warmth, exposure to direct and indirect sunlight, plentiful food, frequent maintenance (feeding, cleaning and observation) and adequately conditioned water and rearing containers.

The majority of the tadpoles were reared (mostly) indoors, at high densities, in preference to the use of artificial ponds outdoors. The artificial ponds appeared to work fairly well in that they provided a lower maintenance, low-stockingdensity option. The slower growth and development rates of tadpoles in the artificial ponds were probably due to logistical difficulties in providing frequent (at least daily) feeding and monitoring. Hence, the artificial ponds did not achieve their full potential as a rearing environment.

Effectiveness of head-starting

Head-starting tadpoles made a significant contribution to the numbers of metamorphic pool frogs in 2013. For eight of the nine monitoring visits metamorph counts from the release ponds were higher than those from the spawning ponds. Furthermore, releasing head-started metamorphs at four ponds, other than the two where spawning occurred naturally, spread transforming froglets over more of the reintroduction site than would otherwise have been the case. The quality and fitness of head-started tadpoles has been questioned (Mendelson & Altig, 2016) and the subsequent survival of head-started metamorphs compared with those left on site is unknown. Nevertheless, the positive early indications have encouraged ongoing development of this approach to provide stock for a secondary reintroduction, which will be subject to long-term monitoring needed to determine the ultimate value of head-starting northern pool frogs.

Logistics and effort

Evaluation of the cost-effectiveness of head-starting tadpoles must consider logistical issues and resources available. Although it has been recommended as a cost-effective means of establishing new populations in one case (Stiles et al., 2016) high costs in terms of labour, finance and other resources have been highlighted in another (Ward & Griffiths, 2015). The head-starting methodology described here for rearing tadpoles indoors was certainly highly labour intensive, but inexpensive in terms of other resources.

Effective biosecurity is a requirement throughout conservation translocations (IUCN/SSC, 2013). Rearing tadpoles in a private home, with no other captive animals present, is an example of how 'modified spaces' can provide effective amphibian rearing facilities (Barber, 2012). This is in contrast to working within an existing facility, such as a zoo, that may house a cosmopolitan amphibian collection. Within such a facility, isolation of reintroduction stock to avoid disease transmission between species (Pessier & Mendelson, 2017) and, ultimately, into the wild with reintroduced amphibians (Walker et al., 2008) may require construction of new buildings or the use of modified shipping containers (Barber, 2012).

In spite of its positive aspects, the current trial yielded only 575 well developed tadpoles/metamorphs. The release of large numbers of animals (>1000) is a significant factor in the success of amphibian translocations (Germano & Bishop, 2009) and to produce more well-grown pool frog tadpoles will require greater investment of financial resources and time.

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Changes in relative population size detection rates of great crested newts (*Triturus cristatus*) over time

ERIK PATERSON

School of Life Sciences, College of Veterinary, Medical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK Author Email: erikpaterson@virginmedia.com

ABSTRACT - Amphibian populations have been shown to be in decline for the past four decades in part owing to habitat degradation and loss. In the United Kingdom, when faced by habitat works through development, consultant ecologists must undertake surveys for European protected species such as the great crested newt under licence following standardised guidance. However, previous studies and survey guidance present an unclear picture in reference to the ideal time of night to undertake sampling for this species. In this paper, an experimental review of the most effective times for detecting population sizes of great crested newts is presented. Sampling was undertaken at Scotland's largest great crested newt population during the peak breeding season repeatedly between sunset and sunrise a total of six times. The results show a significant non-linear relationship between the relative detection rate of great crested newt populations over time with a peak of detectability at 141 minutes after sunset, suggesting that between 60-180 minutes following sunset is the best time to undertake surveys for this species. These data also show a significant peak of great crested newt presence at an air temperature of 3.5 °C which is below the reported critical minimum. However, water temperature which corresponded to peak newt presence was 10.4 °C suggesting this may be a crucial component of accurate great crested newt surveillance.

INTRODUCTION

Cince the 1970s, amphibians have been known to be declining worldwide (Alroy, 2015), with the chief threat being loss and fragmentation of habitats owing to changes in agricultural practices (Vié et al., 2009; Hartel et al., 2010). In Scotland, the great crested newt is protected under schedules 2 and 4 of the Conservation (Natural Habitats, &c.) Regulations 1994 (as amended). More common in England and Wales, Scottish populations are typically smaller and more scattered (Wilkinson & Arnell, 2011; McInerny & Minting, 2016; O'Brien et al., 2017). Gartcosh Nature Reserve in North Lanarkshire is thought to have the largest single population of great crested newts in Scotland with a count of 1,012 adults achieved within the reserve during 2006 (McNeill, 2010). This core site is thought to support between 9 and 29% of the total population of great crested newts in Scotland (McNeill, 2010).

Scottish Natural Heritage licence holders are issued with the English Nature (2001) document entitled "Great Crested Newt Mitigation Guidelines" and must comply with the guidelines set out within. These guidelines do not make any suggestion of the best time of night to undertake surveys, instead stating only that nocturnal torchlight surveys are appropriate for the detection of great crested newts. However, there are a number of conflicting studies and documents, some of which propose that great crested newts are nocturnal (Steward, 1969; Himstdedt, 1971; Dolmen, 1983a; Griffiths, 1996; Kröpfli et al., 2010; Jehle et al., 2011) and others which state that great crested newts are crepuscular (Dolmen, 1983b; Griffiths & Inns, 2003; Kröpfli et al., 2010). Diel patterns of changes in behaviour have been noted amongst the Amphibia (Oishi et al., 2004) including for great crested newts (Himstdedt, 1971; Dolmen, 1983a & 1983b). Behavioural changes over time have been shown to be influenced by a number of factors including the diel rhythm of light and darkness, temperature, and prey availability (Noeske & Nickerson, 1979; Griffiths, 1985; Ranta et al., 1987). This has the potential to introduce errors in the survey of great crested newts, generating conservation concerns for the species. For example, if sample visits were undertaken at inappropriate times, surveyors may fail to detect newts or underestimate population size. If these samples were in support of development proposals, then habitat destruction works may impact newts fatally, or mitigation and compensation measures may fail to account for populations of underestimated size.

Maximising the detectability of great crested newt population sizes by means of determining an appropriate survey window is of chief importance for ensuring that accurate data are gathered. These data can then be collated as a measure of the status of the species to enable effective conservation strategy development and accurate reporting on the status of European protected species can then be undertaken (Wilkinson et al., 2011).

METHODS

Site Selection

As the University of Glasgow has been involved with the Gartcosh Nature Reserve in North Lanarkshire (NS 70 68), which is reported to hold Scotland's largest great crested

newt population (McNeill, 2010; McNeill, 2012; Harper, 2015), this site was chosen as the sampling location. There is currently a development to the north-east of the site and an access road is due to be developed through the narrowest part of the reserve (McCrorie, L. 2016, pers. comm.). Owing to this, access was allowed to only one of the four distinct pond clusters present on site; the Bothlin Burn complex in the north-west of the reserve (Fig. 1).



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

The Bothlin Burn complex of eight ponds was visited on the evening of the 17 March 2017 to identify the suitability of sampling ponds. This was determined in-field by counting the number of great crested newts present in the pond, assessment of the extent of accessible shoreline and by qualitative assessment of the clarity of the water. Ponds B1, B2, B3, B4, B5, B7, and B8 were chosen for sampling. Pond B6 had a thick ring of emergent macrophyte cover and no open water could be seen from the bank; as a result, this pond was excluded from the sampling effort.

Pond Sampling

At the beginning of every pond sample, the water temperature was read approximately 10 cm from the shore and at approximately 15 cm depth in consistently the same location utilising a TPI digital pocket thermometer. Air temperature was measured approximately 50 cm from the ground at a central location between pond B1 and B2 (Approx. NS 70575 68400) using a PeakMeter MS6508 digital thermometer. Cloud cover was estimated by the author as a percentage of visible sky obscured at the beginning of each individual pond sample, and moon % was read from the table given by astronomyknowhow.com (2017).

The seven sample ponds were surveyed by the author with a note taker annotating a recording form using the standard torching methodology given by Griffiths et al. (1996) whereby each pond was sampled at night utilising a high powered spotlight (Clulite Clubman CB3 LED, 1,000,000 candlepower). The accessible perimeter of the pond was traversed on foot from the same starting point and in the same direction for each sample. Torchlight searching was the only method utilised as this is the recommended least-disturbance methodology for sampling in SNH licence conditions and owing to its cost-effectiveness (Kröpfli et al., 2010). During nocturnal sampling key ID features are not always clear, if key features could not be seen during sampling, newts were placed into the category "unidentified newt". These records, which could not be split to sex or species were excluded from statistical analyses (n = 4).

Sample Repetition Rate

Six visits were undertaken at the site during the peak newt season as defined by English Nature (2001). Visits were made on the evenings of 25 March, 8 April, 22 April, 13 May, 26 May, and 17 June 2017. Sampling begun 30 minutes prior to sunset and continued until sunrise of the following day.

Ponds were sampled in a staggered manner repeatedly throughout the evening (Table 1). All seven sample ponds were sampled at differing repetition rates (hourly, two-hourly, and three-hourly) in order to control for the potential effects of repeated torchlight disturbance to newts. Sunset times were read from the table given on the website timeanddate.com (2017).

Ponds were sampled in the order: B1, B2, B3, B4, B5, B8, and B7 and subsets in the same order to ensure relatively consistent timing of visits per pond relative to sunset.

Table 1. Example sampling repetition rate for Bothlin Burncomplex (visit 3 onwards). Example shown is the visitsundertaken on 16th-17th June 2017.

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

Statistical Analyses

Data were scaled to be comparable across survey visits wherein the maximum count achieved of newts per pond per survey evening was assumed to be 100% of potentially detectable newts in that pond on that evening. All other counts at other sample times were then expressed as a percentage of that maximum to provide the proportion of the peak count achieved and acting as a measure of population detection rate. Detectability is used in the context of this work as a measure of the proportion of the maximum count of newts that can be found at a sampling location on a given evening rather than a binary positive or negative establishment of presence. As each individual pond sample lasted up to 20 minutes, the start time for each sample can be placed in the half hour period relative to sunset in which it occurred to provide the explanatory variable "half hour relative to sunset".

Generalised linear mixed models (GLMMs) were built using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages in R v. 3.4.3 (R Core Team, 2017) with the dependent variable being the detected proportion of newts and the explanatory variables being the time relative to sunset in minutes, half hour relative to sunset, moon %, cloud %, air temperature, and water temperature. All models included the random variables of date and pond number. As the data showed distinct curves, polynomial models utilising the same variables were built to the 8th order. Models were nested and so stepwise backwards deletion of explanatory variables via likelihood ratio tests was utilised to choose the best fitted model. GLMMs to test the effects on proportion of newts detected against time since sunset with interaction terms between repetition rate, cloud %, air temperature, and water temperature were also built to ascertain if these explanatory variables interacted and impacted upon survey results.

RESULTS

Great Crested Newt Population Size of Study Site

In all six sample ponds, more male than female great crested newts were detected. Pond B4 consistently had the fewest great crested newts and pond B7 had the largest detected numbers of great crested newts (Table 2). Of the six visits undertaken, the survey beginning on the evening of the 9 April had the peak count of male great crested newts in pond B7, and the sample beginning evening of the 22 April had the highest individual count of female great crested newts in pond B2 (Fig. 2). Male great crested newts were already present in considerable, though unquantified numbers by the time of the first visit on 17 March.

Detection of Great Crested Newts

Figure 3a shows relative detection rates of adult great crested newt population sizes per half hour with a line of statistical best fit arising from a 4th order polynomial model peaking at 141 minutes after sunset ($r^2=0.65$, $F_{(4,105)}=32.71$, p= <0.001). This shows that there is a statistically significant change in the detection rate of great crested newt population sizes over the nocturnal period. The data also suggest that between c.60 and c.180 minutes after sunset, there is a 50% or greater chance that the detected proportion of great crested newts will be above 70% of the potentially detectable great crested newts during a sampling evening.

Male great crested newts had their statistical peak count period at 230 minutes after sunset (Fig. 3b) and were fit

Table 2. Peak counts of great crested newts (*T.c.*) in each of the sampled ponds

Pond No	T.c. Male	T.c. Female	T.c. Peak count
B1	45	9	51
B2	52	27	64
В3	25	9	34
B4	18	5	23
B5	48	4	51
B7	62	19	74
B8	25	6	30



Figure 2. Peak counts of great crested newts in each pond per visit split by sex. The peak count for males was achieved on 9th April in pond B7, and for females the peak count was achieved in pond B2 on the 22nd April. Fitted curves show the statistical best-fit of 5th order polynomial models of detection by sex.

best with a 3rd order polynomial model ($r^2 = 0.55$, $F_{_{(3,108)}} = 23.39$, p= <0.001). In contrast, female great crested newts had a statistical peak count period at 168 minutes after sunset (Fig. 3c) and were also best fit with a 3rd order polynomial model ($r^2 = 0.38$, $F_{_{(3,69)}} = 20.05$, p= <0.001).

The air temperature which statistically corresponded to peak counts was 3.5 °C (Fig. 4a, b) and the data were best fitted with a 6th order polynomial model (r^2 = 0.71 $F_{(6,18)}$ = 22.62, p= <0.001). All maximum counts with the exception of pond B4 were achieved at an air temperature below 5 °C (Fig. 4b). However, water temperature showed a peak of great crested newt counts at 10.4 °C and best fitted with a 3rd order polynomial model (r^2 = 0.71, $F_{(3,26)}$ = 6.25, p= <0.01). Figure 4c shows that as water temperature drops to 5 °C, detection rates of newts fall, and Fig. 4(d) shows that all peak counts with the exception of ponds B5 and B8 occurred above 10 °C water temperature.

The percentage of visible sky obscured by cloud had a significant, negative effect on the proportional counts of great crested newts ($r^2=0.60$, $F_{(1,30)}=8.56$, p=<0.01). However, moon % did not show any significant relation with proportional counts of great crested newts ($r^2=0.61$, $F_{(1,10)}=0.03$, p=0.86).

No significant interaction was found between the repetition rate treatment and the relative detected proportion of great crested newts over time ($r^2 = 0.83$, $F_{(1,138)} = 1.4915$, p= 0.21). However, a significant interaction was found between detection rate of great crested newts over time



Figure 3. (a) Great crested newt detections expressed as a percentage of the maximum count achieved per pond per evening shown as boxplots splitting the detection rates in to quartiles with the thick black horizontal line at each serving as the marker of the median with boxes either side showing the range of the core 50% of data. Dotted lines represent the outer 50% of data and clear dots represent outlying data points. This plot shows great crested newt detection by half hour period relative to sunset. Data show a strong non-linear relation best fitted with a 4th order polynomial (curve) peaking at 141 minutes after sunset. These data suggest that between c.60 and c.180 minutes following sunset, the relative detection rates are at least 50% likely to be c. 70% of the maximum detectable newts per pond per evening. Lower horizontal line corresponds to 70%, middle to 80%, and upper to 90% detection rate. (b) Male GCN expressed as proportion of newts detected per pond per evening plotted against time in minutes relative to sunset. (c) Female GCN expressed as proportion of newts detected per pond per evening plotted against time in minutes relative to sunset. Data show a statistically significant non-linear relation best fitted by a 3rd order polynomial (curve) peaking at 168 mins following sunset.

best-fit 4th order polynomial and air temperature ($r^2=0.83$, $F_{(4,138)} = 4.92$, p = <0.001) as well as water temperature ($r^2=0.61$, $F_{(4,143)} = 5.05$, p = <0.001).

DISCUSSION

The data presented here show a significant non-linear relationship between the relative detection rates of great crested newts and time (Figs. 2-3). The peak time of detection of great crested newts was 141 minutes after sunset dropping gradually to sunrise (Fig. 3a), suggesting that there is a nocturnal pattern of presence in the water column. This pattern of detectability, tending to be more nocturnal, confirms observations by other authors (Steward, 1969; Himstdedt, 1971; Dolmen, 1983a; Griffiths, 1996; Kröpfli et al., 2010; Jehle et al., 2011) and does not conform to a crepuscular pattern of detection wherein newts would be more active at sunset and sunrise with a low point of activity during the core part of the night (Dolmen, 1983b; Griffiths & Inns, 2003; Kröpfli et al., 2010).

The observations of differential detection rates over the nocturnal period suggest that there is a two hour window between c.60 to c.180 minutes after sunset where the chance of achieving a peak count of greater than 70% the available population is more than a half (Fig. 3). The best-fit polynomial curve presented by this data shows broad similarity to that reported by Himstdedt (1971). The guidance for great crested newt surveys determines that great crested newts are most detectable between mid-March and mid-June (English Nature, 2001). The data presented here suggest that in Scotland, great crested newts may be active in large numbers earlier in the season (Fig. 2), and indeed the author has noted great crested newts active at Gartcosh nature reserve in February. A more thorough review of survey methodologies from a Scotlish

perspective may find that the situation is different here at the northern fringe of the range of great crested newts. This trend is known to occur amongst other amphibian species where environmental conditions at the fringe of ranges can lead to plastic developmental responses and ecology that is different to core range populations (Brattstrom, 1968; Orizaola & Laurila, 2009; Orizaola et al., 2010, Muir et al., 2014a; Muir et al., 2014b).

The differential detection peak for males and females (168 and 230 minutes after sunset respectively; Fig. 3b,c) may be due to the breeding ecology of this species. As the breeding system is that of a lek, the males will generally be non-randomly distributed in the open areas of the pond for a prolonged time period as they display and await females for mating. The females spend less time in the lekking arena and following mating they venture in to surrounding vegetation to lay eggs, at which point they can become obscured and detection becomes inherently more difficult (Hedlund & Robertson, 1989). The peak detection time for all adults being earlier than that for males or females at 141 minutes after sunset (Fig. 3a) is counter-intuitive and may be an artifact of modelling. However, this issue remains functionally unexplained and requires further work.

Though the system of peak counts has been shown to be a poor proxy of population size (Griffiths et al., 2015) they are still required by the British statutory nature conservation organisations when designing mitigation and compensation measures at the licensing stage of developments. The data presented here suggest that time relative to sunset will potentially impact not only on the detection of occupied ponds with small populations but also on the assessment of the population size at all occupied ponds. Standardisation of a sampling window for great crested newts, which I propose to be between 60 and 180 minutes following sunset, coupled with the population



Figure 4. Counts of great crested newts over all visits split by pond and plotted against temperatures of air and water with the vertical hashed lines representing the 5 °C idealised detection rate air temperature previously reported. (a) Counts of GCN plotted against air temperature showing a significant non-linear trend best fitted by a 6th order polynomial (curve) with a peak at 3.5 °C (vertical solid line). 66 samples were taken below 5 °C and 97 were taken above 5 °C; (b) shows the peak count achieved for each pond plotted against temperature; only the peak count for pond B4 was achieved above 5 °C. The polynomial from (a) is shown for comparison; (c) shows a significant non-linear relation between GCN detection and water temperature best fitted with a 3rd order polynomial (curve) and peaking at 10.4 °C (solid vertical line); and (d) shows peak counts plotted against water temperature; for all ponds except B5 and B8, the peak count was achieved above 10 °C and the polynomial fit from (c) is shown for comparison.

density system given by Griffiths et al., (1996), would help to ensure that data collected on population sizes across the species range would be comparable. Collation of such data throughout a species range would enable interpretation of the relative importance at both local and national scales of great crested newt populations.

The guidance for surveys of great crested newts and previous literature on the topic suggests that great crested newts become most active above 5 °C air temperature (Verrell & Halliday, 1985; English Nature, 2001; Sewell et al., 2010; Harper, 2015). This study could only be undertaken on specific agreed dates with the land owner and so the temperatures were not always above 5 °C (Fig. 4). However, the results showed that a peak detection of great crested newts was achieved at 3.5 °C air temperature and almost all peak counts per pond were achieved below an air temperature of 5 °C (Fig. 4a,b). This contrasts with observations from prior surveyors (Verrell & Halliday, 1985; English Nature, 2001; Sewell et al., 2010; Harper, 2015), and provides a potentially interesting future research aspect from a Scottish perspective. Indeed, the temperature which seemed to be most relevant to great crested newt activity was the water temperature. This conformed to previous studies where great crested newt presence in the water column reduced as the water temperature dropped towards 5 °C, with a peak of detection at 10.4 °C (Fig. 4c,d). It would be prudent for ecological practitioners to measure water temperature as a regular component of great crested newt surveillance and reporting.

The phase of the lunar cycle has been shown to have a significant impact on the relative detection rates of newts,

wherein detection of newts is significantly lower at a full moon and for up to 10 days following (Deeming, 2008). However, in this study the absolute percentage of visible moon surface was utilised as an explanatory variable and showed no significant effect on detectability. Percentage of visible sky covered by cloud as estimated by the author in the field did show a negative correlation with newt detection rate. This may be related to some aspect of natural light cues and could warrant further exploration.

Though these data present an insight into the relative detection rates of great crested newts over time, there are some areas where the experimental design could be improved. Repeated nocturnal sampling of ponds throughout an evening at different pond clusters on consecutive nights would provide a more statistically robust measurement of newt population detection. In addition, there were no measurements of the light levels at which newts were detected; further studies should seek to measure the ambient light levels at the survey site at each sampling time to ascertain whether this has any impact on the relative abundance of newts in the water column of their breeding ponds. Differences in visibility of and accessibility to the water column on different visits due to growth of algae or macrophytes can introduce differences to the relative detection of newts via torchlight throughout a season (Griffiths et al., 1996). Further work should incorporate these variables during modelling. Finally, third-party verification of identification in-field would be beneficial to ensure that fatigue from prolonged nocturnal surveying did not impact upon the quality of the data being collected.

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Inferring status changes of three widespread British reptiles from NBN Atlas records

TREVOR J.C. BEEBEE^{1*} & SOPHIA RATCLIFFE²

¹Amphibian and Reptile Conservation Trust, 655A Christchurch Rd, Boscombe, Bournemouth, Dorset BH1 4AP, UK ²National Biodiversity Network (NBN) Trust, Unit F, 14 – 18 St Mary's Gate, Lace Market, Nottingham, NG1 1PF, UK ^{*}Corresponding author Email: Trevor.beebee@gmail.com

ABSTRACT - Records from the UK's NBN Atlas for three widespread reptiles (the slow-worm, *Anguis fragilis*, the northern viper, *Vipera berus* and the viviparous lizard, *Zootoca vivipara*) from 1970 to 2017 were investigated to assess relative trends in population trajectories. Unique 1 km² (U1KM) records for slow-worms and viviparous lizards increased over time whereas after the 1970s those for vipers did not. Viviparous lizard U1KM records increased concordantly in southern England and northern Scotland suggesting that climate warming has not influenced population trends for this species in recent decades. In southern and eastern English vice counties with high proportions of arable farming, U1KM records for slow-worms and vipers trended downwards since the 1990s. These declines were not seen in the less intensively farmed west and north of Britain. Problems and limitations of using NBN Atlas records are discussed.

INTRODUCTION

Declines of the widespread British reptiles (slow-worms *Anguis fragilis*, viviparous lizards *Zootoca vivipara*, northern vipers *Vipera berus* and grass snakes *Natrix helvetica*) have been widely investigated since the mid-late 20th century (e.g. Cooke & Scorgie, 1983; Swan & Oldham, 1993; Wilkinson & Arnell, 2013). Two of these species have attracted particular concern. Some observers have noted local disappearances of viviparous lizards, especially in southern England, and this is the only reptile to have declined significantly in the Netherlands since 1994 (Creemers & van Delft, 2009). Vipers have attracted attention in Britain because of increasing evidence that this snake has declined more than any other widespread reptile, an issue discussed at a dedicated conference, 'The Vanishing Viper: Priorities for Adder Conservation', in autumn 2016.

In this paper we report analyses of records from the NBN Atlas (https://nbnatlas.org), a repository for records of all taxonomic groups in UK that extends over several centuries, to assess whether national trends can be identified from this huge data set. For reasons mentioned above we were particularly interested in vipers and viviparous lizards, but included slow-worms as a comparator with less indication of national decline. Grass snakes were excluded because, unlike the other three, their distribution is not Britain-wide and excludes almost all of Scotland. The main problem with using NBN Atlas data for trend analysis is well known; record numbers for most species have increased over time simply because ever more people are submitting their observations. Various methods to compensate for increased recorder effort have been proposed. One uses data from a large number of related species and identifies outliers showing increases or decreases relative to a concordant majority. Species that follow a common trend are attributed to increased recorder effort (Telfer et al., 2002). This could not be applied sensibly to a situation with only three species. Other approaches include random resampling of recent record sets to make numbers comparable with earlier submissions, but these are primarily designed to detect directional range shifts (Hassall & Thompson, 2010) rather than local changes and are therefore not appropriate for the present study. Our major assumption, based on evidence indicated above, is that there has been no true increase in abundance or distribution of any of the three reptiles investigated here, irrespective of increases in records. This is impossible to prove but forms a starting point for interspecies comparisons. The analyses addressed three hypotheses: (1) Vipers have fared less well than slow-worms or viviparous lizards since the 1970s; (2) Viviparous lizard records have increased less in southern rather than northern Britain as a result of climate change; and (3) records of all three species have increased less in the predominantly arable vice counties, mostly in southern and eastern England, compared with the rest of Britain.

METHODS

We downloaded all occurrence records for the three species (northern viper, viviparous lizard and slow-worm) from the NBN Atlas (NBN Atlas occurrence download at https://nbnatlas.org accessed on Sat Dec 09 12:37:17 UTC 2017).The NBN Atlas data resource citations are listed in supplementary material. The data included the Ordnance Survey grid reference and vice county for each record. We aggregated the data, for each species, as the total number of records per time period (1970s, 1980s, 1990s, 2000s and 2010s, up to and including 2017) in each vice county. One hundred and nine vice counties recognised by the NBN

Atlas and with common reptile records are listed in the Appendix. In addition, for each species we calculated the number of unique 1 km (U1KM) grid reference squares with records in each vice county in each decade, where records were available at that resolution. Unique squares were those that were included only once in the analysis, in each decade, even if there was more than one record from that square in a decade. This editing removed about 80% of the total records for each species, which included not only 1 km² duplicates but also all the many records at lower resolution. Although 1 km squares could include more than one locality for a species, this level of resolution was the most accurate basis available for determining record trends over time.

For climate change analysis we compared data from vice counties approximately south of a line between the Severn and Thames estuaries with those north of the Central Belt in Scotland, running approximately between Glasgow and Edinburgh, all as listed in the Appendix. Vice counties with large areas of intensive arable farming were subjectively assessed (no quantitative estimates were available) based on the CEH Land Cover map of 2007 (https://www.ceh.ac.uk/ services/land-cover-map-2007) and these are also listed in the Appendix.

Statistical tests including regression and comparisons of regression lines were carried out using the programme STATISTIX 7 (Tallahassee, USA).

RESULTS

The NBN Atlas accumulated totals of 25,165 records for slow-worms, 16,942 for vipers and 36,213 for viviparous lizards between 1970 and 2017. As expected, total record numbers for all three species increased over time (Fig. 1A). For each of the three reptiles, regression of number against time (1970s – 2010s) was significant (all with rsp<0.002) but the rate of record increases for vipers was less than 60% or 38% of the rates for slow worms and viviparous lizards respectively. In the case of U1KM squares (Fig. 1B) the difference was more dramatic, with no detectable net increase in viper records since the 1970s. However, a majority of records from the 2010s (62% for slow-worms, 56% for vipers and 69% for viviparous lizards) were from U1KM squares not recorded in any of the four previous decades.

U1KM square records of viviparous lizards increased concordantly in the climatically different regions of southern England and northern Scotland (Fig. 2). Regression slopes for the two areas were not significantly different (F = 0.47, df = 1,6, p=0.518). However, U1KM square records from vice counties with large areas of arable farming showed very different patterns of change over time compared with vice counties having generally smaller areas of arable land (Fig. 3). Slow-worms and vipers shared a similar pattern of fewer records since the 1990s in the "arable" vice counties, whereas in less intensively farmed parts of western and northern Britain the records of all three species increased continuously between the 1970s and 2010s.



Figure 1. Reptile record increases over time. **(A)** = based on all records; **(B)** = based on U1KM square records. •, Slow-worm (Af); O, viper (Vb); \mathbf{V} , viviparous lizard (Zv)



Figure 2. Latitudinal variation in increases of viviparous lizard U1KM square records. ▼, Southern counties, total records = 1,729; Δ , north Scottish counties, total records = 1,468

DISCUSSION

There are obvious caveats to analysing and interpreting NBN Atlas record data in the way that we have attempted to do. The continuous increase in recorder effort means that in most cases it is impossible to quantify absolute changes in abundance, and interpretation is necessarily restricted to relative variations among species or circumstances. Focusing on the most geographically precise (1 km²) records came at the cost of ignoring the remaining 80%, the hope being that the most geographically accurate data provide a realistic view of overall trends. There are also some differences among the three reptiles with respect to survey methods. Viviparous lizards are mostly seen basking, slow-worms are usually under refugia while vipers are found by both methods but most often when out basking. The snake, being higher up the food chain than the lizards, is likely to be inherently the rarest of the three species. A notable preliminary result of the analysis was a substantial turnover of records, with many previously unrecorded U1KM squares appearing in the 2010s commensurate with overall declines. This implies probable heavy losses from previously documented U1KMs during the recording period.

How, then, do the three starting hypotheses stand up in the face of the NBN Atlas data analysis? In the first instance, is there evidence of vipers faring worse than the other two reptiles? The answer seems to be yes. In the 1970s and 1980s total numbers of records for all three species were quite similar, as shown in Figure 1A. After that, despite a general increase in recorder effort, viper records increased more slowly than those for either of the lizards. Trends based on the U1KM square records (Fig. 1B) reinforced evidence of the viper's special predicament, with numbers flat-lining since the 1970s. This result is concordant with field-based studies of vipers (e.g. Gleed-Owen & Langham, 2012). In a wide-ranging synopsis of viper monitoring across Britain, more than 90% of over 100 populations studied for at least three years were in decline (Gardner & Baker, 2018). The NBN Atlas data therefore support the viper decline hypothesis.

Local declines of viviparous lizards have been noticed by several observers. Experiments in enclosures subjected to differing temperature regimes have suggested that an overall average increase of 2 °C, within the range predicted by some models of climate change, would dramatically increase mortality rates of adult viviparous lizards ultimately leading to local extinctions (Bestion et al., 2015). However, the second hypothesis, that climate warming might be causing viviparous lizard declines in Britain, was not supported. U1KM square record increases between the 1970s and 2010s were essentially identical at the two extremes of latitude in Britain, notably southern England and northern Scotland (Fig. 2). It may be too soon to expect climatic effects on viviparous lizards, even in southern Britain. Such warming as has occurred in recent decades has mostly been manifest in winter, with much smaller effects in summer (Beebee, in press). Local viviparous lizard declines are perhaps more likely due to factors such as habitat change or destruction.

Comparing records from heavily arabilised vice counties with those from western and northern Britain broadly



Figure 3. Reptile U1KM records and land use. **(A)** = trends in 35 "arable" vice-counties, total records = 2,538; **(B)** = trends in 74 "non-arable" vice counties, total records = 8,939.•, Slowworm (Af); O, viper (Vb); $\mathbf{\nabla}$, viviparous lizard (Zv)

supported the hypothesis that intensive agriculture has damaged reptile populations. Remarkably, from the 1990s onwards U1KM square records for slow-worms and vipers actually decreased substantially in arable regions (Fig. 3A) despite the overall increases in recorder effort documented earlier. In sharp contrast, U1KM square records for all three reptiles, including the viper, increased continuously in western and northern vice counties (Fig. 3B). "Arable" vice counties constituted about 32% of the UK total vice county area, whereas they had only about 22% of the widespread reptile records since 1970 (almost identical percentages for all three species) despite having some of the warmest summers in Britain. The "arable" region records just since 2010 were even lower, averaging about 15% of the UK total. Reptile fates therefore matched those of many other declining species of British wildlife for which agricultural intensification stands out as the major cause (Burns et al., 2016). Nevertheless, it would be wrong to attribute all the widespread reptiles' problems to arable farming. The differences between arable and non-arable regions could be coincidental, perhaps due to factors other than farming practices. Vipers, for example, have declined dramatically in much of central England and even pastures have widely been 'improved' to the detriment of wildlife. Among other things, urbanisation and increasing disturbance by walkers in the countryside have also impacted adversely on this snake (Gardner & Baker, 2018). However, albeit with the limitations alluded to earlier, it seems that the NBN Atlas records can be useful as providers of evidence about the recent fate of wildlife despite complications from increased recorder effort.

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APPENDIX

The vice counties of Great Britain that were the origin for the NBN Atlas reptile records in this study, indicating those used in climate change analysis (Southern England v. Northern Scotland) and those used for assessing the impacts of a high incidence of arable land (Arable v. the rest)

	Listings for specific a	analyses	
Vice County	Southern England	Northen Scotland	Arable
Anglesey			
Ayrshire			
Banff		Х	
Bedfordshire			х
Berkshire	х		
Berwickshire			
Brecon			
Buckinghamshire			х
Caernarvon			
Caithness		х	
Cambridgeshire			х
Cardiganshire			
Carmarthenshire			
Cheshire			
Cheviotland			
Clyde Islands			
Cumberland			
Denbighshire			
Derbyshire			
Dorset	х		
Dumfrieshire			
Dunbarton			
Durham			
East Cornwall	х		
East Gloucestershire	-		x
East Kent	х		x
East Norfolk	-		x
East Perth		х	
East Ross		х	
East Suffolk			x
East Sussex	х		x
East Sutherland	-	х	-
Easterness		х	
Edinburgh			х
Elgin		х	
Fife		х	
Flintshire			
Forfar		х	
Glamorgan			
Haddington			x
Herefordshire			x
Hertfordshire			x
Huntingdonshire			х
Isle of Man			
Isle of Wight	х		
Kincardine		х	x
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Kirkudbrightshire			
Lanarkshire			

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Relative clutch mass in four exotic reptile species from southern Florida

WALTER E. MESHAKA, JR.1*, FRANK J. MAZZOTTI², & MICHAEL R. ROCHFORD²

¹Section of Zoology and Botany, State Museum of Pennsylvania, 300 North Street, Harrisburg, PA 17120, USA

²Fort Lauderdale Research and Education Center, University of Florida, Davie, FL 33314, USA *Corresponding author Email: wmeshaka@pa.gov

INTRODUCTION

Florida is home to 57 exotic species of amphibians and reptiles (Krysko et al., 2016; Meshaka, 2011). Reproductive cycles and clutch characteristics are known for some of these species in Florida (Meshaka, 2011); however, relative clutch mass (RCM) is poorly known. RCM expresses clutch or litter mass as a proportion of the combined mass of the female body and the clutch or litter. It provides a measure of female reproductive investment, putting clutch or litter size into a broader context.

Four exotic species established in Florida, Oustalet's chameleon (*Furcifer oustaleti*), the Argentine black and white tegu (*Salvator merianae*), Nile monitor (*Varanus niloticus*), and Burmese python (*Python bivittatus*), lay relatively large clutches of eggs (Krysko et al., 2008; Meshaka, 2011; Hanslowe et al., 2016; Smith et al., 2016; Wolf et al., 2016). Feeding habits of the latter three species threaten a wide range of vertebrates (Snow et al., 2007; Dove et al., 2011; Meshaka, 2011), and they are dispersing rapidly in the state (Meshaka, 2011). Here, we used clutch data from these four species to calculate RCM and interpret our findings within the context of predictions associated with their body form and life history traits (Shine, 1992) and what these values could mean with respect to colonisation success.

MATERIALS AND METHODS

Relative clutch mass (RCM) was estimated by the equation: Clutch mass/clutch mass + female body mass. Data for Oustalet's chameleon were derived from Smith et al. (2016). For the remaining three species, masses were measured to 0.1 g. We measured snout-vent length (SVL) to the nearest 0.1 cm in lizards and to the nearest cm in the Burmese python. Means are followed by ± 1 standard deviations. All statistics were calculated on Excel 2016. Statistical significance was recognised at p< 0.05.

RESULTS

Oustalet's chameleon (*F. oustaleti*) – Data from 10 females were available from Smith et al. (2016). To calculate pre–oviposition mass, we added clutch mass to the unpublished post–laying mass of the female. Body size (SVL) was available for nine females (mean = 16.5 ± 1.6 cm SVL; range = 14.5–19.5 cm). For 10 females, clutch size averaged 43.4 eggs (\pm 9.0; range 34–62), and RCM averaged approximately one–third of the mass of ovigerous females (mean = 0.35 ± 0.03 ; range = 0.32–0.43).

Argentine black and white tegu (*S. merianae*) – Three females from Florida City, Miami-Dade County, were available for estimation of RCM. The first two were captured alongside their nests at which time we recorded their clutch characteristics. A 39.4 cm SVL female weighing 1950.0 g was captured on 13 May 2015. The nest contained 27 eggs weighing 621.0 g. We calculated an RCM of 0.32 for this female. A 35.9 cm SVL female weighing 1550.0 g was captured on 14 May 2015. The nest contained 27 eggs weighing 646.0 g. We calculated an RCM of 0.42 for this female. Neither date nor clutch size was recorded for a third female that measured 36.6 cm SVL; however, we calculated an RCM of 0.33 for this female.

Nile monitor (V. niloticus) – A 41.6 cm SVL female Nile monitor was captured on 30 June 2014 from along a canal in Palm Beach County. Once thawed, we found the female to contain 20 shelled eggs; 11 eggs in the left oviduct and 9 eggs in the right oviduct. We calculated an RCM of 0.05 for this female.

Burmese python (*P. bivittatus*) – Data were available to calculate RCM from four ovigerous female Burmese pythons captured during June–July 2013 and in July 2014. Number of eggs in left and right oviduct, respectively are shown in parentheses. A female of 251 cm SVL containing 22 eggs (14 and 8) had an RCM of 0.15. A female of 239 cm SVL containing 24 eggs was calculated to have a RCM of 0.22. A female of 296 cm SVL containing 41 eggs (27 and 14) had an RCM of 0.38. A female of 252 cm SVL containing 13 eggs had an RCM of 0.28. For all four females, the RCM averaged 0.26 (\pm 0.1). A female of 264 cm SVL from Everglades National Park having laid 22 eggs lost 54% of her body weight (Wolf et al., 2016). Among captives in a study, 30.0–38.1% of body weight was lost in nesting females (Brashears & DeNardo, 2013).

DISCUSSION

The RCM values of the three lizard species we examined conflicted in-part with predictions associated with foraging strategy and predator escape (Vitt & Congdon, 1978; Vitt & Price 1982). As expected, Oustalet's chameleon, a

cryptic sit-and-wait or ambush predator, produced a high RCM. Counterintuitively, however, RCM values of the Argentine black and white tegu, a widely-foraging species that takes flight from predators, were similar to those of Oustalet's chameleon and were much higher than those of other widely-foraging teiid lizards (Vitt & Congdon, 1978; Vitt & Price 1982). Furthermore, RCM of the Nile monitor measured in our study, although conforming to predictions of low RCM associated with their ecology, was contrary to findings in this species elsewhere (Buffrénil & Rimblot-Baly, 1999). We proffer that the Argentine black and white tegu, and possibly the Nile monitor, adjust their behaviour to accommodate a high RCM as reported for the broadheaded skink (Plestiodon laticeps) (Cooper et al., 1990). Females of this skink adopt sit-and-wait cryptic behaviour when gravid, thereby offsetting the danger of compromised locomotor escape associated with a high RCM (Cooper et al., 1990).

The Argentine black and white tegu and broadheaded skink share two relevant aspects in their ecologies associated with reproduction. Both species produce a single large clutch annually, and parental solicitude is developed strongly in both species. For a highly seasonally-active species like the Argentine black and white tegu, an ovigerous female has little if any room for food and has somatic fat stores (WEM, FJM, and MRR unpubl. data). Moreover, the eggs are laid in a nest of the female's making and guarded, which would enforce reduced movements as the female approaches oviposition and is most vulnerable to a predator. To that end, captures of ovigerous females in southern Florida are uncommon in standardised trapping (WEM, FJM, and MRR unpubl. data), thereby corroborating an inability or unwillingness to forage actively at that reproductive stage. The Nile monitor likewise produces a single large clutch with a high reproductive investment with only a portion of adult females laying eggs each year (Buffrénil & Rimblot-Baly, 1999). Abdominal body volume: body mass ratio is a strong predictor of RCM (Shine, 1992), and a high value in the Nile monitor would be indicative of a high RCM. However, unlike the broad-headed skink and Argentine black and white tegu, the Nile monitor does not guard the clutch. Thus, it remains to be seen if and in what way females of the south Florida population adopt a cryptic and sendentary disposition prior to oviposition.

Snakes generally produce a higher RCM than lizards (Seigel & Fitch, 1984), and Shine's (1992) findings corroborate that pattern in association with an abdominal body volume: body mass ratio of snakes being nearly twice that of lizards. The RCM values of our Burmese pythons ranged widely. Because female Burmese pythons can easily exceed the body sizes in our study and because our sample size was small, a larger sample that includes larger–sized females would provide a stronger assessment of RCM of the Florida population.

High fecundity is a correlate of successful colonisation (Baker, 1965). This ecological correlate, generally measured by clutch size and frequency, is associated with many established exotic species of amphibians and reptiles in Florida (Meshaka, 2011), including the four species of

this study. Interspecific differences in RCM in our study conformed to predictions associated with behaviour. These differences underscore the importance of RCM as an important measure of fecundity, apart from clutch size and frequency, when evaluating fecundity as a correlate of success in members of Florida's exotic herpetofauna.

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Incidence of snake bite and corresponding compensation payments in the Kannur district of Kerala, India

RAMESH. ROSHNATH^{1,2*}, EDAKKEPURATH KUNHIRAMAN³ & CHANDERA V. RAJAN⁴

¹Ecology and Evolutionary Lab, Department of Animal Sciences, Central University of Kerala, Padannakad, Kerala, 671314
²Malabar Awareness and Rescue Centre for Wildlife, Kannur, Kerala, ³Pappinisseri Visha Chikilsa Kendram, Kannur, Kerala
⁴Kerala Forest Department, Kannur, Kerala
^{*}Corresponding author Email: roshnath.r@gmail.com

ABSTRACT - Data on snake bite cases were collected from a hospital that is the main centre for the diagnosis and treatment of envenomation and poisoning in the Kannur district of Kerala (India), from 2012-2015. Data on financial compensation for snake bite was collected from the Kannur Forest Department in the same period. A total of 770 snake bites were recorded, of which 487 were from *Hypnale hypnale* (hump-nosed pit-viper), 281 from *Daboia russelii* (Russell's viper), and one incidence each of bites by *Naja naja* (Indian spectacled cobra) and *Bungarus caeruleus* (common krait). Snake bites were most frequent in summer and during the wet season and most common in individuals aged 40-50 years. Although 770 snake bite cases were recorded in the period, Kannur Forest Department paid snake bite compensation in only 452 cases (58%). This amounted to US\$0.63m for snake bite mortality and US\$1.081m for morbidity. We suggest that increasing the knowledge and awareness of the general public about venomous snakes could reduce the risk of snake bite.

INTRODUCTION

The World Health Organization estimates that India has the highest snake bite mortality in the world (Singh & Singh, 2013). This is due to the large number of medically significant snake species in and around human habitations, lack of medical facilities, improper first aid and treatment, inadequately trained clinicians, and reliance on traditional snake bite treatment (Whitaker & Whitaker, 2012). Acquiring snake bite data is difficult and the data are likely incomplete (Ahmed et al., 2008). Nevertheless, Kerala appears to be among the Indian states with a high incidence of snake bite (Philip, 1994).

About 60 species of venomous snakes are present in India, of which four venomous species, *Naja naja* (Indian spectacled cobra), *Bungarus caeruleus* (common krait), *Daboia russelii* (Russell's viper) and *Echis carinatus* (saw-scaled viper) are well known as major threats to human life in most parts of the Indian mainland (Whitaker & Captain, 2004). More recently a fifth species, *Hypnale hypnale* (hump-nosed pit-viper) which is often misidentified as *E. carinatus* (Simpson & Norris, 2007) has been considered as no less important and causes serious complication such as acute kidney injury, hematological manifestations, and other organ involvement, in some cases leading to death if not treated (Kularatna & Ratnatunga, 1999; Shivanthan et al., 2014).

Under the Kerala Rules for Compensation to Victims of Attack by Wild Animals 1980, (latest amendment in 2014), compensation is paid for both mortality and morbidity following snake bite. Snakes come under the Schedule of Indian Wildlife Protection Act 1972 administered by the Forest Department and thus even though snake bites occur

al manifestations, and 80% occurs during the south-west monsoon.

Collection of data

with compensation.

We collected snake bite data for the period 2012 to 2015 from Pappinisseri Visha Chikilsa Kendram (PVCK), a hospital exclusively for the diagnosis and treatment of envenomation and poisoning in Kannur district. The data included the names, ages, and gender of snake bite victims, and the month and year of the bite. The species of snake implicated was identified from the bite marks

mostly in agriculture areas, the Forest Department is tasked

undertaken to document the species involved, seasonality,

METHODOLOGY

Kannur district of Kerala lies between latitudes 11° 40' to

12° 48' N and longitudes 74° 52' to 76° 07' E, with elevation ranging from sea level to the highest point in Paithalamalla

Grasslands (1372 m above mean sea level). In a 2011

survey, Kannur had a population of 2,523,003 which gives

a human density of 852/km². The district can be divided

into three regions; highlands comprising mountains with

major plantations such as coffee, rubber, tea, cardamom

and timber; midlands of undulating hills and valleys with

agricultural activity; and lowlands with rivers, deltas and

seashore. The district has a humid climate with a hot season

from March to the end of May followed by the south-west

monsoon that continues until the end of September. The annual average rainfall is 3438 mm of which more than

frequency, and associated compensation costs.

Geography of Kannur district

This study of snake bite in Kannur district was

by the experienced medical practitioners in PVCK and observations of victims. We also consulted doctors and some victims to understand the circumstances of snake bite. In addition, information on compensation paid to victims of snake bite mortality or morbidity in the period 2012 to 2015 was obtained from the Forest Divisional Office in Kannur to give an understanding of the economic impact of snake bite.

RESULTS

Snake bite data

A total of 770 snake bites were recorded in PVCK during the year 2012-2015 (Table 1) of which 63% of the bites were reported from *H. hypnale* (hump-nosed pit-viper, Fig.1B), and 37% from *D. russelii* (Russell's viper, Fig. 1A). Of the total of 281 bites considered to be by *D. russellii*, 169 were suspected to be from adult snakes and 112 from juveniles. There was one incidence of a bite each by Indian spectacled cobra, *N. naja* and common krait, *B. caeruleus*.

Snake bites varied seasonally (Table 1). Bites from *H. hypnale* average 14/month but rose to an average of 23/ month during September to November. Most bites were of laborers working in rubber or cashew plantations and of people living adjacent to forest areas. Monthly variations were also observed in *D. russelii*, with an average of 8 bites/ month, but rose to an average of 14/month during the months of May to August, when bites by juvenile snakes were more frequent. The single incidence of bites by *N. naja* and *B. caeruleus* were reported in the months of May and October respectively.

Significant variation was observed in sex ratio of snake bite victims. During 2012-2015, men had 70% of the bites by *D. russelii* ($\chi^2 = 47.06$, df=1, p<0.001) and 64% of bites by *H. hypnale* ($\chi^2 = 38.54$, df=7, p<0.001). Significant difference was observed in the number of snake bites among different age class (Fig. 2; $\chi^2 = 282.15$, df=7, p<0.001). People belonging to age class 40- 50 had the highest bite rate compared with other classes.

Compensation data

During the three year period, the Kannur Forest Department made 452 compensation payments for snake bite mortality or morbidity (Table 2). In each of the financial years (April-March) of 2012-2013 and 2013-2014, 12 death cases were reported and in the year 2014-2015 this rose to 16, giving a total of 40 deaths in all (Table 2). This was verified from the postmortem reports. A compensation payment amounting to about US\$1590 (Rs 1 lakh) was given by the Forest Department in each case of mortality; amounting to a total of about US\$0.63m (Rs 40 lakh) paid in the three year period. Over the three year period, a total of 412 cases of compensation of morbidity were applied for and backed up with a formal doctor's certificate resulting in a total payment of about US\$1.081m (Rs 67.99 lakh).

DISCUSSION

The snake species responsible for bites treated by the PVCK hospital were almost exclusively victims of *H. hypnale* and *D. russelii*. The annual rate of bites recorded from this



Figure 1. Examples of major species of snakes involved in snake bite cases in Kannur district of Kerala (A) *H. hypnale* Humpnosed pit-viper; (B) *D. russelii* Russell's viper



Figure 2. Number of snake bites in relation to the age of the victim in Kannur district (2012-2015)

main treatment centre amounts to roughly nine people per 100,000 of the population in Kannur district. However, the rate would be expected to be considerably higher than this as some victims would seek treatment from other clinics/ hospitals, from traditional healers or even no treatment. The bite frequency peaked during the summer and monsoon season, reflecting agricultural activity, flooding, increased snake activity, and abundance of their natural prey (Ahuja & Singh, 1954; Sawai & Honma, 1976; Kasturiratne et al., 2008).

Hypnale hypnale is terrestrial, nocturnal, and usually

Table 1. Number of snake bites cases reported during 2012-2015 at the Pappinisseri Visha Chikilsa Kendram hospital Kannur

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Indian spectacled cobra					1								1
Common krait										1			1
Hump-nosed pit-viper	28	21	27	23	45	31	31	35	61	83	62	40	487
Russell's viper	15	14	11	9	51	64	33	22	18	13	15	16	281
Total	43	35	38	32	97	95	64	57	79	97	77	56	770

Table 2. Compensation paid by the Forest Department in Kannur District to snake bite victims suffering mortality or morbidity (Indian rupees)

Year	Mortality		Morbidity	
	No.	Compensation Amount (Rs)	No.	Compensation Amount (Rs)
2012-2013	12	1,200,000	160	689,000
2013-2014	12	1,200,000	76	1,450,000
2014-2015	16	1,600,000	176	4,660,000
Total	40	4,000,000	412	6,799,000

sluggish but will bite when threatened or provoked (Das, 2002). It is more abundant in Parassinikadavu, Mayyil and Mallapattam areas of the district and the victims were mainly the people working in rubber tapping and other plantations. Daboia russelii is found widely across the district, mainly in agricultural lands, open forests, and gardens and is mostly a nocturnal forager. However, in cooler conditions it becomes active during daylight hours and spends more time thermoregulating (Daniel, 2002). A high incidence of bites by D. russelii in Kerala has been recorded previously (Mahadevan & Jacobsen, 2009). This species mates during the months of December- January and produces young mainly during May to July (Mallow et al., 2003, Whitaker & Captain, 2004). The highest frequency of bites by this species was during the months of May to August (14/month) and these were more often delivered by juveniles. Juvenile D. russelii are more nervous and active than adults (Warrell, 2010) and are likely to be present in greater numbers than adults in this period.

The victims of snake bite recorded at the PVCK hospital, were more likely to be men than women. As more men are engaged in work in the fields their chances of being bitten by a snake are greater, similar results were reported elsewhere (Suchithra et al., 2008; David et al., 2012). We found that the 40-50 years age group was more prone to snake bite compared to other age classes, again we believe this is due to their higher encounter rate with snakes. This contrasts with two other studies where risk of death from snakebite was greater at the age 5–14 years and 15–29 years (Sawai & Honma, 1976).

Echis carinatus (saw-scaled viper) has been reported to be the most serious cause of snake bite morbidity and mortality in South Asia (Chand 1990; Alirol et al., 2010). In Kannur district *E. carinatus* is restricted to the Kannur-Kasaragod border (Chemmeni) and no bites were reported during the study period, although a few cases have been reported previously (Dr. Murali, PVCK, Kannur). The other two species reported as minor causes of snake bite in Kannur, *N. naja*, and *B. caeruleus*, are distributed widely across the entire district although *N. naja* is apparently absent from the area of Kannur town (Riyas Mangad, Snake rescuer, Malabar Awareness and Rescue Centre for Wildlife).

Economics of snake bite

Enormous effort, money and time are involved in the management of snake bite cases. Costs associated with a snake bite begin with travel to the hospital and end in to post treatment expenses. Hospital charges vary according to the hospital and location. Generally local people in Kannur rely on the PVCK hospital and Pariyaram Medical College. The cost of treatment in these institutions is lower than in private hospitals. A vial of antivenom may cost US\$10 to \$16 (Rs 600 -1000). Antivenom administration depends on the species and degree of envenomation. In some snake bite cases, 20 vials of antivenom is prescribed. Kerala Forest Department pays compensation for snake bite treatment which is a major support to such victims. However, only 58% of the cases known to this study appear to have received compensation. The reason for this may be that a few victims can easily pay for themselves while others may have been unaware that they could claim compensation from the Forest Department.

Mitigation

The incidence of snake bite may be minimised in a number of ways. Regular rodent control is advised to avoid snakes in human habitations. During periods of high snake activity (May-July for *D. russelii* and Sept- Dec for *H. hypnale*), more precaution are to be taken during agriculture/ gardening practices, grass/wood collection etc. Especially people working on agricultural lands or in rubber/cashew plantations have to take greater care. Rural inhabitants should avoid sleeping on the ground. Isolation of snake habitat from human living space is essential to reduce human-snake conflict. For example, plants pots must be kept away from courtyards and branches of trees near windows must be cropped to help prevent snakes entering houses. An education programme on the conservation of non-venomous snakes in Kerala has achieved positive attitudinal change among the local people (Balakrishnan, 2010), a similarly focused approach towards an awareness of the general public towards venomous species would be expected to reduce the frequency of snake bite morbidity and mortality.

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The movements and habitat preferences of a Malayan krait (*Bungarus candidus*) in an agrarian landscape

TYLER K. KNIERIM¹, BENJAMIN M. MARSHALL¹, LUCY HAYES², SURACHIT WAENGSOTHORN³, PONGTHEP SUWANWAREE¹ & COLIN T. STRINE^{1*}

¹Suranaree University of Technology, Nakhon Ratchasima, Thailand ²Sakaerat Conservation and Snake Education Team, Thailand ³Sakaerat Environmental Research Station, Nakhon Ratchasima, Thailand ^{*}Corresponding author Email: Colin_Strine@sut.ac.th

ABSTRACT - Little is known about how south-east Asian snakes respond to the conversion of natural areas to humandominated land uses. We radio-tracked a *Bungarus candidus* (Malayan krait) in an agricultural zone of the Sakaerat Biosphere Reserve in Thailand for 68 days. The snake exhibited nocturnal activity and remained within a 3.23 ha 100% minimum convex polygon (MCP) home range during this period. Using Duncan's Index of Preference, we found the snake preferred less disturbed habitats such as agricultural field margins (0.90), and to a lesser extent, a Eucalyptus plantation (0.75).

INTRODUCTION

S outh-east Asian landscapes are undergoing rapid anthropogenic change, posing threats to the region's unique biodiversity (Hughes, 2017). To date, most ecological research in the region has been conducted in protected areas or in patches of contiguous habitat (Martin et al., 2012); despite protected areas only representing a small portion of the total global land area (Juffe-Bignoli et al., 2014). To better understand how snakes can persist in human-dominated landscapes, we need a more comprehensive understanding of their space use.

The Malayan krait (*Bungarus candidus*) is distributed across much of south-east Asia and thought to dwell primarily in forests and plantations (Chan-ard et al., 2015; Wogan et al., 2012). Two studies have assessed the spatial ecology and habitat use of *B. candidus* by radio-tracking. One radio-tracked a single individual for 22-days (Mohammadi et al., 2014), the other tracked an individual that only survived 14 days, before being captured and killed in a fish trap within 1 km of our study site (Crane et al., 2016). The objective of our study was to investigate the ecology of a *B. candidus* living within a highly-disturbed landscape, where the risk for human-snake conflict exists. We report our observations on the movements, habitat preference, and shelter site use of an individual *B. candidus* from an agricultural habitat in Thailand.

METHODS

We captured a *B. candidus* at approximately 21:30 h on 8 July 2016, as it crossed a dirt path, dividing a Eucalyptus plantation (*Eucalyptus camaldulensis*) and a cassava field, outside the boundary of the Sakaerat Biosphere Reserve's protected Core Area (14.51° N, 101.95° E; WGS84; Fig. 1A). The individual was male, likely a juvenile, weighing

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113.5 g and measuring 77.0 cm snout to vent length (SVL) and 97.6 cm total length. We transported the individual to our field laboratory where we anesthetised and surgically implanted the specimen with a 1.8 g radio transmitter (Holohil Systems model SD-2, Carp, Ontario, Canada). We followed the surgical implantation methods described by Reinert and Cundall (1982), which are believed to not cause physiological or behavioural changes in snakes (Reinert & Cundall, 1982). Later that day at dusk, we released the *B. candidus* at its site of capture.

To receive the transmitter's signal and locate the snake, we used an R410 ATS radio receiver connected to a Telonics RA-23K VHF antenna. On the days that we did track the snake, we tracked it for some hours during daylight and at night-time. In total, we located the snake 21 times during daylight tracks and 13 times at night. During daylight tracking (07:00 h to 19:00 h), we used triangulation to approach the snake, attempting to identify its shelter site while minimising disturbance. We recorded the location of the snake using Universal Transverse Mercator (UTM) WGS 84 projection on a Garmin 64S GPS device. While at each site, we recorded the habitats the snake used, the habitats surrounding the snake's location and attempted to identify the specific shelter site being used. During nighttime tracking (19:00 h to 07:00 h), we estimated the snake's position using wide-arcing triangulations at a minimum distance of 10 m from the snake to limit disturbance while it was potentially active. Therefore, we were unable to accurately identify specific shelter sites or cover used at night.

Working in R (R Core Team, 2017) with the software packages adehabitat' (Calenge, 2006), 'rgdal' (Bivand et al., 2017), and using all observed locations, we calculated the home range as 100% and 95% minimum convex polygons (MCP). These were displayed using QGIS (Quantum GIS Development Team, 2017). We opted to use MCPs



Figure 1. The study area and the 12 habitats located within it. Also displayed are the locations that the *B. candidus* was located as well as the 95% and 100% MCP areas. The map scale corresponds to the 47N UTM region (m), with each grid square equating to 100 m².

to measure the home range of our snake instead of more sophisticated estimation methods due to the short tracking duration of our single individual and our limited number of locations. Similar studies on related species have also used MCPs (Mohammadi et al., 2014; Barve et al., 2013; Croak et al., 2013). This allows comparisons with our data.

Habitat preference was based on daytime shelter site locations and estimated using the Duncan's Index which we log normalised between 0-1 (Duncan, 1983). We defined the study area used in our habitat analysis as the region within a 265 m buffer combined with the 95% MCP home range to eliminate bias from areas unused by the *B*. candidus. We derived the width of the buffer from the distance between the nearest edge of the 95% MCP to the furthest outlining observation of the snake. The buffer's width is an estimate of the longest distance the snake could have moved away from its 95% MCP core home range area before returning, i.e. a generous estimation of the area available. We classified 12 habitat types within the study area: roads, highway, human settlement, cassava field, maize field, rice paddy field, fallow field, eucalyptus plantation, mixed fruit orchard, field margin, pond, and dry dipterocarp forest (DDF; Fig. 1). The DDF was within the protected Core Zone, all other habitats were in the reserve's unprotected transitional and buffer zones.

RESULTS

We radio-tracked the *B. candidus* for 68 days (15 July - 21 September 2016) and obtained 34 confirmed locations, until we were unable to obtain a signal due to transmitter failure, or less likely, the individual abruptly leaving the study area. By listening to fluctuations in the signal, we determined that the *B. candidus* was moving during four of the 13 night tracks and one of the 21 day tracks. The sole day time movement occurred at 16:37 h, when the snake appeared to be moving beneath rocks at the location of its previously used shelter site. The ambient temperature was 27.1° C and there was 100% cloud cover, following light rains during the movement observation.

The 100% MCP home range size was 3.23 ha and the 95% was 1.80 ha (Fig. 1). We identified the specific shelter site used by the *B. candidus* on eight of the 19 relocations. The snake sheltered in animal burrows during five tracks, termite mounds during two tracks, and beneath rocks during two tracks. We were unable to identify the specific shelter site during 11 tracks because of dense vegetation.

The Duncan's Index revealed a strong preference for field margins (0.90), the Eucalyptus plantation (0.75), and to a lesser extent, a maize field (0.22). The preference for maize results from a single shelter site location in which the

snake was in a weedy patch of the field, several meters from the field's densely vegetated margin. All other habitat types show no preference and absolute avoidance.

All identifiable shelter sites used by the *B. candidus* during day tracks (animal burrows and termite mounds) were located on field margins or within the Eucalyptus plantation. At one of its shelter sites, the individual sheltered in an animal burrow complex located within 1 m of the four-lane highway at the eastern edge of its 100 % MCP home range. However, we located most shelter sites along a single margin, approximately 3 m wide, and characterised by sparse mature trees, unmanaged herbaceous vegetation, and a dry irrigation ditch running lengthwise through the site. This margin was primarily bordered by maize fields on either side.

We did not observe the *B. candidus* using the DDF, rice fields, fallow fields, orchards, or human settlements (Fig. 1). However, several of the locations within the field margin brought the snake within 10 m of the nearest household. Movements during night tracks showed a similar pattern, suggesting a preference to move along vegetated field margins. The snake was either on or within several meters of a field margin during tracks in which it was moving. The snake made use of a narrow field margin, approximately 1 m in width, to move between the adjoining main field margin and the Eucalyptus plantation.

DISCUSSION

Our juvenile male individual had a smaller 100% MCP home range (3.23 ha) by the end of our 68 day tracking period than the adult male radio-tracked by Mohammadi et al. (2014), (12.30 ha) from their 22 day tracking period. Both snakes were tracked at the apex of the second rainy season between June-September. Our study also confirms nocturnal activity in B. candidus which was not reported by either Mohammadi et al. (2014), nor Crane et al. (2016). Additionally, our observations highlight the potential importance of certain landscape features in agricultural landscapes for B. candidus. We suspect that both undisturbed raised field margins and irrigation ditches serve as essential shelter sites and movement corridors for B. candidus. Choosai et al. (2009) found that dikes and termite mounds hosted higher soil macrofaunal biodiversity than the surrounding fields in north-east Thailand's agricultural rice systems. At our study site, agricultural fields undergo at least two crop rotations per year, subjecting the soil to frequent disturbances. Eucalyptus plantations, which are harvested on a 3-5-year cycle, and field margins, that are left undisturbed long enough to accumulate animal burrows and termite mounds, likely provide a stable source of shelter for B. candidus. Discovering when kraits are active and whether preference for field margins and irrigation ditches continues throughout the year for other individuals will aid in identifying areas where B. candidus and humans are most likely to encounter each other.

We suggest future research on *B. candidus* in agrarian landscapes to focus on determining activity periods and assessing microhabitat features, such as the availability of shelter sites between margins and adjacent anthropogenic habitats. Identification of specific habitat features will aid the conservation of *B. candidus* in anthropogenic landscapes by providing land managers with explicit features to maintain. Combatting human-snake conflicts in anthropogenic landscapes is critical to the survival of snakes (Whitaker & Shine, 2000) and further research into the movement patterns of this medically important species may provide insight into how this could be achieved. We also suggest incorporating more individuals from varying age classes to investigate whether spatial and activity patterns differ across age classes.

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The diet of African house snakes (*Boaedon* spp) revealed by citizen science

ERIK PATERSON

205 Telford Road, East Kilbride, G75 0DG Author Email: erikpaterson@virginmedia.com

frican house snakes (Boaedon spp) are nocturnal Aconstricting predators, and as their name suggests, are commonly found around human habitation (Briscoe, 1949). It is generally remarked that house snakes will feed on the rodents and other pests which are known to gather and feed around urban settlements (Gratz & Arata, 1975). Occurring throughout sub-Saharan Africa, there has been a recent increase in researchers aiming to decipher phenotypic crypsis using molecular techniques, so that there are now 13 recognised species (Kelly et al., 2011; Greenbaum et al., 2015; Trape & Mediannikov, 2016). Very little is known of their ecology. With the availability of publicly shared information on social media sites and online recording platforms, data which are of research interest and relevance in the study of wild fauna can be utilised to generate insights into the ecology of species via citizen science (Newman et al., 2012).

METHOD

Interested members of the general public in Southern Africa are encouraged to submit images of reptiles and amphibians to the Facebook group "Predation Records – Reptiles and Frogs" (Predation Records, 2017): all records submitted are freely available for use by interested individuals and researchers. A review of all images posted within the group which clearly depicted a *Boaedon* house snake as a predator was conducted on 25 November 2017 encompassing all publicly accessible records submitted. A scan of the images tab was undertaken, each record was opened and the prey item was noted. As house snakes present a number of cryptic species (see above), no attempt was made to distinguish exact species. Prey items were grouped as bats, rodents, birds, frogs, lizards or snakes.

RESULTS & DISCUSSION

There were 23 images submitted between 15 September 2015 and 23 November 2017 which clearly depicted a *Boaedon* sp as a predator. Ten showed a lizard as prey, 5 showed a bird, 4 a rodent, 2 a bat and 1 each a frog or a snake. These data suggest that *Boaedon* are opportunistic predators which might take a wide range of prey, but that lizards might form an important part of the diet, at least for some species. In the past, *B. geometricus* has been recorded to feed on the invasive oriental garden lizard *Calotes*

versicolor (Matyot, 2004), and *B. lineatus* is said to feed primarily on lizards and frogs, although this observation is not supported by hard data (Akani et al., 2008). Whether any, or all, of the species of *Boaedon* are specialist predators must at present remain an open question.

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The work reported here also demonstrates that data derived predominantly from amateur observations, which are not part of a structured research project but obtained from publicly shared information on social media sites or online recording platforms (citizen science), can have value and contribute to an understanding of the ecology of snakes, and possibly of other predatory animals as well.

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Dietary notes and foraging ecology of south-east Asian water monitors (*Varanus salvator*) in Sabah, northern Borneo, Malaysia

JOSHUA P. TWINING^{1*} & ANDRÉ KOCH²

¹Queen's University, Belfast, School of Biological Sciences, MBC, 97 Lisburn Road, BT9 7 BL, Northern Ireland, UK ²Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany *Corresponding Author Email: joshuaptwining@gmail.com

The South-east Asian water monitor (*Varanus salvator*) is one of the largest and most widely distributed lizard species in the world occurring from Sri Lanka to the Moluccas (Koch et al., 2007; Somaweera & Somaweera, 2009; Koch & Böhme, 2010). Despite its widespread distribution, and being considered an apex predator in many of the ecosystems it inhabits, particularly east of the Sunda shelf where large carnivorous mammals are generally absent (Sweet & Pianka, 2007), little is known about the functional ecology of water monitors. Typically regarded as generalist carnivores, they have been observed feeding on a huge variety of prey ranging from invertebrates, crustaceans and vertebrates including frogs, snakes, birds, fish, squirrels, deer, crocodiles, rats and tortoises (Gadow, 1901; Smith, 1932; Losos & Greene, 1988; Gaulke, 1991), to carrion, human refuse, fermented coconut and even human corpses (Uyeda, 2009; Guawardenam, 2016; Koch & Arida, 2017). From such records it can be deduced that water monitors provide integral ecosystem services through regulation of prey populations and carrion removal which is important in disease dynamics and nutrient cycling (Wilson & Wolkovich, 2011; Moleon et al., 2015; Twining et al., 2017). However, the effects of land-use change on ecological role of the species remains unclear, as wildliving water monitors are typically highly mobile and alert making direct observations difficult.

During a study of land-use change on vertebrate scavengers in Sabah, northern Borneo (Twining et al., 2017) water monitors were sampled using large cage traps (150 x 50 x 50 cm), baited with native fish, rodents, half domestic chickens and pig. Individuals were injected with a unique passive integrated transponder (PIT) tag in the thigh of the left hind leg, injecting it between muscle and skin pointing downwards (Ariefiandy et al., 2014; Twining et al., 2017). Due to the physiological stress response observed in water monitors in which they regurgitate their stomach contents when the trap is approached by the researcher some opportunistic observations were made on the diet of V. salvator macromaculatus in areas of varying land-use intensity. Typically, only the bait provided in traps was expelled, however, three observations of non-bait items were made from two individuals along riverine margins in oil palm plantation and selectively logged forest.

The first and second observations were on the 3rd May 2014, and 6th December 2014 from the same individual, a large female trapped along a riverine margin in an oil

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palm estate, which had ingested human refuse (in the form of instant noodle packets) on two separate occasions. This individual had a snout-vent length of 51.2 cm, a tail length of 88.5 cm and a mass of 1.39 kg. The third observation was made on the 18th December 2014, from a small adult male in selectively logged forest, with a snout-vent length of 39.8 cm, a tail length of 62.9 cm and a body mass of just 700 g. This lower body condition individual on its second capture regurgitated fourteen 3 - 4cm long, thick keratin coated quills, the internal organs, and upper cranium of a juvenile Malayan porcupine (Hystrix brachyura) Although having been reported to eat other rodents, to our best knowledge this is the first record of any varanid consuming a porcupine (Henry, 1912; Harrison, 1955; Sharma & Vazirani, 1977, Auffenberg & Ipe, 1983; Rao & Rao 1984; Losos & Greene, 1988; Gaulke, 1991; Traeholt, 1994; Uyeda et al., 2009; 2015). Even on recovery, the quills remain hard, and very sharp. The presence of such a number of quills, and the intact gastro-intestinal tract suggest the prey was consumed whole, a particularly tricky prey item to ingest in such a way. Such a prey item is indicative of an active hunting strategy, perhaps a greater necessity in order to meet high metabolic demands of varanids in lower intensity land use areas due to elevated competition for carrion with mammalian counterparts (Twining et al., 2017; Wearn et al., 2017).

Greater abundance and size of varanids in habitats with high anthropogenic presence have been linked to the utilisation of human trophic subsidies previously (Uyeda et al., 2009; Jessop et al., 2012). The female, which regurgitated human refuse on two occasions, had a high body condition, despite the presence of six buccal nematodes inside its mouth. This observation provides another record of water monitors adapting to human inhabitancy, which may be indicative of a switch to life history more dependent on scavenging of refuse (Uyeda et al., 2009; 2015). Such dependence on human refuse has also been observed in many small islands in the Indo-Malay Archipelago, including the Wakatobi Islands southeast of Sulawesi, the Gili Islands northwest of Lombok, Pulau Tiga and Pulau Gaya east of Sabah (JT, personal observation; H. Bernard, pers. comm., 2017). However, despite the presence of highly calorific food sources, and the low energy cost in attaining them, the benefits of this energy trade off, in conjunction with increased abundance resulting in increased sexual competition, biased sex ratios

and increased parasitism has been questioned previously (Uyeda et al., 2009; 2015; Jessop et al., 2012; Twining et al., 2017)

Although three observations are not sufficient to make any concrete conclusions from, when considered with the reported significant declines in biodiversity and abundance of typical prey items of varanids in oil palm estates, they may represent a shift in feeding ecology. Anurans, large and small mammals, fish and invertebrates were all reported to decline in the highest land-use intensity areas compared to forested sites within the same experimental landscape (Wilkinson et al., 2003; Konopik et al., 2015; Gray et al., 2016; Wearn et al., 2017). This decline in abundance and biodiversity of prey species, in conjunction with observed greater abundance, masses and body conditions of water monitors in oil palm estates (Twining et al., 2017), may be indicative of a distinct switch in foraging ecology observed in populations in forested sites compared to those in oil palm estates. Although observations were scarce, those made suggest an ecological switch from active hunting in natural environments to a scavenging life history in oil palm.

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Reproductive biology of *Humerana miopus* (Anura: Ranidae) from Kedah, Peninsular Malaysia

SHAHRIZA SHAHRUDIN

School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia Author Email: shahriza20@yahoo.com

Humerana miopus is an intermediate to large-sized frog, with a snout-vent length reaching 73 mm (Berry, 1975). This forest species is distributed in southern Thailand and Peninsular Malaysia, and occurs at low altitudes (IUCN, 2014). It can often be encountered in primary rainforests, along logging tracts and in secondary growth forests. Additionally, it can be found along the river banks, forest edges, and overgrown rubber plantations, and is known to breed in pools (Grandison, 1972; Berry, 1975). It is the only species of frog from genus *Humerana* that existed in the forest of Peninsular Malaysia.

On 8 October 2017, between 21:30 and 22:30 h, an amplectic pair of *H. miopus* was collected at Sungai Sedim Recreational Forest, Kedah, Peninsular Malaysia (5°25'N, 100°46'E; elevation about 150 m a.s.l.). It was captured at the edge of a forest pool after heavy rainfall (2.5 hours rain). The temperature and relative humidity of the sampling site was 25 °C and 85%, respectively (Thermo Hydro meter, Fisher Scientific). The pool (approximately 3.5 m long, 3 m wide, and 0.02-0.4 m deep) was shaded and surrounded by low vegetation and was located along a trail within a lowland dipterocarp forest. The pool had a muddy bed and contained turbid water, with leaf litter, twigs, and tree branches accumulating at the bottom. In addition to the amplectic pair of H. miopus, five H. miopus (Fig. 1) and three Fejervarya limnocharis were detected at the same pool. This indicated that the pool served as a breeding site for both species of frogs.

The amplectic pair was captured and brought back to the laboratory for further inspections. Snout-vent length (SVL) and head width (HW) of male (SVL=68 mm, HW=19 mm) and female (SVL=77 mm, HW=23 mm) were measured by using digital calliper. Frogs were placed in a glass aquarium (60 x 30 x 30 cm), consisting of tap water (15 cm deep), sands, leaf litter, and drift woods. Frogs remained in amplexus, until the female deposited its eggs on 9 October 2017 (approximately 12 hours after captured). The eggs were sphere in shape, black in colour, coated by viscous jelly, and floating on the surface of water (Fig. 2). The clutch, which consisted 856 eggs were in a single layer. Twenty eggs were randomly selected and measured using a microscope with an ocular micrometer. The mean \pm SD $(\min{-}\max, N)$ egg diameter was $1.1 \pm 0.09 (0.9 - 1.2, 20)$ mm. After the study period, both frogs were released back to their original location.



Figure 1. An adult male of *H. miopus* from Kedah, Peninsular Malaysia



Figure 2. Egg clutch of H. miopus

The egg clutch was kept in the same aquarium until hatching (10 October 2017, approximately 28 hours post oviposition). An aerator was provided, to supply sufficient oxygen to the embryos. Only 472 (55%) of the eggs successfully hatched and became free-swimming tadpoles. Tadpoles were small, slender, and black in colour. Most of the tadpoles stayed at the bottom of the aquarium and only some at the surface of the water. At this stage, the mean \pm SD



Figure 3. Tadpole of H. miopus

(min-max, N) length of the tadpole was 4.9 ± 0.93 (3.0-6.0, 20) mm (Gosner's stage 19, Gosner 1960). Tadpoles were fed small fish pallets and rotten dead leaves. On 30 October 2017 (20 days post hatching), the mean \pm SD (min-max, N) total length of the tadpole was 18.9 ± 2.3 (16.0-23.0, 20) mm (Gosner's stage 25). The tadpole body was oval shaped and light to dark-brown in colour, with a light brown/yellow tapering tail, a white belly, and black spots on the body and tail (Fig. 3). Tadpoles had dorso-lateral located eyes, with pinkish colouration behind its eyes, and nostril at the end of a rounded snout. The tadpoles grazed on rotten leaves and spent most of it times at the bottom of the aquarium (usually under leaf litter). However, sometimes tadpoles would emerge to the surfaces of water to breath. The order of larvae development was followed Gosner's stages (Gosner, 1960).

Previously, reproductive biology of several frog species from Peninsular Malaysia have been studied and documented. These included *Fejervarya limnocharis* and *F. cancrivora* (Ibrahim et al., 1999), *Chalcorana labialis* (Shahriza et al., 2010, 2016), *Ingerophrynus parvus* (Shahriza et al., 2012, 2015), *Sylvirana nigrovittata* (Shahriza, 2016) and *Rhacophorus prominanus* (Shahriza, 2017). This observation will increase the current knowledge and understanding of the ecology and breeding biology of tropical frogs.

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First report of death feigning behaviour in the yellow collared wolf snake (*Lycodon flavicollis*)

SANATH KRISHNA MULIYA^{1,2*}, ANUKUL NATH² & ABHIJIT DAS²

¹Zoo Hospital, Bannerghatta Biological Park, Bannerghatta, Bengaluru – 560 083, Karnataka, India ²Wildlife Institute of India, Chandrabani, Dehradun – 248 001, Uttarakhand, India ^{*}Corresponding author Email: sanath@wii.gov.in

eath feigning or thanatosis is a state of tonic immobility, exhibited by certain species of snakes as a response to external stimuli, including predation attempts (e.g. Bhattarai et al., 2017). Currently, this behaviour is reported from Xenochrophis piscator, Coelognathus radiates, Lycodon aulicus and Pseudoxenodon macrops (Vogel & Kam Han, 2010; Mirza et al., 2011; Bhosale & Thite, 2013) among snakes from the Indian subcontinent. On 25 March 2015, the first author (SKM) rescued an adult male specimen (Snout to vent length - 296 mm, tail length -76 mm) of *L. flavicollis* (Fig. 1) from a house in the vicinity of Bannerghatta Biological Park (12.812°N, 77.578°E; WGS 84, 929 m elev) around 22.00h IST (Indian standard time). The individual was identified based on the 'type' description by Mukherjee & Bhupathy, 2007. On handling, the individual exhibited no aggressive behaviour and was bagged quickly. Fifteen minutes later, the snake was placed on the ground for documentation and photography, during which it made several attempts to escape. After approximately five minutes the snake stopped moving and started rolling upside down revealing its ventral scales, remaining in this position for approximately five to ten minutes (Fig. 2). SKM located and palpated the heart, revealing an active pulse. On manually inverting the snake to stimulate, the individual moved haphazardly, possibly in a vague attempt to escape. The snake was bagged again and kept under observation at Bannerghatta Zoo Hospital. The snake was released the next day near to the capture location, uneventfully.

For snakes, death feigning includes tonic immobility often coupled with mouth gapping and hanging/protrusion of the tongue (Bhosale & Thite, 2013). However, in this observation we only recorded immobility and rolling of body, without mouth gapping, similar to observations of death feigning behaviour in other Lycodon spp (Mirza et al., 2011).

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Figure 1. L. flavicollis from Tumkur, Karnataka



Figure 2. *L. flavicollis* from Bannerghatta, Karnataka exhibiting death feigning behaviour

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Oxybelis aeneus: aggressive interactions with the clay-colored trush (Turdus grayi)

MARCO D. BARQUERO

Sede del Caribe, Universidad de Costa Rica, Montes de Oca, San José, Costa Rica, 2060 *Corresponding author Email: marco.barquero_a@ucr.ac.cr

In this note an aggressive encounter between a brown vine snake (*Oxybelis aeneus*) and a clay-colored thrush (*Turdus grayi*) is reported. The observations took place in the campus of the University of Costa Rica, Limón port city, Costa Rica (9° 59'4" N, 83° 3'40" W), which includes an open, grassy area, and a secondary tropical rainforest (Gutiérrez Calvo, 2012). Observations were carried out 15 m away from the interacting animals, such that individuals were not disturbed. Animals were not captured at the end of the observation period and were identified through photographs and video recorded during the interactions. *Oxybelis aeneus* is a long, slender species with a pointed head, brown dorsal coloration, and black-purplish lining of the mouth (Savage, 2002; Solórzano, 2005), whereas *T. grayi* is a medium-sized, brown bird with a yellowish bill (Stiles & Skutch, 2007).

The encounter occurred on 25 March 2017, when an adult T. gravi was observed actively exploring a hollow metal beam (rectangular C-shaped cross-section) that supports a side section of the roof of one building (Fig. 1A). The thrush was first seen at 12:40 h, approaching the roof and repeatedly looking inside the hollow beam. The bird then introduced its head into the beam and the adult brown vine snake emerged from the open side. The bird stayed on top of the roof for about 1 minute and then flew away, while the snake remained immobile with its mouth open showing the dark lining for several minutes after the bird was gone. The snake then returned to the beam and disappeared from sight. This first encounter was not videotaped; however, after approximately 10 minutes the bird returned to the same place and resumed searching around the beam. This time the event was filmed using a HDC-HS60 Panasonic camcorder. The interactions described above were repeated, with the snake again opening its mouth and remaining immobile (Fig. 1A). The thrush was mobbed by a hummingbird (most likely a rufous-tailed hummingbird, Amazilia tzacatl), which flew against the thrush repeatedly (Fig. 1B), and the thrush aborted its search after 2 minutes. The clay-colored thrush returned to the roof after c. 11 minutes and was mobbed again by the hummingbird, flying away after 30 s. During this time period, the snake remained in almost the same position and moved towards the beam 39 minutes after the second encounter with the thrush. When the snake was approaching the beam, the thrush reappeared and pecked the snake at its mid-body. No other encounter occurred after this direct attack.



Figure 1. Snapshots obtained from a video that recorded the interactions between T. grayi and O. aeneus, with the bird exploring the place where the snake was hidden (A), and the interactions being halted because of the dives made by a hummingbird (red circle) towards the thrush (B).



Figure 2. Pictures showing the beam of the roof where a brown vine snake was hidden and a clay-colored thrush nest above the roof (A), and the chicks observed in that nest two weeks after the interactions between the thrush and the snake (B).

Since the snake was hidden from the bird's sight and not moving, the aggressive behaviour of the thrush towards the snake was a puzzle. The diet of *O. aeneus* is composed mainly of lizards, especially *Anolis* sp., although it also includes insects, fishes, small mammals, and small birds (Henderson, 1974, 1982; Grant & Lewis, 2010). Adult *T. grayi* are too large (23 cm in length [Stiles & Skutch, 2007]) to be potential prey of *O. aeneus*, but chicks could be easy targets. After exploring the surroundings where the encounter took place, a nest of *T. grayi* was found 3 m above the beam (Fig. 2A). Two weeks after the aggressive interactions, two chicks were observed on that nest (Fig. 2B). The proximity of the snake to the nest could have been perceived as a threat to the chicks and might have prompted the direct attack by the thrush, even when this represents a risky anti-predator strategy.

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First record of male-male combat in Xenopeltis unicolor

JESSE GOODYEAR¹ & EDWARD GILBERT^{2*}

¹Tambon Lak Hok, Pathum Thani, 12000, Thailand ²Duncan Drive, Guildford, Surrey, GU1 2NR, UK *Corresponding author Email: edd1eg@live.co.uk

The sunbeam snake (*Xenopeltis unicolor*), is one of two **I** species in the family Xenopeltidae. It is distributed throughout south-east Asia where it is often encountered in open areas close to bodies of water, particularly agricultural areas (Cox, 1993, Karns et al., 2005). It is a medium-sized (approximately 1 m) fossorial snake, characterised by a wedge-shaped head and highly iridescent smooth scales. Male-male combat in snakes, is sometimes misidentified as opposite sexes courting, but is a key behaviour that may drive life history strategies (Shine et al., 1981). Not all snake species exhibit male-male combat. Evolutionary relationships between these behaviours show that body bridging and downward anterior pushing may be ancestral traits amongst snakes. Some clades show more complex behaviours such as neck biting, while others exhibit none at all (Senter et al., 2014).

Herein we present the first known recorded observation of male-male combat within Xenopeltidae. On the 13 February 2018 at 21:40 h, in Pathum Thani, Bangkok, Thailand (13.9691°N, 100.5760°E, Datum 47P) during a herpetofaunal survey we witnessed two adult male *X. unicolor* engaging in male-male combat (Fig. 1). The snakes were observed on a roadside verge consisting mainly of tall grass, displaying body bridging behaviour, twisting continuously while moving forward. After approximately 10 minutes they coiled around each other and bit each other (holding) for approximately 1 minute (Fig. 2). They continued body bridging behaviour and once aligned, simultaneously pushed their anterior downwards on to each other. They disregarded the presence of observers and combat continued onto a road, for an additional 10 minutes. They coiled around each other and bit each other again, locking for a further minute. Combat concluded with 1 minute of body bridging and simultaneous anterior downward pushing, until 22:03h when they separated and the smaller individual began moving away. The larger individual displayed a jerking head-raise behaviour before moving away in the opposite direction.

Despite their wide distribution and relative abundance within certain habitats, no form of intrasexual competition has been recorded previously in *X. unicolor*. Schuett et al. (2001) however, predicted that male-male fighting should be expected in the Xenopeltidae based on their defence behaviour and the present observation confirms this prediction. As the family Xenopeltidae belongs to a basal lineage in snake phylogeny, the present observation contributes to a further understanding of the evolution of intrasexual competition (e.g. Senter et al., 2014).

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Figure 1. Two male X. unicolor engaged in combat, exhibiting body bridging on a road next to a grass verge



Figure 2. The two male *X. unicolor* subsequently coiling and biting each other

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Australia's Dangerous Snakes: Identification, Biology and Envenoming

Peter Mirtschin, Arne R. Rasmussen & Scott A. Weinstein

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Being married to an Australian and having visited her home country almost annually over 25 years, my personal experience is that there is a widespread distrust and indeed fear about snakes amongst many Australians. As a visitor interested in Australia's amazing natural history most locals are incredulous that I want to see snakes and other reptiles in their beautiful country.

Such a "climate of fear" can in part be explained by the fact that Australia does (in)famously posses some very, very venomous snakes: the inland taipan *Oxyuranus microlepidotus* is one of the world's most venomous snakes with its venom being 50 times more toxic than that of the Indian cobra *Naja naja*. But the reality is that in an increasingly urbanised country many Australians seldom encounter snakes. Furthermore, snakebites of humans are rare and deaths from snakebites even rarer. Australia's Dangerous Snakes notes that there are 200-500 snakebites per year in the country with just 2-3 resulting in fatalities. To put this in perspective about 1,300 people died on Australian roads in 2016.

Clearly much needs to be done to overcome this atmosphere as snakes play a crucial part in the biodiversity and ecology of the country and can co-exist with humans. One route is through education, understanding and appreciation. This is where the publication of *Australia's Dangerous Snakes* is timely and helpful.

This comprehensive book describes the venomous snakes of Australia defined as "medically important (dangerous)". These include 39 terrestrial species (out of an Australian total of 100), 24 species (out of 34) of sea snakes including two species of sea kraits (*Laticauda*).

In various chapters the book covers the identification of each snake species with keys, text and photographs (though of the latter more later). It also has large sections on the production, action and use of venoms and the clinical management of snakebites. Chapters on the conservation of snakes along with their interactions with humans are included. Finally, there is an appendix describing the history of snake venom production in Australia, a useful glossary and an extensive reference list.

I found the text comprehensive and authoritative with the complementing contributions from all three authors very good. The authors should be congratulated on the work they have done to bring together for the first time so much significant information. This will make *Australia's Dangerous Snakes* an essential reference for many years to come.

A major part of the book is the identification of the snake species: this is done through a key, a table and individual species accounts including photographic images. Though the text is well written, in many cases the photographs of snakes are of medium to sometimes low quality. These are of either live animals or museum specimens and have a curious (and old-fashioned) look as in each case the background has been cropped, leaving a uniform and contrasting white surround. Though I understand the motives for wishing to show a photo for each species and the practical reason for doing this as presented (some species are rare or difficult to photograph) it is not successful. In an era of high-resolution digital cameras and modern book design and production the photos are sometimes below standard. This is a shame as for many readers the images of snakes are the first thing that they will look at when opening the book. High quality images of these beautiful creatures would go a long way to increasing appreciation of them.

Unfortunately such criticism also feeds into the general production of the book. Again it has an old-fashioned feel. Many schematic figures are copied directly from other sources sometimes with too much detail included. These should have all been redrawn for this book to make a uniform style. Other figures/tables specifically made for the book are very basic. Some photographs are superfluous and seem be added to fill space. I feel uncomfortable making these criticisms because the authors have put a great amount of high quality content in this important book, but it is shame that the publishers have not invested more money and time to match this in the production.

However, with this proviso, I hope that *Australia's Dangerous Snakes* is widely circulated and used as it offers much essential information about venomous snakes in Australia which should be disseminated. As for the goal to reduce the "climate of fear" and increase understanding and appreciation in the general public of these creatures, this will be best served in a book containing high quality photographs and drawings to display these wonderful animals and their role in the Australian natural environment.

CHRISTOPHER J. MCINERNY

School of Life Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

Email: Chris.McInerny@glasgow.ac.uk

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Ruud de Lang

The Snakes of Java, Bali and Surrounding Islands

Ruud de Lang

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The Snakes of Java, Bali and Surrounding Islands ▲ of Sumatra, Java and Borneo have snake faunas of particular interest. By virtue of size, these islands support many species and furthermore being in the shallow seas of the Sunda shelf they have at various times been connected or isolated from adjacent land masses. Consequently, the Greater Sundas possess a range of species with affinities to either the Malayan fauna,

The Greater Sunda Islands

which mostly favour humid tropical climates, or to the continental Asian fauna that is more tolerant of tropical, but seasonally dry, climates. With an overlay of time this has created islands with distinctive blends of similarity and difference (endemism). In recent years, academic interest has focused on the affinities of the faunas of not just these islands but throughout the whole Indonesia archipelago.

For many years, the most comprehensive source of information on reptiles of the Archipelago was the two volume series by the Dutch herpetologist Nelly (Pertonella) de Rooij; her second volume covers snakes (De Rooij, 1917). In the 1980/90s, this essential reference was difficult to obtain and I recall having to make do with bits photocopied from relevant sections. Things have changed. Starting from just before the new millennium there has been a flurry of books/field guides on the snakes of the region; so that now there are texts covering Papua New Guinea, Sumatra, Borneo, Sulawesi, Bali, the Lesser Sunda Islands, and the Moluccas. The missing piece to the puzzle was a field guide to Java. It is therefore fitting that the latest volume is a guide to the snakes (excluding sea snakes) of Java and Bali by the Dutch herpetologist Ruud de Lang. The inclusion of Bali is logical since the fauna of this island has strong affinities with its neighbour Java: both are on the Sunda shelf and so are on the same side of the Wallace line. Some smaller islands off the coast of Java are also included but, as might be expected, have rather limited snake faunas.

I was delighted to receive a free copy of this field guide as a result of contributing some photos and data on East Java species. On first opening, the most striking feature is the wealth of photographs. There are 288 of them to illustrate 91 snake species and some associated habitats. The photos are generally of a high standard and beautifully reproduced, six are full page illustrations and 13 presented as double page spreads. Some of the species photographs are rare such as the two Java endemics, the brown blind snake (*Argyophis fuscus*) known from a single specimen, and Frustorfer's mountain snake (*Tetralepis frustorferi*) for which there appears to be no other photographs take in life. The first part of the guide deals with the geographical and biogeographical features of the area but dwells more on island affinities than on the origins of the diverse fauna. It provides details of conservation organisations, and presents a checklist of species. This is followed by an identification key that links to the species accounts that occupy the greater part of the text. It is a pity that an Indonesian language version of the key was not included, as it was with the guides to the Lesser Sundas (De Lang, 2011) and Bali (McKay, 2006).

The species accounts vary widely in detail depending on how well a species is known. Clearly, there is still a lot that keen field naturalists could contribute to our knowledge. Interestingly, scattered throughout the book there are text boxes with pictures and biographical details of five European herpetologists who have devoted their energies to Javanese snakes. Notable by her absence is Nelly de Rooij, but then her data came from examining pickled specimens in the Museum of Zoology in the University of Amsterdam. Apparently she never set foot in Indonesia, unlike Ruud de Lang who has travelled extensively in pursuit of his passion but also has to hand the Leiden Museum's extensive collections of Indonesian snakes that date back to the colonial era.

Towards the end of the guide there are three useful tables. One of geographical names and co-ordinates of the many places quoted in the text, which are also plotted as numbers on a series of maps. The second table shows the distribution of species in Java by province (West, Central and East) and by other islands. The third table gives details of lepidosis and pupil shape. Taken together with the rest of the text this presents a wealth of information and offers a good chance of fulfilling Ruud de Lang's stated objective that "...this guide makes it possible to identify all snake species known today from the area. It is meant to be of value to professionals as well as amateurs". As expected the taxonomy of some of the species described has potential for change and warnings are given about uncertainty of Ahaetulla species, the difficulty of separating Trimeresurus albolabris from T. insularis in Central Java, and possible new species of Dendralaphis. There is also a short listing of species of doubtful presence in the area.

In the guide's Foreword it is stated that ".. snake enthusiasts will hopefully recognise species that are unknown when they are found.." and it suggests that "many snakes may have successfully evaded the damaged environments and still survive somewhere on the

island waiting to be discovered by science". This claim is not too farfetched and can be demonstrated with reference to two species; the Indochinese sand snake (Psammophis indochinensis) and the eastern Russell's viper (Daboia siamensis). In continental Asia, both species are known from central Thailand, they are not part of the fauna of peninsula Malaya nor are they known from Sumatra. However, both have been recorded from Java but only from a relatively small patch of northern East Java where there is a seasonally dry climate. Daboia siamensis was confirmed in Java only in the 1930s despite being very noticeable for its behaviour when disturbed, inflating the body and producing a loud continuous hissing, and being a serious cause snakebite morbidity. Further populations of this species are known from some of the Lesser Sunda islands, at a distance of at least 700 km to the east of the Javanese locality, where there are similar seasonally dry climates. What the new guide reveals is that there is a tentative record of D. siamensis from one location in west Bali. This turns out to be more or less the same location from which P. indochinensis it is also known in Bali. Given an active snake trade in Indonesia, the record of D. siamensis on Bali needs confirmation, but Bali aside the two species have remarkably similar distributions. These are presumably based on shared habitat preferences that relate to seasonal climates. P. indochinensis is not known

from any of the Lesser Sunda Islands. Is it possible that one day it may also be recorded from the very same far flung Lesser Sunda islands as *D. siamensis*? *Psammophis indochinensis* may yet surprise us, it was only recorded for the first time from Cambodia in 2011 (Hartmann et al., 2011).

The Snakes of Java, Bali and Surrounding Islands is a 'must have' for anyone interested in the fauna of south-east Asia or snakes in general. Prices vary but typically around $\pounds 54 \ (\leqslant 61.00)$.

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RICK HODGES

66 Marlborough Crescent, Sevenoaks, Kent TN13 2HJ, UK Email: rickhodges123@gmail.com

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American Snakes

Sean P. Graham

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s the author says, this Acharming volume deals with "Snakes as American as apple pie." Being a reader of the Herpetological Bulletin, you are probably someone fascinated by amphibians and reptiles. This is the only qualification needed for reading this excellent book. No special interest in American snakes is required, neither is any technical background since

from the start the author abandons Latin names, almost all technical jargon, and when discussing snake trunk musculature states "... these muscles, movements, and the diagrams that accompany them make my eyes glaze over ...". This is a good starting point for writing an absorbing, personal account that will have you turning all the pages. Nevertheless, without detail and insight a book of this sort would not convey the real interest of American snakes. The biological detail is there; for example, when covering reproduction the author doesn't shy away from explaining sperm competition, or when describing the ectothermic way of life he makes informative comparisons with endotherms. Furthermore, details of snakes are accompanied by vivid descriptions of important habitats interwoven with personal experiences communicated with excitement. In places you feel as if this is travel writing in the spirit of Theroux.

The book is just a little smaller than the standard coffee table volume and is beautifully illustrated with more than one hundred and fifty, well printed, highquality photographs. Many of the images show snakes in fascinating micro-habitats, against interesting vegetation, or displayed against beautiful American vistas. In eleven chapters, this volume covers all you might expect about American snakes but there are also 14 special boxes describing inspiring herpetologists of the author's acquaintance. These are young and old, male and female, famous and not so famous, and are role models from the past and for the future. The book's eleven chapters slip seamlessly from one to the next and each one starts with a story based on the author's or another's experiences. After the 'Introduction' follows 'Form and Function' then two chapters devoted to first 'A day' then 'A year' in the life of snakes. Other chapters cover sex, food, snake eaters, defense and dangerous snakes. In connection with the latter, it was interesting to read that "There really is no such thing as a dangerous snake. But boy there are some stupid

people."; a parody of the National Rifle Association? Then comes a chapter on snake invaders, mostly Burmese pythons in the Everglades. Finally, there are thoughts about snake conservation especially the efforts to protect America's largest species, the Indigo snake, and the charming story of the New Mexico ridge-nosed rattlesnake, the only venomous species with federal protection. Towards the end there is an epilogue, an extensive reference section arranged by chapter, and finally an index. I learnt a lot from the book and special favourites of mine include the narrow headed garter snake that has adopted the way of life of a water snake and by convergent evolution has been shaped to look just like one, and the short-tailed king snake that eats only Florida crowned snakes.

The book is graced with a Foreword by Rick Shine, the distinguished Australian herpetologist. This is appropriate not least because the author was inspired to write 'American snakes' following the example of Rick Shine's 'Australian snakes – a natural history'. After Shine completed his doctorate he spent a few years in the USA which seemed not to have worked out that well, he admits to not understanding what American snakes were doing. Later he mistook a cottonmouth for a harmless water snake and spent a few days in hospital after which he says he came out with a renewed appreciation of the diverse and fascinating nature of American snakes. He concludes that readers of 'American Snakes' can develop a similar appreciation but without spending time in hospital. The retail price for this hardback book is around £22. I would recommend you put it on your birthday or Christmas list, whichever comes sooner, unless of course you are planning a stay in hospital.

RICK HODGES

66 Marlborough Crescent, Sevenoaks, Kent TN13 2HJ, UK Email: rickhodges123@gmail.com

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HERPETOLOGICAL BULLETIN REPORT

February 2018

All four issues of *Herpetological Bulletin* were published on time during 2017 with almost 100% of manuscripts published within 3 months of acceptance. The target for 2018 is a continuation of publication of papers in the next available issue once they have been reviewed and accepted.

During 2017 we received 108 submissions with an additional 5 (not included in the table below) rejected without review. This represents a slight increase in submissions compared to 2016. Full paper submissions remained high at around 32 % of total submitted manuscripts but with higher acceptance rates than previously. Acceptance rate overall was 44.4%. We began a new manuscript category *Short Communication* to take into account submissions that were neither a NHN (single unusual observation) or Short Note (single data set). However, in the table below these are grouped with NHN for simplicity. Full details are shown in the table below with comparable figures for 2016 shown in parentheses.

	Submitted	Accepted	Percent accepted
Full papers	34 (42)	19 (13)	55.9 (30.9)
Short Notes	13 (11)	7 (7)	53.8 (63.6)
Natural History Notes	61 (52)	22 (13)	36.1 (25.0)
Total	108 (105)	48 (33)	44.4 (31.4)

We published one review in 2017, a multi- authored description of the activities of the Zoological Society of London; *Zoological Society of London: contributions towards advancing the field of herpetology through conservation, research, captive management and education* (issue 139).

The following people gave their time and expertise reviewing manuscripts for *Herpetological Bulletin* during 2017: Kevin Arbuckle; Daniel Ariano; Roger Avery: John Baker; Andrew Buxton; Chris Barratt; Trevor Beebee; Xavier Bonnet; John Buckley; Michael Cota: Roger Downie; Eleanor Drinkwater; Carl Ernst; Helen Fearnley; Joan Garcia-Porta; Philippe Geniez; Ron Gerlach; Frank Glaw; Gunter Gollman; Chris Gleed–Owen; Stuart Graham; Richard Griffiths; Jihène Ben Hassine; Rick Hodges; Ivan Ineich; John B Iverson; Laurence Jarvis; Robert Jehle; Brett Lewis; Todd Lewis; Anita Malhotra; Christopher McInerny; Roger Meek; Robert Mendyk; Konrad Mebert; José Antonio Mateo Miras; Joseph T Mitchell; Sinlan Poo; Sara Rocha; Jay Redbond; Elva Robinson: Suzanne M. Simpson; Tahar Slimani; Ben Tapley; Christine Tilley; Audrey Trochet; Rob Ward; Romulus Whitaker & John Wilkinson.



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THE HERPETOLOGICAL BULLETIN

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