

Research Article

Basking Behaviour and Ultraviolet B Radiation Exposure in a Wild Population of *Pelophylax lessonae* in Northern Italy

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ABSTRACT - Amphibians are facing catastrophic global declines under pressure from a variety of threats. Many of these are so acute and poorly understood that ex-situ conservation has been employed where in-situ efforts may be unable to act quickly enough to save species in the wild. However, our knowledge of the captive requirements of most amphibians is unknown or poorly understood and this knowledge gap jeopardises the success of ex-situ programs. A lack of data from the habitat of wild populations underpins many husbandry failures in captivity, as without it husbandry is based on best guess. Ultraviolet B radiation has been shown to be critical in the care of many reptile species, but its importance to amphibians is not well understood. We present the first data on UV-B exposure and basking behaviour in wild amphibians, using *Pelophylax lessonae* as a model species. We show that wild frogs inhabit a UV-B microclimate defined by physical features of their habitat and by the basking behaviour of the animals themselves. This data may encourage the future gathering of such wild environmental data, which could be fed directly into ex-situ programs.

INTRODUCTION

With growing threats to amphibians worldwide and more than a third of known species threatened by extinction (IUCN, Conservation International and NatureServe, 2008) ex-situ conservation is becoming more important in the long term survival of amphibian species (Gascon, 2007). As such, a number of species have been ‘evacuated’ from the wild and installed in captive breeding programs, with the eventual aim of reintroduction. However, knowledge of the captive requirements of many species is poor and this deficit of knowledge has the potential to undermine captive conservation efforts (Gascon, 2007; Gagliardo et al., 2008). Without a good understanding of captive requirements, evacuated populations may be no more likely to survive than their wild counterparts and ex-situ conservation efforts may fail.

One approach to addressing this lack of knowledge is to study amphibians and their

environment in the wild, with the aim of replicating wild parameters in captivity. By aligning the captive environment more closely to wild conditions, the success of captive husbandry may be improved. Furthermore, replicating wild conditions in the terrarium may help to address the issue of adaptation to captivity. Where captive conditions differ from those to which organisms have evolved (i.e. those in the wild), this may either relax selection pressures that existed in the wild (e.g. predation pressures), alter the direction of selection pressures (e.g. fear of humans, foraging behaviour) or exert entirely new ones (e.g. dealing with crowding or novel pathogens) (Woodworth et al., 2002; Frankham, 2008). This phenomenon has been identified in captive colonies of *Alytes muletensis*, which, after 9-12 generations in captivity, showed little or no response to predator cues as tadpoles (Kraaijeveld-Smit et al., 2006). Reproducing wild conditions more faithfully in the captive

environment can help to address this problem to some extent (Frankham, 2008; Williams & Hoffman, 2009).

Ultraviolet B (UV-B) radiation is a form of solar radiation that many organisms, including at least some amphibians, use in the synthesis of vitamin D₃, which is vital in calcium uptake, as well as for other biological functions (Antwis & Browne, 2009). Although the understanding of UV-B requirements in captive lizards and chelonians has revolutionised the way these animals are maintained in captivity and has resolved many previously common pathologies, including metabolic bone disease (e.g. Ferguson et al., 1996; Divers, 1996; McArthur & Barrows, 2008), the importance of UV-B radiation for captive amphibians is very poorly understood (Antwis & Browne, 2009).

Anecdotal, and some experimental (e.g. Verschooren et al., 2011), evidence suggests that UV-B may be important for some amphibians (Antwis & Browne, 2009), particularly those that are known to expose themselves to solar radiation by basking (e.g. *Trachycephalus resinifictrix*; Verschooren et al., 2011). Conversely, UV-B radiation has been suggested as possible driver of amphibian extinctions (e.g. Kiesecker et al., 2001) and some studies have found impacts of UV-B radiation on mortality in captive amphibians (Blaustein et al., 2005).

However, neither set of captive studies (either those finding a benefit or those finding detrimental effect) used data from the field and, more specifically, from the microclimate of these amphibians to inform UV-B exposure levels in the laboratory, so leaving their results open to interpretation. Nor did either study refer to data on basking behaviour in the wild. For example, (Blaustein et al., 2005) found mortality associated with ‘ambient’ levels of UV-B exposure in *Anaxyrus (Bufo) boreas*. UV-B levels were measured at the site where study animals were collected and replicated in the lab. However, UV-B levels were not measured within the microclimate where the toads live and so the exposure in captivity may not reflect levels of UV-B to which these toads are normally exposed. Furthermore, basking behaviour was not recorded and so any effect of behaviour on actual UV-B exposure in the field could not be taken into account. While this study certainly

shows that UV-B radiation can have negative effects of amphibians, it should not be used to justify a lack of exposure in captivity. This study also did not make use of earlier work (Lillywhite et al., 1973), which defined the thermal optima of *A. boreas*, dependent on nutritional status. The UV-B-associated mortality may, therefore, have been influenced by laboratory temperatures being up to 10°C lower than the thermal optimum for this species.

As such, until thorough research reveals the true extent of amphibian dependence on UV-B radiation, or any detrimental effects thereof, the safest course of action may be to provide captive amphibians with levels of UV-B radiation similar to those that they are exposed to in nature, within a spectrum of microclimates allowing for downwards regulation of exposure.

Meteorological data, including UV-B Indices, are readily available for many regions and are sometimes used as a guide for designing captive conditions. However, amphibians live in and use microclimates within the larger environment (e.g. McClanahan et al., 1978; Kluber et al., 2009; Heath, 1975; Seebacher & Alford, 1999; 2002), so meteorological data for the wild range of a species is unlikely to be representative of conditions within the microclimate. Therefore data taken from the wild microclimate must be gathered, rather than using anthropocentric data as a proxy.

The actual amount of UV-B radiation to which wild amphibians are exposed will be dependent on many aspects of species biology. The maximum levels of UV-B exposure that a species can be exposed to will be limited by the micro-climate in which the animals live. Amphibians are not simply passive objects within the environment, however, and UV-B exposure can also be regulated through basking behaviour. This has been demonstrated in reptiles (e.g. Karsten et al., 2009) and there is no reason to suspect that it is not the case in other taxa. Many amphibians are known to bask to thermoregulate (e.g. McClanahan et al., 1978; Wells, 2007) and this behaviour will inevitably also regulate exposure to UV-B radiation, the environmental gradient of which mirrors that of sunlight and solar heat.

Water, or green, frogs (*Pelophylax* spp.) are well-known for their basking behaviour. They exist throughout Europe and Asia and inhabit

still or slow-moving bodies of water from which, in contrast to the brown frogs of the genus *Rana*, they do not stray far (pers. obs.). Several species of this genus are threatened in the wild, according to the IUCN Red List (IUCN, 2012; Uzzell & Isailovic, 2009). The common pool frog, *Pelophylax lessonae* (the focal species of this study) represents a common and readily accessible water frog species, which is itself held within several institutional collections (e.g. Horniman Museum and Gardens and Slimbridge Wildfowl and Wetlands Trust, both in the UK), as well as being used in laboratory research and the culinary industry, for both of which they are collected from the wild (although they are often advertised as ‘farmed’) (pers. comm. J. Bentley).

We present data on basking behaviour and exposure to UV-B radiation in the wild, basking amphibian *P. lessonae*. Although the measures and observations presented are conceptually and practically simple, they represent the first such study in amphibians and provide a baseline for comparison with future studies. We identify the levels of UV-B radiation to which this frog was actually exposed during one day in the breeding activity season (late May) and demonstrate that this species inhabits a UV-B microclimate while basking. Our data, although limited to a single day of sampling, has implications for the captive husbandry of water frogs (*Pelophylax* spp.) and for the way that environmental data should be collected for wild amphibian populations.

METHODS

Study site

The study site, shown in Fig. 1, is a small, mature, artificial pond in a private residential/agricultural setting in Savorgnano, San Vito al Tagliamento, Pordenone, Friuli-Venzia Giulia, NE Italy (N 45°53' 848" E 012°51'180"). It is a rough oval in shape, measuring approximately 2 m in maximum length and 1.5 m in maximum width, with a maximum depth of around 1 m. The pond is located in full sun, with a fringe of long grass. Adult pool frogs (*P. lessonae*) are resident in the pond and were observed to bask in four major positions (marked with ‘x’s in Fig. 1) on the bank near a fringe of long grass running round the perimeter of the pool; henceforth referred to as ‘basking sites’. All

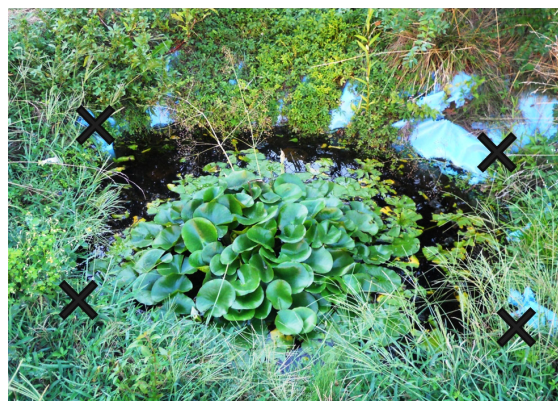


Figure 1. Study site, a small pond inhabited by *P. lessonae* in northern Italy.

readings were taken on the 20/5/2012. During sampling, at least seven adult frogs were present in the pool. All of the following readings were taken on a day of full sun with high visibility and a maximum ambient temperature of 23°C. Frogs were active, and males called during the evening and early morning. Spawning took place 2 weeks after sampling.

UV Index (UVi) readings

Ultraviolet Index (UVi) readings were taken using a Solarmeter 6.5 UV Index meter (Solartech Inc.) from the four identified basking sites. A Solarmeter is roughly cuboid in shape, being 10.5 x 6 x 2.2 cm in size, with the sensor positioned on one of the small end faces of the device. Readings were taken by placing the Solarmeter upright, with the sensor on top, on the basking sites. By placing the meter flat on the ground, the sensor was orientated in the same direction as the dorsal surface of frogs basking on the same site (i.e. vertically perpendicular to the slope of the bank). This ensured that the UV Index measured by the meter was as similar as possible to the UVi encountered by a basking frog. At each time point, mean UVi was calculated across the four positions. Ambient UVi was recorded from an exposed, elevated (head height) position full sun in the vicinity of the pond, again taking the mean from four readings. Readings were taken hourly from 07:00 (local time) until 18:00 (local time); however, no data were collected at 16:00.

UVi is a unitless measure of the amount of UV-B radiation reaching the surface of the planet, weighted for the erythema (sunburn) action spectrum, which quantifies the differing

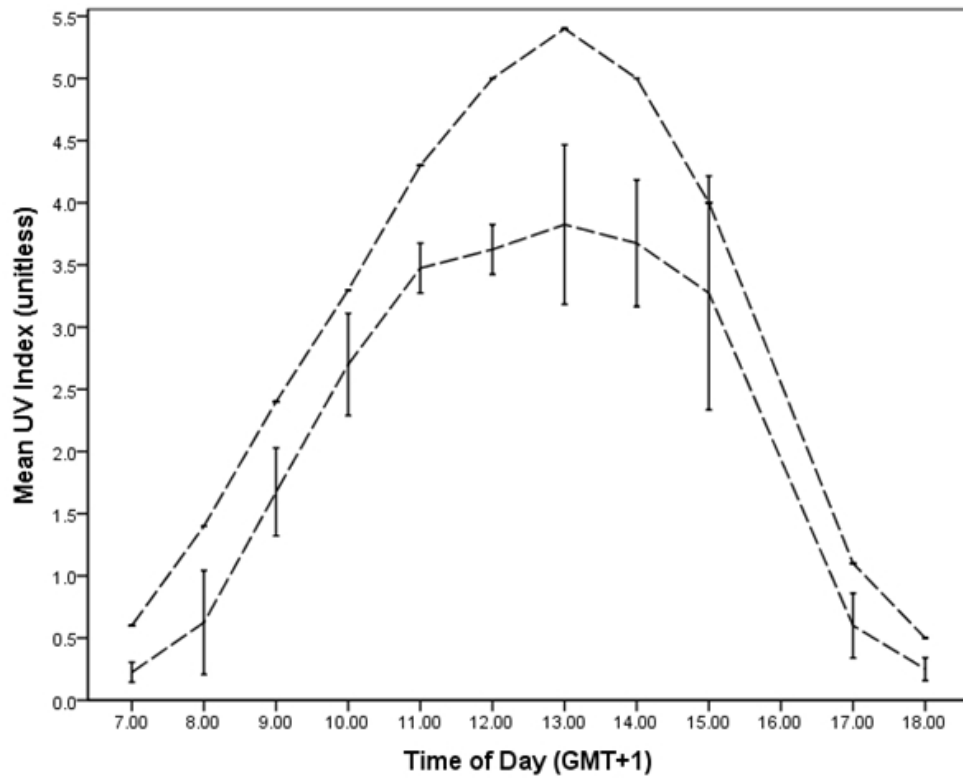


Figure 2. Ambient (broken line) and Basking-site (unbroken line) UV Index readings. Ambient UVi is significantly higher than Basking-site UVi (Paired t-test; $t=-6.384$, $P<0.001$). Error bars represent 95% confidence intervals (these are present, but tiny, for ambient data as repeated measurements had the same values).

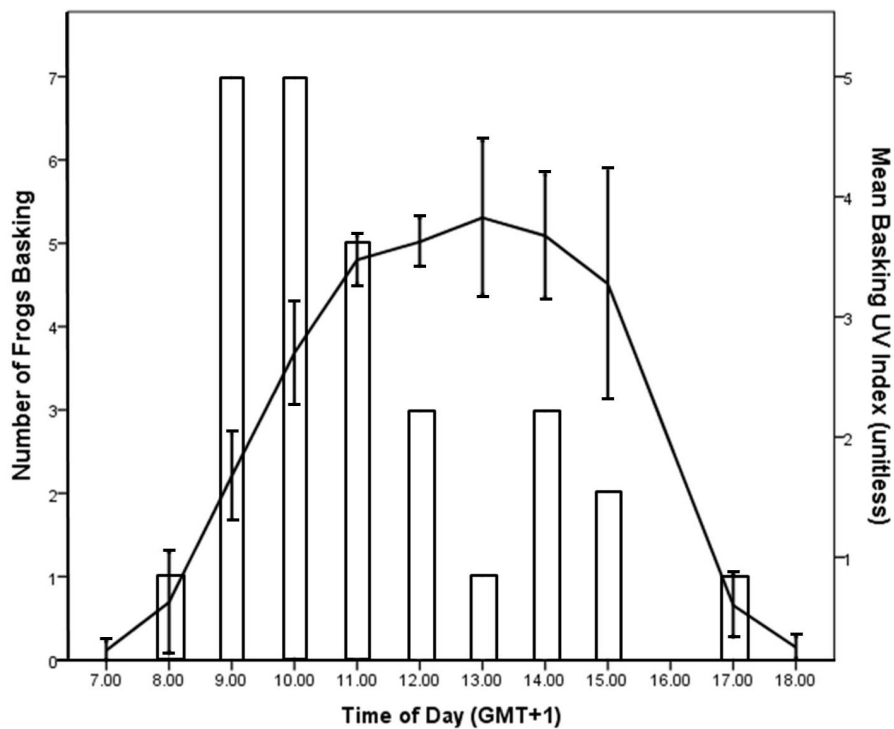


Figure 3. Numbers of basking frogs (bars) and mean UVi (line) at basking sites around the pond. Error bars represent 95% confidence intervals.

biological impact of wavelengths. This value, in W cm^{-2} , is multiplied by an arbitrary value of 40 to give an index value of biologically significant UV irradiance. Although this last transformation is not strictly necessary, it brings values in line with the established UV index scale and makes comparisons more intuitive. This differs from a straightforward measurement of UV-B irradiance, as measured by other devices, which does not correct for biological significance of wavelength. UV indices are widely understood and are thus used to quantify biologically relevant UV irradiance for herptiles by the Reptile and Amphibian Working Group (RAWG) (part of BIAZA) in their UV-Tool, a database designed to compile information relevant to the provision of UV-B radiation to captive reptiles and amphibians. Although this value is used to quantify erythema risk in humans, which is not necessarily relevant to amphibians, it is still a useful representation of the intensity of UV light known to have biological importance in all vertebrates. Furthermore, as well as being linked to the erythema action spectrum, it also describes the action spectrum for the synthesis of vitamin D3 in the skin, with 96% overlap between wavelengths of 290 and 320 nm (F. Baines, pers. comm.), which is of direct relevance to amphibians.

Frog basking counts

When approaching to take UVi readings, the number of frogs basking around the pond was counted. The relatively small size of the pond allowed numbers to be counted easily, with a maximum count of seven frogs.

The pond was left undisturbed between readings so that frogs emerged to bask. Frogs were observed to return to basking positions after 5-10 minutes, so hourly intervals provided sufficient time for normal basking behaviour to be manifested.

Statistical analyses

Data was analysed in SPSS 16.0, specific tests are reported alongside results.

RESULTS

Comparison of basking and ambient UVi; evidence for a UV-B microclimate

Fig. 2 shows mean ambient and mean basking-site UV Indices throughout the day. Error bars are present for ambient readings, but as all four readings were identical, they are very small. Ambient UVi was significantly higher than basking-site UVi (Paired t-test; $t=-6.384$, $P<0.001$). The maximum basking UVi never exceeded the ambient UVi (despite the overlap of the 95% confidence intervals at 15:00).

Frog basking counts and UVi at basking sites

The number of frogs basking on basking sites in relation to UVi is shown in Figure 3. Basking behaviour was bi-phasic; numbers of basking frogs peaked first in the morning, between 09:00 and 10:00 while a second, lower, peak in basking behaviour occurred in mid-afternoon (between 14:00 and 15:00). There were very few basking frogs early in the morning, late in the evening and around the solar zenith (13:00, due to summertime hour change).

DISCUSSION

Many amphibians use environmental microclimates within the broader climate of their geographic range. These microclimates include relatively cool and damp downed wood environments used by plethodontid salamanders (Kluber et al., 2009) and relatively warm microclimates sought out by some juvenile bufonid toads (Lillywhite et al., 1973).

This is the first time, to the authors' knowledge, that a UV-B microclimate to which amphibians are subjected has been identified. Our data suggests that *P. lessonae*, despite being 'sun worshipping' amphibians that appear to bask in full sun, in fact use a UV-B microclimate with significantly lower levels of radiation than ambient exposure. This is perhaps surprising, given the apparently open and un-shaded aspect of their wild habitat. Forest or crop cover reduce UV-B penetration (e.g. Mazza et al., 1999; reviewed by Paul & Gwynn-Jones, 2003), so the reduction in UV-B radiation in basking sites compared with the ambient exposure in this study are likely to be the result of the sparse long-stemmed grasses growing around the pond creating shade.

In this study, while basking, *P. lessonae* are

exposed to a UVI of between 1.7 and 3.5 (rounded to 1d.p.; the limit of accuracy for the UV Index meter). Through their bi-phasic basking behaviour, pool frogs are exposed to only a relatively narrow portion of the full UVI range of their habitat, being concealed during the most intense periods of UV-B exposure (at the solar zenith, when only one frog was observed basking) and being generally protected by the low-UV-B microclimate around the pond banks. In reptiles, basking behaviour is often driven by thermoregulatory needs (Huey, 1982) and the pool frogs in this study are likely to be doing the same, with UV-B exposure correlated with, but incidental to, thermoregulatory basking behaviour (pers. comm. F. Baines). However, this cannot be tested with this data.

Despite the detection of a low-UVI microclimate used by basking pool frogs, our findings also highlight the fact that at times, these amphibians are exposed to relatively high levels of UV-B radiation. This reinforces the need for further experimentation into the dependence of amphibians on UV-B radiation. It is to be expected that organisms should exploit a free energy resource to their own metabolic ends and, as with reptiles, UV-B radiation may be important for a number of aspects of amphibian wellbeing, perhaps most importantly vitamin D3 synthesis and calcium metabolism.

Although this species of *Pelophylax* is not a conservation or commercial target, several species of this genus living in very similar habitat are threatened, including *P. shquiperica* (Uzzell & Isailovic, 2009), for which *P. lessonae* may act as an analogue. Other species (e.g. *P. bedriagae*) are part of the frog meat and laboratory animal trade, particularly those imported from Turkey (pers. obs. C. Michaels of laboratory animals imported from Turkey to the University of Manchester via the culinary trade). *P. lessonae* is maintained as part of several educational live collections. This data, collected from a readily accessible and common species, may be used to inform the captive husbandry of other water frog species that require ex-situ conservation breeding or are maintained as part of research, educational or commercial enterprises.

However, any application of this data should be made with the caveat that, given the limited

scope in both space and time of sampling, there are likely to be limitations to this dataset. Variation in weather conditions from day to day and solar altitude across the year, as well as variation in the frogs' behaviour across time, will all affect the interaction between the frogs and UV-B radiation. There is also likely to be variation in exposure with the geographic origin of this wide-ranging species. Further investigation to elucidate the nature of this variation, the roles of thermoregulation in determining basking behaviour and the physiological effects of UV-B radiation on this species are all warranted.

The authors suggest that this data is therefore used to provide an indication of the level of exposure within the natural range for this species, which can be used in designing basking microclimates within captive enclosures. If and when further data becomes available, captive conditions can be modified to provide even more natural UV-B exposure.

Our data also draws attention to the issue of detecting microclimates in amphibian habitats when designing captive husbandry protocols, rather than relying on meteorological records. In this case, the maximum ambient recorded UVI would provide excessive (relative to exposure recorded here) levels of UV-B radiation if animals were unable to escape it in the confines of captivity, whereas the assumption that amphibians are not tolerant of UV-B radiation at all could lead to under-exposure. Both over- and under-doses of UV-B radiation can have significant health implications for amphibians (Antwis and Browne, 2009).

CONCLUSIONS

P. lessonae, despite being a basking species of amphibian, appears to use a basking microclimate with significantly lower levels of UV-B exposure than the ambient environment. This, combined with the timing of basking activity, means that this species, as well as in all likelihood other *Pelophylax* frogs, is exposed to relatively lower levels of UV-B radiation in comparison to the maximum available amount of UV-B radiation in its habitat.

These findings may inform the design of captive husbandry protocols for *Pelophylax* species and also demonstrate the importance of collecting microclimate, rather than ambient,

data when sampling amphibian habitats, including exposure to UV-B radiation.

Further investigation into temporal and spatial variation in UV-B irradiance and in basking behaviour, as well as other factors that may influence this interaction, are required and encouraged.

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Research Article

The Husbandry and Captive Reproduction of The Gliding Leaf Frog *Agalychnis spurrelli* (Boulenger 1913)

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INTRODUCTION

The recently reviewed genus *Agalychnis* (Faivovich 2010) comprises fourteen species of charismatic leaf-sitting nocturnal hylids distributed throughout most of Central and northern South America. Usually characterised by brightly coloured flanks and irises, all members of this genus are also arboreal and deposit their eggs above temporary or permanent bodies of water attached to overhanging vegetation or roots. Although found to reproduce more frequently during the rainy season, *Agalychnis* are opportunistic and in some instances spontaneous and explosive breeders (Scott & Starrett, 1974; Roberts, 1994). It appears some may emerge to reproduce given any opportunity; *A. callidryas* has even been observed reproducing after periods of no rain at all, providing there is a suitable body of water to utilise (pers. obs.). However, besides the Red Eyed Tree Frog, *A. callidryas*, this genus is relatively poorly represented within captivity. This paper describes captive reproduction in *A. spurrelli*.

A. spurrelli (Boulenger, 1913) occurs over a large range of Central and northern South America including Costa Rica, Colombia, Panama and Ecuador, from sea level to 880 m (Duellman, 1970). Known for its parachuting, or gliding ability, it has been reported to breed in unusually large aggregations around temporary or permanent bodies of water after periods of heavy rainfall (Scott & Starrett, 1974). In the wild, the tadpoles of this species

have been observed schooling like fish and congregating in areas that receive more light (Gray, 1997). *A. litodryas* is now considered a synonym of *A. spurrelli* after phylogenetic research (Faivovich, 2010); it is now considered a variant of *A. spurrelli* which lacks orange coloured flanks. This species is identified as having a maroon coloured iris, usually orange flanks, a green dorsum and a white ventral surface. Also characteristic are irregular white spots on the dorsum, bordered with black, unique to each individual although some may lack it altogether. Full webbing of the hands and feet is used to perform the gliding behaviour typical of this species (Duellman, 1970). The following husbandry and captive breeding recommendations have been produced following a number of successful reproductions throughout 2012.

METHODS

The adult group of *A. spurrelli* consisted of six males and four females that were housed within a well-ventilated glass vivarium measuring L 90 cm x H 60 cm x W 45 cm. Temperatures ranged between 22°C - 27°C. A humidity of 65% - 75% when being maintained outside of breeding conditions was ideal. A large shallow water dish and a heavy misting in the evening shortly before the lighting was switched off achieved this. A 12/12 photoperiod was given and the frogs were maintained using 6% UVB T5 lighting. Appropriately sized crickets were fed 3-4 times per week, providing up to two or three



Figure 1. Amplexant pair of *A. spurrelli* within the rain chamber.

food items per individual. All food items were dusted with a vitamin and mineral supplement such as Nutrobal or Repashy Calcium Plus and gut loaded using fresh fruit and vegetables.

The vivarium was furnished with live potted plants such as *Monstera deliciosa*, *Spathiphyllum* sp. and *Ficus* sp.. These plants provided resting areas and shelters for the frogs as they sleep during the day (although they often used the glass walls of the vivarium). The long stems of *M. deliciosa* also provided walkways for these highly arboreal amphibians. Additional branches were also included to provide more climbing space.

Although it is possible to maintain these frogs in natural planted vivaria with a soil based substrate, it was decided to leave the base of the vivarium bare. Instead of a substrate, the frogs were provided with an area of moist paper towel placed next to a large shallow water dish. This was replaced daily along with cleaning all surfaces within the vivarium and the removal of any waste. A routine such as this is the key to maintaining and reproducing *A. spurrelli* long term. The enclosure was fully cleaned daily with little or no disturbance to sleeping frogs, which provided the opportunity for close

observation, and any problems were likely to be noticed almost immediately.

Upon deciding that the frogs were in adequate condition to attempt breeding, the group was moved into a large rain chamber measuring L 125 cm x H 76 cm x W 60 cm. Adequate space was required to create distance between calling males. The rain chamber was furnished with climbing branches and *Monstera* sp.. The base of the rain chamber was filled with 10 cm of cool water; this was to aid a brief cooling period of two days which created air temperatures of between 15°C - 20°C. During this period the frogs were not fed and water in the chamber was replaced daily. Activity was decreased during this time.

After the brief cooling period, on the morning of the third day, a water heater was switched on within the chamber, heating the water to 25°C. This coincided with partially covering the ventilation on the roof of the vivarium. As the water warmed and the humidity began to increase, a pump within the rain chamber was switched on circulating the water as artificial rain. This rapidly increased the temperature to approximately 25°C and the humidity to above 80%. This artificial rain remained during daylight hours and was switched off during the night when the frogs became active. Electronic timers may be used to control heaters and pumps but in this instance they were controlled manually.

RESULTS

During the period of observation the frogs maintained a high level of health and over time came into breeding condition. Indicators of this were the possession of nuptial pads in males and females looking particularly large. After being exposed to the above environmental changes, males became incredibly active, vocal and competitive. They were observed competing amongst one another and followed females around the vivarium throughout the night. Amplexus between at least one pair was achieved after the first night (Fig.1), but may have taken up to four days of consecutive rain for amplexus and/ or spawning to be achieved. In some instances pairs spawned after only one day of rain.

The eggs were laid in a single layer and during spawning were deposited in single or



Figure 2. Eggs deposited on a *Monstera* leaf.

paired rows close together. They lacked the large gelatinous mass seen in other *Agalychnis* sp.. Instead, they were encased within a thin but rather rubbery and tough membrane (Fig. 2) Eggs produced by one female in a single spawning numbered as many as 110. On one occasion, egg deposition was observed during the early hours of the morning, beginning at approximately 5 am and continuing to 7.30 am. When pairs finished, they immediately separated. Eggs were deposited on any smooth and stable surface within the rain chamber including the *M. deliciosa* leaves, the glass walls of the chamber and on the sides of plant pots. During the process of spawning the pair, guided by the female, repeatedly visited the water in the base of the chamber to submerge her ventral surface before returning to the egg mass. Interestingly, upon returning, they then continued to deposit eggs adding to the row that they had previously left, giving the impression of a continuous row of eggs.

The eggs developed and hatched into free-swimming tadpoles within 7 – 9 days. Tadpoles were generally unproblematic to raise to metamorphosis. Water temperatures from 23°C - 26°C were provided, an air stone was also provided to gently aerate the water. No filtration was required and regular partial water changes were made. Metamorphosis was achieved at approximately six to nine weeks, with individuals of the same group leaving the water at varying times. Young frogs were housed in small plastic faunariums furnished with a *M. deliciosa* leaf, climbing branches, a substrate of moistened paper towel and a shallow water dish.

Food was provided up to twice a day in amounts that would be eaten within a short

period of time. The tadpoles had particularly large appetites and were fed a mixture of tropical fish flakes, sera micron, spinach leaves, and small amounts of bloodworms were also eaten on occasion. Tadpoles were raised in groups in an attempt to replicate the natural behaviour of the species. The young frogs began feeding 7-10 days after leaving the water and full metamorphosis was completed. They varied in size from 1.5 cm – 2.5 cm and were capable of consuming 1st and 2nd instar crickets from their first feed.

DISCUSSION

A common misconception regarding captive breeding in most tropical amphibians is the requirement for distinct wet and dry seasons. Although this may be true for many species it appears not to be the case with *A. spurrelli*. Creating a 'wet season' in order to stimulate *A. spurrelli* to reproduce in captivity would maintain the frogs within a stressful environment for a prolonged period of time. This may eventually lead to weight loss and bacterial infections due to the constant wet conditions and high level of activity.

A. spurrelli is potentially a large species, with females being larger than males. Although size may vary greatly between individuals from different localities, a large female may measure 92.8 mm snout to vent (Duellman, 1970). This should be taken into consideration to ensure appropriate captive management of the species. It is possible that males from one locality may not be capable of reproducing with females from another locality due to the huge discrepancy in size. Keeping multiple males and mixed sexes together did not seem to be problematic when they were being maintained outside of breeding conditions. Competition or aggression between males was not observed during regular conditions; only rarely would a male amplex with a female outside of the rain chamber (see below) and should this occur the pair would separate within a day or two.

An instance where any *Agalychnis* sp. have spawned when outside of the rain chamber environment has not been noted. A pair joined in amplexus during regular conditions only served as an indicator of willingness to breed (and usually just of an over eager male!), or perhaps that they were being maintained slightly

too wet. Keeping *A. spurrelli* too wet gives the frog's only half of the cue they require to reproduce; which can subsequently result in a pair ready to spawn but unable to find an appropriate area to do so.

Due to the spontaneous nature of reproduction in this species, breeding may be attempted at any time of the year providing that the animals are in adequate condition and that breeding poses no risk to their health. Successful reproduction relied upon creating a clear distinction between the usual environment and a warm, humid and wet rain chamber. A sharp increase in humidity was essential in encouraging reproductive behaviour.

Once spawning was accomplished all frogs were removed and placed back into their regular vivarium. In most instances the eggs remained within the rain chamber for the duration of their development. It was an option to remove leaves containing eggs and suspend them in containers above a small amount of water for the hatching tadpoles to drop into. Although it seemed more beneficial to leave the eggs where they were deposited within the rain chamber where the conditions remained humid, warm and more constant. Egg scraping behaviour performed by competing males as observed by Scott and Starrett (1974) has not yet been observed in the captive environment.

The care guide for the young was generally the same as for the adults. Housing and food size were adjusted according to growth; it was found that young frogs had large appetites and required feeding almost daily to maintain a healthy body condition. Adult size and maturity can be expected to be achieved from 12 – 18 months of age when raised in optimum conditions. The young of this species lack the full webbing of the hands and feet as seen in the adults; this is developed during growth.

The method described here is ideal for breeding species, which do not require distinct seasons, as it takes advantage of the spontaneous nature of their reproduction. The frogs need only to be within a rain chamber for seven days or less. In attempts at breeding several *Agalychnis* sp. the frogs have never been subjected to more than five days of rain before spawning; if no eggs are deposited after this time they are simply placed back into their normal routine and tried again some weeks later. In the long term these methods are beneficial to the health and longevity of *Agalychnis* sp. in captivity.

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Research Article

Surgical wound management and healing time in *Iguana iguana*

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ABSTRACT - Wound healing in reptiles occurs within the same phases noticed in mammals. Our objective was to record the surgical wound healing of an *Iguana iguana*, registering the management steps and the healing time. The animal had a firm mass at the submandibular region, which was surgically removed, and the phases of healing were registered by photographs. After surgery, the individual showed a great improvement in its health. The healing time was in accordance with publications, but not the length of time nor the degrees of the phases. These results could be justified as a stress response.

CASE REPORT

A green iguana (*Iguana iguana*), male, measuring SVL = 39 cm and CL = 90 cm, weighing 2.015 kg, from the woods of the Campus of the Universidade Federal do Ceará (UFC), was brought by students to the Núcleo Regional de Ofiologia of the same university (NUROF-UFC). The animal had a normal body condition, but it was apathetic, with a left head tilt and a serosanguineous discharge in the left eardrum. It was unable to climb any tree and it seemed unbalanced, because its stance was abnormal. In the submandibular region, a firm mass was detected, measuring 29.6 x 31.3 x 24.6 mm, which appeared to be an abscess (Fig. 2A). It was prescribed systemic antibiotics and anti-inflammatory drugs to improve the clinical signs.

MATERIALS AND METHODS

After one week, the mass was surgically

removed, with the surrounding integument. Macroscopically, it was found not to be an abscess but most likely a fibroma, which was sent for histology for subsequent analysis. Postoperatively, it was prescribed systemic antibiotics and anti-inflammatory drugs, topical therapy with penicillin and urea balm once a day, and an occlusive bandage, changed daily, for five days. During recuperation, it was offered fluid, nutritional and thermal care as well. A well-balanced assisted-feeding diet was given (greens, fruits, vegetables; kale, mango, banana, sweet potato, Leucaena's leaves and water) along with sun bathing (8 hours light/day, on average), in which the patient would reach the optimum temperature of 34.5°C. After the five days, the occlusive bandage was removed and the healing process continued with the lesion exposed, being registered by daily photographs. The healing time was analysed.

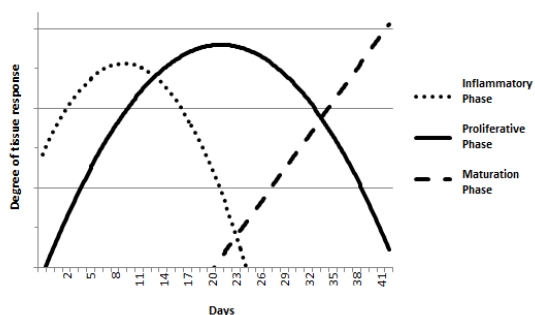


Figure 1. Phases of surgical wound healing in *I. iguana*. Note the overlapping phases.

RESULTS AND DISCUSSION

The surgical wound measured nearly 10 cm² (Fig. 2B). It was not sutured but cauterised, leaving anterior and posterior pterygoids partially exposed. To remove the submandibular mass, surgical techniques described by Mader (2006) were utilised, in which the overlying skin is removed and the wound is cauterised, instead of being sutured. According to these techniques, the preservation of the integument could allow the development of a new abscess, due to the permanence of the fibrous capsule. As the primary approximation of the surgical edges is not possible in this technique, second intention healing was chosen, in which the wound is left open, delaying healing three or four times more than by first intention. In the absence of interference factors, such as underlying diseases or husbandry defects, the normal healing process should be completed in four to six weeks (Mader, 2006).

The use of an occlusive bandage was a temporary option, in order to stabilise the inflammation, remove debris, and protect against desiccation and secondary infection until the onset of granulation. The use of this bandage technique was suggested by Mitchell & Diaz-Figueroa (2004) for a limited period of two to four days. After abandoning the occlusive bandage, the wound was kept open, being washed with saline solution daily.

The healing process in reptiles depends on high environmental temperatures, as well as appropriate nutrition (Smith & Barker, 1988; Cooper, 2006). Maintenance of the patient in the upper end of its optimal range has been shown to promote healing, with cranial to caudal oriented wounds healing faster than transverse wounds (Mader & Bennett, 2006). It

took 42 days for complete fibroplasia.

The wound evolution was classified in minimum, medium and maximum degrees of inflammation, proliferation and maturation, considering the macroscopic features observed in each phase, based on Clark & Denver (1985), represented in Fig. 1. During the inflammatory phase, the wound was erythematous and exudative, with vestigial blood clots (Fig. 2C). The inflammation reached the maximum degree around the 14th day after surgery, showing the maximum edema, exudation and erythema (Fig. 2D). The maximum degree of inflammation contradicts the publications (Maderson & Roth, 1972; Smith & Barker, 1988), which have shown the inflammatory response in lizards is minimal.

The length of the inflammatory phase, which was expected to be approximately three days (Contran et al., 1996; Tazima et al., 2008), was observed to be longer in this individual. The inflammatory findings on the 14th day after the injury are in contrast with French et al. (2006), who found the total healing length was no longer than 17 days, and this could have been justified by a stress response (excessive manipulation, restraint, confinement). The inflammation phase is the most stress-sensitive, and when the animals are submitted to environmental and husbandry changes it increases the blood corticosteroid concentration, delaying the biochemical events of healing (French et al., 2006).

At the proliferative phase, the development of the granulation tissue, pale and finely granular, was visualised, along with the gradual enlargement of the desiccated fibrin clot in the wound centre, while the fibroplasia started to develop on the periphery, centripetally (Fig. 2E,F), which is consistent with other works (Tazima et al., 2008; Isaac et al., 2010). The maturation phase is characterised by wound contraction and scar remodelling.

The initial wound area, which measured approximately 10 cm², had been reduced to nearly 4.5 cm² at the 38th day of observation (Fig. 2G, H, I). The peripheral tension should reduce after eight weeks, enlarging the scar area, since the inclination of the maturation curve is more acute in the first six to eight weeks after the injury (Tazima et al., 2008). In this case report, reepithelialisation would not be



Figure. 2. Photographic registration of surgical wound healing in *I. iguana*. (A) Submandibular mass, possibly abscess. (B) Day 0 = surgery moment. (C) Day 1 = start of inflammatory phase, exudation and blood clots. (D) Day 14 = maximum degree of inflammatory response. (E) Day 19 = granulation tissue and peripheric fibroplasia. (F) Day 24 = desiccated fibrin clot. (G) Day 32 = maximum degree of wound contraction; decrease of granulation tissue, start of maturation. (H) Day 36 = wound contraction, maturation. (I) Day 42 = Total wound fill with matrix of collagen, maturation.

noticeable, most likely due to wound depth and the destruction of the epidermal stratum germinativum, stopping the production of new keratinocytes.

With regard to the general condition of the individual after the surgery, there was a great improvement in its health status. After the first two weeks, the green iguana had recovered its normal balance, gait, and state of consciousness. It was alert and sensitive to external stimuli and started to move and climb regularly. The second intentional healing in *I. iguana*, with the correct fluid, nutritional and thermal care, developed within the period described in publications. Although the healing process and its phases occur in biochemical and physiological similarity to mammals, the dependence on environmental temperature is one of the reasons for the differences in the process.

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Research Article

Effect of bait in funnel-trapping for great crested and smooth newts *Triturus cristatus* and *Lissotriton vulgaris*

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ABSTRACT – Aquatic funnel traps (collapsible, mesh construction) set for newts were baited with beef and compared with non-baited traps. Baited traps captured great crested newts *Triturus cristatus* more than twice as frequently as traps with no bait, yielding more than three times as many newts. Females were more frequently attracted to baited traps than were male great crested newts. The addition of bait to traps did not significantly affect their efficiency in trapping smooth newts *Lissotriton vulgaris*. Baiting traps is worthy of further consideration in great crested newt survey and/or surveillance work. Nevertheless, the trapping methodology described here is suitable only for specific conditions and is best regarded as an option within a range of survey techniques.

INTRODUCTION

Aquatic funnel-trapping has long been used in the study of newt ecology (e.g. Bell, 1977; Dolmen, 1983a; Griffiths, 1987; Griffiths & Mylotte, 1987; Baker, 1999). In the UK bottle traps have been the most commonly used design and are recommended for survey work (Griffiths et al., 1996; English Nature, 2001; Sewell et al., 2013). Nevertheless, other types of funnel trap have also been used in newt research and monitoring work, including specially constructed box traps (e.g. Dolmen, 1983a; Baker, 1999), and more recently a ‘Dewsbury’ trap has been designed (Dewsbury, 2011). Mesh fish traps have been recommended and used in North America (e.g. Olson et al., 1997) and specifically for newt surveys in continental Europe (Bock et al., 2009; Kröpfli et al., 2010). Illuminating traps has improved trapping efficiency for some amphibians (e.g. Grayson & Roe, 2007) but has had mixed results for great crested newts; glow sticks were ineffective in one study (Kröpfli et al., 2010) but light emitting diodes improved trap efficiency in another (Beckmann & Göcking, 2012). In most cases, however, traps set for amphibians, including newts, rely on the

animals’ tendency to enter spontaneously – that is without the use of bait.

The current study tested the effectiveness of traps baited with small pieces of beef in capturing great crested and smooth newts.

MATERIALS AND METHODS

The traps used (BO traps supplied by Interex International Ltd.) are constructed of plastic mesh supported by a frame which is collapsible for storage. They measure 46 cm long x 25 cm x 25 cm and have funnel entrances at both ends. The trapping trials were carried out in natural ponds (pingos) in Norfolk, eastern England. Ten traps were set during each trial. Five traps in each trial were baited with small cubes of stewing steak (approximately 3 g) held in a zippered pocket set in the roof of the traps. Traps were set along the pond edges in pairs, each comprising a baited and non-baited trap, with at least two metres between traps.

Twenty-five trapping trials (125 pairs of traps) were carried out over six years, in seven ponds known to support both great crested and smooth newts. The traps were set during the daytime on dates ranging from 11 March to 20 July. Water temperatures, taken at the pond

	Great crested newt		Smooth newt	
	Bait	No Bait	Bait	No Bait
Traps capturing newts	49	21	17	13
Traps not capturing newts	76	104	108	112

Table 1. Numbers of traps with and without bait capturing/not capturing newts.

surface, ranged from 9 to 27°C. Each trap was set approximately 1.5-2.0 m from the shoreline, and attached to the pond bank by rope tied to a stake. Traps were thrown into open water, allowing them to sink to the pond bottom (depths of approximately 0.3-1.0 m). Each trial lasted for three to four hours after which the traps were removed from the water and inspected for newts.

RESULTS

Baited traps captured great crested newts more frequently than did traps with no bait ($\chi^2 = 15.56$, 1 d.f., $p < 0.01$); there was no such effect for smooth newts ($\chi^2 = 0.61$, 1 d.f., $p = 0.44$) (Table 1).

Baiting traps captured more than three times as many great crested newts (all stages) as using traps with no bait (Fig. 1). This effect of baiting was most marked among females – more than seven times as many were captured in baited traps.

Great crested newt larvae were captured only in low numbers making it difficult to

determine whether they were attracted to baited traps. No smooth newt larvae were captured.

DISCUSSION

For great crested newts baiting mesh funnel traps with small pieces of beef increased trapping efficiency, more than doubling the number of traps capturing newts and more than trebling the number of newts captured. Hence the use of bait in funnel traps is worthy of further consideration.

Field trials of mesh funnel traps, similar to those used in the current study, indicate that they are more effective than bottle traps (Madden & Jehle, in press). An additional advantage of these traps is that they can be set on the pond bottom in deeper water than is practical for bottle traps. Bottle traps are confined to relatively shallow water, often close to the shoreline of larger ponds. Great crested newts (although not their larvae) tend to live on the pond bottom and for much of the aquatic season spend the daytime in deeper parts of the pond (Steward, 1969; Dolmen, 1983b; Griffiths

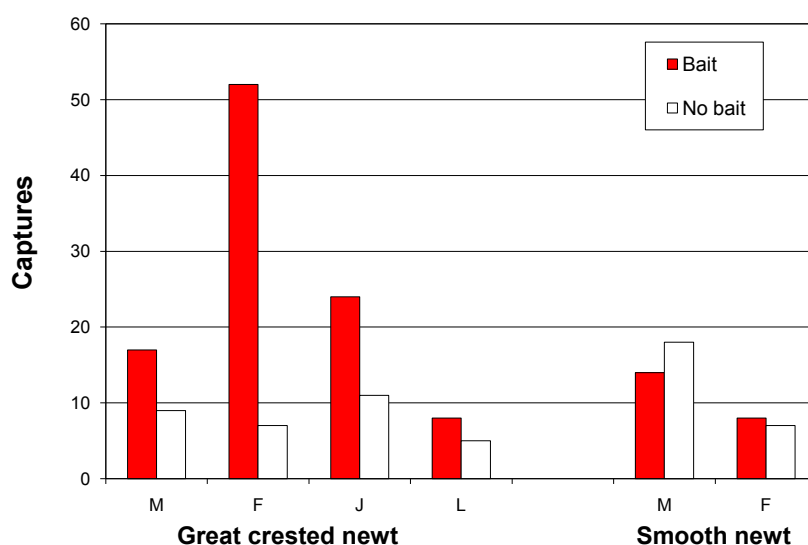


Figure 1. Numbers of great crested and smooth newts captured in 125 pairs of traps with and without bait. M = male, F = female, J = juvenile, L = larva.

& Mylotte, 1987) so trapping here is likely to be productive (e.g. Kröpfli et al., 2010).

Use of traps in the current study differed from convention. Great crested newts are primarily nocturnal (Dolmen, 1983a; Dolmen, 1983b) and consequently funnel traps are more effective at night (Bock et al., 2009) whereas in the current study trapping was carried out during the daytime. This was because fully immersed funnel traps risk killing newts which are prevented from reaching the water surface. Hence traps were deployed for only three to four hours rather than leaving for a longer period typical of overnight trapping. The short trapping period also permitted the use of traps at water temperatures higher than regarded as safe for prolonged submersion. It should be noted that the ponds in the current trial were relatively deep and supported plentiful submerged aquatic vegetation. Trapping in other conditions should be undertaken with caution to determine whether the water is sufficiently oxygenated to hold newts in submerged traps without asphyxiation.

Additionally, some of the trapping trials were carried out relatively late in the year. This did not seem to reduce trapping efficiency. Trapping rates (proportion of traps capturing newts) were 0.57 for the 35 baited traps which were set in June and July, compared with 0.32 for baited traps set from March to May. The success of late season trapping may be in part a geographic effect (seasonal amphibian activity being later in eastern England than in southern or western areas) but it would be worthwhile investigating whether traps that capture from the pond bottom in deep water (e.g. fish traps and Dewsbury traps) extend the period over which great crested newts can be trapped beyond the newts' breeding season.

Baited mesh funnel traps used in the current study would certainly not be suitable for all situations. It would be difficult or impossible to use these traps in ponds packed with dense beds of aquatic vegetation, for example. Taking a wider perspective, the future use of traps in amphibian survey work will likely be dependent on developments in the field of environmental DNA, which has been used to detect great crested newts and may even be able to quantify population density (Thomsen et al., 2012). Less reliance on traps should be welcomed given the

risks to wildlife entailed (Denton, 2002; Klemish et al., 2013). The effectiveness of any particular trap type is likely to vary according to survey conditions. Baited mesh traps should be regarded as an option within a range of techniques considered by the surveyor, who should choose according to the pond conditions in hand, survey objectives and financial constraints.

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Short Note

Climbing behaviour of terrestrial bufonids in the genus *Rhinella*

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INTRODUCTION

One of the most effective ways to prevent predation is to avoid direct contact with the predator, because it does not require energy expenditure for agonistic encounters and/or extra production of defensive substances, prevents injuries and, ultimately, mortality (Madison et al., 2009). In anurans, the behaviour of climbing and resting on the vegetation at night may be to avoid predators (Granda-Rodriguez et al., 2008). Studies on anti-predatory behaviour in anurans are mostly focused on the use of colour and morphological characteristics and, data on specific behaviour are rare and scattered in the literature (Toledo et al., 2011). Furthermore, many behaviours still lack specific names, hindering their recognition and description (Toledo, 2007). This paper reports on climbing behaviour observed in terrestrial bufonids of the genus *Rhinella*.

METHODS

Observations were conducted at São Nicolau Farm (09°49'09.9" S, 58°15'31.1" W) and Cristalino State Park (9° 32' 47" S, 55° 47' 38" W), located in Cotriguaçu and Novo Mundo, respectively, both in the state of Mato Grosso, Brazil. Individuals were visualized and their behaviour described during eight field trips conducted from December 2010 to November

2012. Five observations of arboreal behaviour were made, all at night between 19:00 and 23:00 hours. The toads were photographed and their height above the ground recorded. To rule out that individuals were in search of food, we looked for possible sources of food in the micro-habitat where individuals were found.

RESULTS AND DISCUSSION

Four individuals of the species *Rhinella margaritifera* and one individual of *R. castaneotica* were recorded above ground level, on vegetation (Fig. 1). The first individual was spotted on a low tree, near a water course, 130 cm above the ground (Fig. 1A). The second was seen 32 cm above the ground on a root (Fig. 1B). The third was observed on a palm (*Attalea* sp.) 75 cm above the ground (Fig. 1C) and the fourth was seen on a clump of *Olyra latifolia*, 45 cm above the ground (Fig. 1D). The individual of *R. castaneotica* rested on a palm (*Attalea* sp.), 102 cm above ground level (Fig. 1E). No food sources, such as termites or ants, were detected near the individuals sighted above ground level but this does not exclude opportunistic predation on passing flying insects.

The traditionally arboreal anuran species (e.g. hylids) have specialized structures on their fingertips (adhesive discs), which provide



Figure 1. Individuals of the bufonids *R. margaritifera* (A, B, C, D) and *R. castaneotica* (E) observed on vegetation above ground level, state of Mato Grosso, Brazil.

adhesion climbing (Hanna & Barnes, 1991). But these *Rhinella* species are terrestrial and have no obvious morphological adaptations that favour climbing (Gosá, 2008). According to Lindquist et al. (2007) and Granda-Rodriguez et al. (2008) terrestrial bufonids may select arboreal resting sites to avoid predators. In addition, the tactile perception of an approaching predator, generated by the movement of the vegetation, would be an additional benefit to resting on saplings and shrubs (Liquisind et al., 2007) and palm trees (this study).

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Natural History Notes

LAMPROPHOLIS DELICATA (Common Garden Skink): **COPROPHAGY.** *Lampropholis delicata* is a small terrestrial lizard, common and widespread on the coast and mountain ranges of southeastern Australia (Cogger, 2000). It is common in anthropogenic urban habitats, and with well-developed arboreal capabilities is sometimes observed on vertical surfaces such as brick and concrete block walls, wooden fences, the lower trunks of rough barked trees, and shade cloth netting (pers. obs.). Its reported diet comprises terrestrial, arboreal and flying arthropods (e.g. Crome, 1981). This note reports an instance of coprophagy by *L. delicata*, hitherto unreported, in an anthropogenic urban habitat.

On 6 January 2013, 15:36 h (Australian Eastern Standard Time), in Werrington (a western suburb of metropolitan Sydney), New South Wales, Australia, at 33°45'35.0"S, 150°45'25.5"E (WGS84 grid), 28 m elevation, ambient temperature 33.1°C, 0/8 cloud cover, medium gusts of easterly breeze, the author observed an adult (total length ~80 mm) *L. delicata* with a complete original tail resting in the shade on the base of an old lamp in the covered area next to the author's dwelling. The lizard held a dried scat (length ~11 mm, maximum diameter ~2.5 mm) of a subadult treefrog *Litoria dentata* transversely in its jaws; the scat was comprised of compacted arthropod exoskeletal elements and recognisable from its characteristic shape and size. *L. dentata* is also common on the property. Approached closer, the lizard leapt off the lamp base on to

the concrete, and moved briskly away from the observer until lost from view; hence ingestion of the scat was not observed. Neither the *L. delicata* nor the scat were collected or photographed. It is likely that the lizard discovered the scat whilst foraging on old furniture and other objects, and subsequently descended to where it was encountered by the author. Motion is the primary cue eliciting predatory feeding responses in most lizard species including *L. delicata* (pers. obs.), although in the above case olfaction was presumably involved, perhaps aided by visual recognition of the item as potential food which would imply memory based on local experience. Notable was the retention of the scat by the lizard despite pursuit by the observer. In observations of large numbers of active *L. delicata* at many localities over an extensive period, this is the only case of coprophagy noted by the author.

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PHILODRYAS PSAMMOPHIDEA (Günther's green racer) **DIET.** Snakes of the genus *Philodryas* are medium to large bodied species distributed across the countries of Argentina, Bolivia, Brazil, and Paraguay (Thomas, 1976). They are considered to be generalist predators, preying upon a wide variety of taxa including birds, mammals, anurans, lizards, and snakes (Funk et al., 2003; Lopez, 2003; Franca & Araujo, 2007; Lopez & Giarudo, 2008; Quinteros-Muñoz et al., 2010; Schalk, 2010). Herein we report a new prey item for *Philodryas psammophidea* from the inter-Andean dry valleys of Bolivia.

On 29 March 2012, a dead adult *P. Psammophidea*, that had been killed by a villager, was found (SVL = 425 mm; TL = 145 mm; sex unknown) in the town of Becerro, Florida Province, Santa Cruz Department, Bolivia (GPS 18° 1'20.12"S, 64°11'19.75" W; datum WGS 84). Upon dissection in the field, the remains of an adult *Pleurodema cinereum* (Anura: Leptodactylidae, sex unknown) were found.

Amphibians and reptiles have been reported as constituting the largest part of the diet in other species of *Philodryas*. However, previous studies have only documented lizards (Franca & Araujo, 2007) and a rodent (Quinteros-Muñoz et al., 2010) in the diet of *P. psammophidea*. Quinteros-Muñoz et al. (2010) suggested that mammals may constitute a larger part of the diet of *P. psammophidea* in the inter-Andean valleys of Cochabamba because they are a more abundant as prey species than amphibians or reptiles. Whether or not prey exhibit a similar abundance pattern in this region is unknown, but our report provides support for this species possessing attributes of a generalist predator, similar to other members of its genus.

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EUROLOPHOSAURUS NANUZAE

(calango): **COURTSHIP AND COPULATION.**

Courtship and copulation are important characteristics of animal behaviour since both displays and morphology are involved in these processes and might be used as cues for reproducing individuals to select their mates. Understanding behaviour during sexual interactions should illuminate the understanding of morphology and/or behaviour from an evolutionary perspective. The lizard *Eurolophosaurus nanuzae* is a saxicolous small bodied species with an adult average body size of 50 mm (Galdino et al., 2003). The species is endemic to the rocky fields of the Espinhaço Mountain Range in Brazil (Rodrigues, 1981), and is assessed as “Near Threatened” (IUCN, 2012). Despite aspects of its ecology being relatively well known there are few studies regarding its behaviour.

Behaviours were observed by ad libitum sampling (Altmann, 1974). Courting lizards were observed between late August and early September 2010. We observed a total of six *E. nanuzae* pairs. Courting males were first seen performing push-up displays in the presence of females. Males then approached females and climbed upon the females’ dorsum while biting and holding the female’s neck (Fig. 1), this interaction lasts c.a. 3 min. During this interaction females carried the biting male, attached to her dorsum, a short distance (c.a. 10 cm). Afterwards, the males passed one of their hind-limbs up towards the female’s tail base while hugging the female’s trunk while still biting her neck (Fig. 1). Females were seen carrying the attached males for short distances in this position. Receptive females were passive and lifted their tails enabling the courting male to introduce his hemipenis in female’s cloaca. In two of the observations males seemed to ‘force’ copulation as females seemed not to be receptive and resistant to males. Females were seen attempting to resist biting males that eventually forced the hemipenis in the females’ cloaca. Two females were able to expulse the males.

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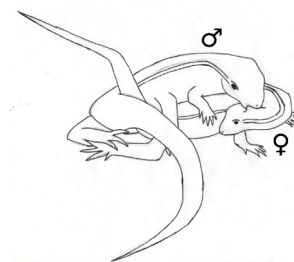


Figure 1. Illustration of copulation in *Eurolophosaurus nanuzae*.

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NEW RECORD OF *PELOBATES VARALDII* IN THE REGION OF BEN SLIMANE (NORTHERN MOROCCO) The family Pelobatidae comprises four species distributed in the western Palearctic region (Amphibiaweb, 2012). Only one species, *Pelobates varaldii*, is present in North Africa, where it is endemic to the coastal plains of northern Morocco (Salvador, 1996). Its distribution is limited to the region between Tangiers to the north, Oualidia to the south, and Ouezzane to the east (de Pous et al., 2012) (Fig.1). This species is threatened by habitat destruction and the recent introduction of exotic fishes (for example, *Gambusia* and *Lepomis* species; García et al., 2010) and for this reason *P. varaldii* is classified as endangered in the IUCN Red List categories (Salvador et al., 2012). Recently, the distribution and the regional niche occupied by this species have been revised (de Pous et al., 2012). These authors were unable to confirm the presence of this species in the region of Ben Slimane, and considered this region unsuitable for *P. varaldii*, specifically due to the absence of sandy soils and the prevalence of “thick bushy undergrowth” (de Pous et al., 2012). In March 2013 we discovered a new population of *P. varaldii* in the Ben Slimane region, at coordinates 33.69 ° N, 7.15° W (Fig.1). A tadpole (Fig. 2) was found in a temporary pond, along with two other

species of amphibians: *Hyla meridionalis* and *Pleurodeles waltl*. The distinctive traits of the *P. varaldii* tadpole were identified following Schleich et al. (1996). This pond had a surface area of 2,530 m² and an average depth of 26 cm. The surroundings were agricultural fields with some remnants of natural vegetation (mainly shrubs of dwarf fan palm *Chamareops humilis*) (Fig. 3), on a sandstone/schistous substrate (Grillas et al., 2004).

Our finding confirms that *P. varaldii* occurs in absence of sandy soils, contrary to assertion of de Pous et al. (2012). In this sense, the sister species *P. cultripipes* is also not limited to sandy soils (García-París et al., 2004), although it favours this type of substrate (Tejedo & Reques, 2002). *P. varaldii* is likely to be scarce in the region, given that it was only found at a single pond out of 21 ponds surveyed, in the Ben Slimane area. The existence of previous records both south and north of the discovered locality (Fig.1), suggested that this population would be part of a continuous range that would extend until recently through northern Morocco’s Atlantic coastal plain. It is unlikely that it is result of an introduction, since this species has no value for the local people. The discovery of this new population indicates that small isolated populations can survive in the area surrounding Ben Slimane, (although they might be difficult

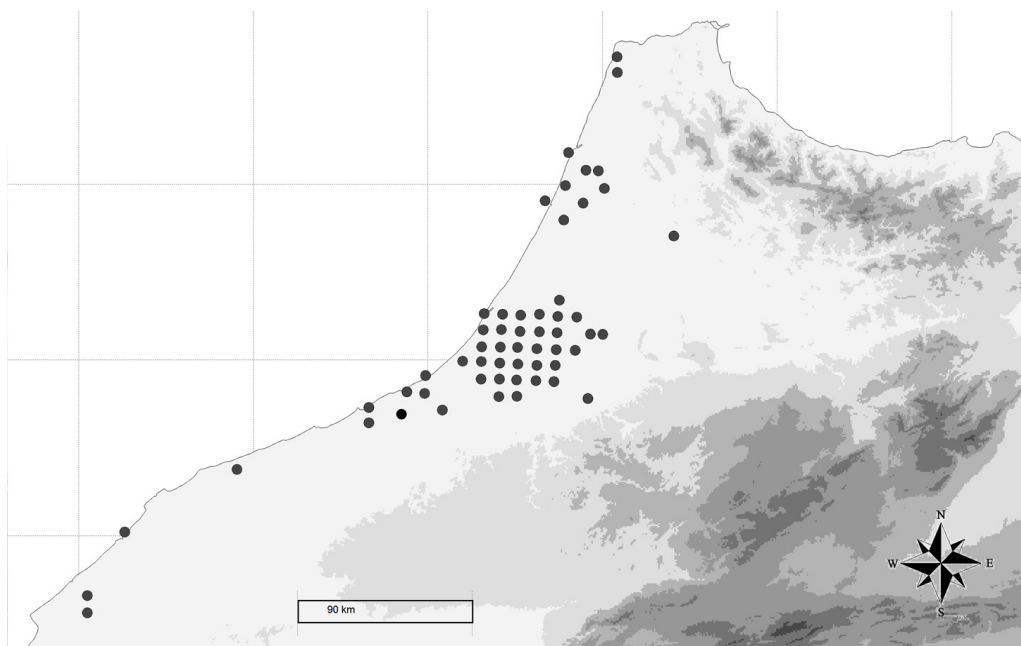


Figure 1. Distribution of *P. varaldii* in Morocco according to de Pous et al. (2012): grey circles. The new location described in this article is shown in a black circle.

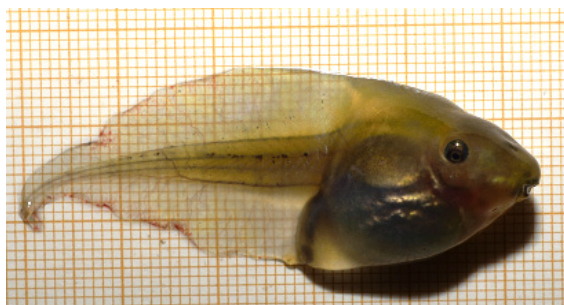


Figure 2. Photograph of the tadpole of *P. varaldii* captured in the Ben Slimane area, March 2013.



Figure 3. Temporary pond on sandstone/schistous substrate. Breeding habitat of *P. varaldii*, *H. meridionalis* and *P. waltl*.

to detect), and that they are possibly very vulnerable to extinction.

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PHRYNOPS TUBEROSUS (NCN): DEFENSIVE BEHAVIOUR. Animals use various strategies to remain less visible and to avoid predation, such as camouflage, aposematism, thanatosis, among others (e.g. Stevens & Merilaita, 2009). The high mortality of juveniles and hatchlings is an important part of turtles' life history, since the young are preyed on by ants (Parris et al., 2002), fishes (Gyuris, 1994) and birds (Janzen et al., 2000). Behaviour that enables predator evasion is therefore key for survival; this note reports an example of a cryptic behaviour in a juvenile *Phrynops tuberosus*.

The observation was made in May 2012, during a field expedition to Cedro's dam, located in the municipality of Quixadá, Ceará, Brazil. A juvenile *P. tuberosus* (carapace length = 6.44 cm) was captured under a bank of floating macrophytes, marked by removal of a small triangular section of the marginal scute to enable identification (Cagle, 1939) and photographed. When returned to where it was captured, the turtle made alternating movements of its legs, which threw sand and gravel over its carapace. When we attempted recapture, the movements were increased and although more debris covered the turtle it was never completely buried. The sand and sludge accumulated mainly in the neck and at the intersection of epidermal shields of the carapace (Fig. 1).

Jennings (2007) noted that *Terrapene carolina* individuals used a similar strategy, in which they hid between leaves and buried themselves. This cryptic behaviour may be a strategy for escaping from predators, for example, as birds and carnivorous fishes.

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Figure 1. Juvenile of *P. tuberosus* partly buried. It is possible to observe the less evident orange spots mainly on the animal's left side and the accumulated sludge and sand over the margins of the carapace and the neck. Note the right forelimb throwing substrate over the shell.

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ITAPOTIHYLA LANGSDORFFII (ocellated tree frog): **ANURAN PREDATION ATTEMPT.** Amphibians are essentially predators, being mostly generalists and opportunists. The items of their diet are determined by available prey and its size, which are limited by the predator's mouth and body size (Caramaschi, 1981). Anurans tend to target elongated prey such as crickets and insect larvae (Schad, 2007). Hylids attempting to predate other frogs are less commonly observed (Toledo et al., 2007). This note describes one such event.

The ocellated treefrog *Itapotihyla langsdorffii* Duméril & Bibron, 1841 is a widely distributed hylid habiting the Atlantic Rainforest from the state of Sergipe to the state of Rio Grande do Sul. The species also occurs in eastern Paraguay and north-western Argentina (Frost, 2011) and is nocturnally active in arboreal microhabitats (Haddad et al., 2008). *Scinax x-signatus* Spix, 1824 is widely distributed through South America in Colombia, Guyana, Suriname and Venezuela. In Brazil it occurs in the north-east, south-east and southern regions where it inhabits tropical savannas, forest edges and open areas, and riparian zones (Rodrigues et al., 2004).

On May 4th, 2011, a distress call of a *S. cf. x-signatus* drew our attention to a *I. langsdorffii* attempting to predate a *S. cf. x-signatus* in the Reserva Sapiranga (12°56'813" S, 38°01'398" W). Both frogs were on a shrub located above a pond. The *I. langsdorffii* had the legs of the *S. cf. x-signatus* in its mouth and was holding it with both front feet. The *S. cf. x-signatus* continued performing the distress call and was struggling to break free as the predator tried to swallow it by the legs. Eventually, the *S. cf. x-signatus* managed to free itself and escaped (Fig. 1). This is the first record of attempted predation on *S. x-signatus* by this species.

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Figure 1. Sequence of events during predation attempt. (A) The moment of sight of the event, (B) *I. langsdorffii* trying to swallow its prey, (C) *S. cf. x-signatus* attempting to escape. Photograph by M.L.T. de Oliveira.

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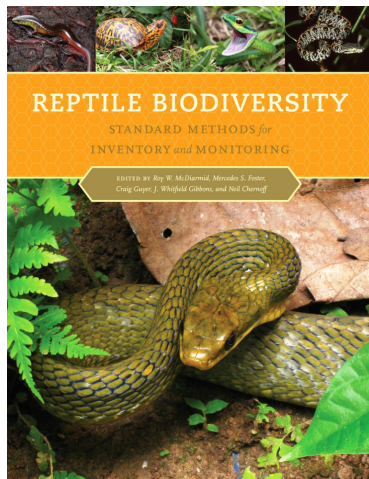
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Book Reviews

Reptile Biodiversity. Standard Methods for Inventory and Monitoring

McDiarmid, R.W., Foster, M.S., Guyer, C., Gibbons, J.W., Chernoff, N. (eds.)

2012. University of California Press, Berkeley. ISBN 978-0-520-26671-1 (cloth). Xii +412



Probably once every ten years I receive a book that is a true milestone in the field. This is one such book. Initiated at a workshop in 2002, the volume has had a long and difficult gestation, but its birth has been well worth the wait. In his foreword, Rick Shine sums it up: ‘This is the book that I desperately needed at the beginning of my scientific career’. I’m sure that many experienced herpetologists will echo that sentiment.

With five editors, 70 authors from six countries, four parts and 17 chapters, this is a big book in every sense. One reason this book review is appearing rather late is because of the time it took for the reviewer to read it! After two scene-setting natural history chapters in part one, ‘Planning a Diversity Study’ is comprehensively covered in part two. This section comprises eight multi-authored chapters discussing study design and analysis, capture, handling, marking, sexing, ageing and voucher specimens. Also included here is a very useful section on practical aspects of data collection and management, including dataloggers, databases and hand-held computers.

In part three the volume moves on to consider sampling reptile diversity. This section contains a comprehensive chapter that describes some

familiar (e.g. pitfall and drift fences, funnel traps, noosing) and not so familiar (e.g. helium balloons, projectiles, laser pointers) methods for sampling reptiles. This is followed by a brief, but thoughtful chapter that discusses statistical issues such as bias, precision, efficiency and validation, and the factors that influence reptile detectability. At 67 pages the next chapter on standard techniques for inventory and monitoring is almost a book within a book. Once again, the approach here very neatly integrates practical aspects such as trap construction and data sheet format with more fundamental material relating to survey design and analysis. Regional issues are neatly dealt with in separate boxes, and the literature on artificial cover objects is concisely summarised in a very useful table spanning two and a half pages. A concise chapter follows on methods for analysing diversity, and then an illuminating review of methods for estimating population size and demography. The latter chapter embraces occupancy modelling, capture mark recapture methods and density estimation, with sound advice on the applicability of software such as PRESENCE, CAPTURE and MARK to reptile populations. This leads into a discussion of life table analysis and the pros and cons of population viability analysis. Part three concludes with a self-contained chapter dealing with issues associated with exploited species. Part four consists of a concluding ‘Where do we go from here’ chapter plus appendices listing collections of reptiles and useful websites.

The whole volume is well-illustrated throughout, with clear diagrams and photographs depicting practical aspects. The conceptual material that deals with design and analysis is liberally complemented by case studies, and self-contained topic boxes deal with related issues. The nature of the book means that some topics crop up more than once in different

chapters and sections, but careful editing has ensured that this results in complementarity rather than redundancy. The book is ambitious in the breadth of taxa that it covers, as clearly the tools needed for sampling aquatic turtles are quite different from, for example, those for burrowing lizards. Nevertheless, the well signposted chapters mean that the reader seeking subject or taxon-specific information is able to find the relevant sections efficiently.

All too often general books on assessing diversity and abundance skim over issues relating to reptiles. This can be frustrating, as dealing with animals that are cryptic, difficult to detect and that live in challenging habitats can present difficulties when trying to apply

standard protocols. This book doesn't resolve all these issues, but its pages are full of useful advice, examples and pointers for fieldworkers who are wrestling with them. In this respect, I found the book a very reassuring read, and one that I will be recommending as essential for any budding researcher about to embark on a project that involves field assessment of abundance and diversity of reptiles.

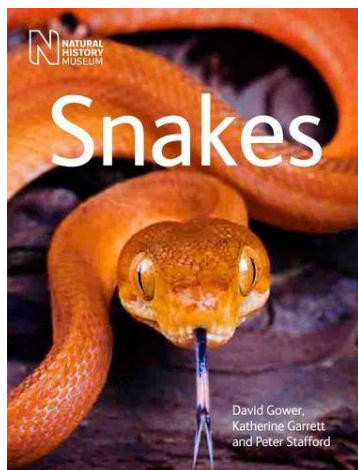
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Snakes

David Gower, Katherine Garret & Peter Stafford 2012.

Natural History Museum, London, 144 pp.



On acquiring 'Snakes' I was pleasantly surprised by the expertly taken photo of the vivid orange South American tree boa (*Corallus hortulanus*) on the front cover. This stunning picture combined with a read through the preface and authors section, highlights how professionally this book is written.

Snakes is divided into three parts, where only part one and three are divided into appropriate subsections and only these will be explained in any detail. Part two 'The evolutionary tree and classification of snakes' is only four pages long; however it is not an insignificant part of the book - it holds useful information on the phylogenetics of the various snake families. The book is peppered with grey 'boxed' texts, which contain snippets of information on a variety of subjects.

Part one 'Structure and lifestyle' is divided into eight sub-sections, which are further subdivided; however, due to the amount of information, I will pluck out the more interesting ones for this review.

Although brief 'The origins and fossil record of snakes' will give the reader an introduction into the main environmental hypothesis that promoted the modern day snake anatomy, which is coupled with information on prehistoric snakes.

The generalised structure of the modern day snake, is explained in 'the anatomy' section; page 11 holds a detailed annotated drawing of a male snake, showing the typical organ layout which is a consequence of the evolutionary elongation of the body. This diagram couples extremely well with the detailed information provided. The more striking alteration produced by the elongation of the body, is the reduction or complete loss of the left lung and the possible addition of the tracheal lung as compensation. Information and diagrams on pages 12 to 14 provide a beginners guide to skull structure and dentition. Particularly informative is the illustration on types of dentition, with sketches showing the variety of biting mechanisms and teeth arrangement in venomous and non-venomous snakes.

The 'boxed text' on page 16 highlight the horrifying numbers of snakes that are used by humans, particularly for consumption/medicinal use. For instance in China alone 7 to 9 million kilograms of snakes are harvested annually. Other major threats mentioned include the pet trade and fashion industry.

The 'feeding and diet' section encapsulates the varying feeding mechanisms of snakes; however, the gem of information is the different diets, which portrays the more obscure dietary habits. For example, adult striped swamp snakes (*Regina alleni*) feed exclusively on crayfish, whereas juveniles feed on dragonfly nymphs and shrimp.

In 'reproduction' the generalised events that occur during courtship and the strategies that increase the chances of paternity are described. For instance, in many promiscuous species, males will produce a 'copulatory plug' to prevent insemination by rival males. It is finalised with 'eggs and hatching', which introduces the reader to the importance of temperature regulation and the strategies undertaken by various oviparous and viviparous

species to produce a fine balance for successful embryo development.

Part three is clearly the largest and covers snake diversity. As one would assume the families Boidae and Pythonidae, Viperidae, Elapidae, and Colubridae dominate. Due to the amount of information, I will only mention snippets from these families, leaving the rest to be discovered by the reader. Particularly interesting is the 'behavioural secrets of the water python' that describes the exceptional adaptability of the Australian water python (*Liasis fuscus*), which is able to mirror its dietary requirements to the season and prey abundance, such as rats during the dry season and water birds and eggs in the wet season. This strategy maximises its survival in the harsh and variable billabong environment.

The 'True vipers' section, projects the family's global importance, and is nicely represented by *Vipera berus* (adder). The adder is the most successful terrestrial living species of snake, inhabiting a geographical range from the British Isles, throughout Eurasia, and east to the Pacific Ocean. The adder has a considerable colour dimorphism, illustrated by the three brilliant pictures on page 82.

The authors delve into great detail and obscurities in the section on the marine elapids, focusing mostly on the physiological modifications of these unusual snakes. Examples of sea snake evolutionary changes are highlighted in the olive sea snake (*Aipysurus laevis*) that has light sensitive cells (photoreceptors) within their tail, to ensure that they are not exposed during the day. Furthermore,

an attribute common to all sea snake species is that they shed more frequently (2-6 week intervals), to possibly keep their skin barnacle and parasite free. The section on egg eating snakes describes the similarities and differences in egg eating strategies between the African egg eating snakes (*Dasypeltis*), and other egg eating species from around the world.

Some of the smaller, more obscure groups and families are not overlooked, for instance the group Scolecophidia (thread, worm and blind snakes), with their lack of or reduced dentition and their unique skull structure. The families in this major order may look insignificant; however, they have representatives of the smallest snake (*Tetracheilostoma carlae*), which grows to a maximum of 10 cm, and one of the very few parthenogenetic species (*Ramphotyphlops braminus*). The section on Texas thread snakes (*Rena dulcis*) describes its role as a parasite regulator in the nest of screech owls (page 45).

Overall *Snakes* is a brilliant read from start to finish and will captivate anyone who has a latent interest in snakes. David Gower, Katherine Garret & Peter Stafford have put together a magnificent piece of work that holds a plethora of balanced information, coupled with high quality photographs and annotated drawings.

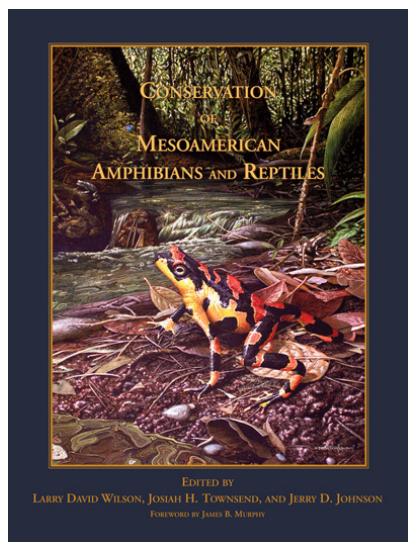
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Conservation of Mesoamerican Amphibians and Reptiles

Larry David Wilson, Josiah H. Townsend and Jerry D. Johnson

2010, Eagle Mountain Publishing, Utah, 812 p.



Mesoamerica (or Middle America) is one of the world's major herpetofauna hotspots with over 1,900 species of which about 1,000 are endemic. It is a hugely complicated region with megadiverse habitats and complex conservation conundrums. *Conservation of Mesoamerican Amphibians and Reptiles* is the result of dedicated collaboration and collates an impressive 21 contributions from 43 authors. It is razor sharp, concise, hugely informative and has exquisite attention to editorial detail.

The book begins by introducing the region, its history of global amphibian assessment, recent taxonomic changes, diversity and endemism. For any student of Neotropical herpetology these three chapters alone provide an essential backdrop to the region's herpetofaunal history, wading through masses of important literature.

The book is then divided into logical chapters for each country in Middle America. Each of these sections provides distributional analyses, physiography, elevation, vegetation zones, conservation biology, zoo biology, environmental problems and governmental policy structure in relation to conservation. Mexico differs slightly in being treated in four

separate regions, but this is deservedly so due to the incredible diversity within each. Each country specific chapter is titled differently and this reflects content and variation in conservation history, research conducted, deployed conservation strategies and current progress.

Of particular interest to me was the chapter by Mahmood Sasa and colleagues on Costa Rica. This chapter, like other geographically divided sections, provides not only an extensive list of species for the country, but also distributional and habitat inferences that shed interesting light on many species' biogeography and ecology. It is interesting to note the massive diversity (189 amphibians, 231 reptiles) and extreme levels of endemism (52 amphibians, 19 reptiles). With such numbers it is easy to realise that Costa Rica, a country the size of South Carolina, holds 3% of the world's amphibians and 4% of its reptiles. Further to the species distribution is an in-depth praise on habitat degradation that also reviews the socio-political influences on rainforest threats to Costa Rica and the historical successes and issues with the countries' biological reserve network. Attention to detail and extensive referencing also helps any reader grasp the importance of agriculture and urbanisation that has affected natural lands across Costa Rica. The analysis of reduced distribution for a number of species provides sobering reading. The reviews then continue with pesticide impacts, emerging infectious diseases, invasive species, overharvesting and trade in herpetofauna - all concisely treated. A very useful table provides the identity of all the major herpetofauna institutions and collections that are active in the country - something any student would need to know! No stone is left unturned in the chapter; sea-turtles receive extensive summary treatment; crocodilians, global amphibian declines, environmental education and conservation laws round off its

review content. Then, an essential and even-handed appraisal of the threat levels and priorities for future directions ends the chapter.

It would be impossible to comment on every country chapter content in this single review for the *Bulletin* but I hope by offering a taste of its massive encompassing content it will enthrall readers to buy a copy. For students and researchers, the species lists, and systematic addendum, within this tome provide the starting point for Mesoamerican taxonomy and are clearly a must read for anyone monitoring emerging taxonomical changes in the Neotropics.

The breadth of concepts addressed in this book have inspired me to review my research directions in Middle America and in my opinion the information within it serves to function as the baseline reference for conservation of


herpetofauna in the region for many years. Its price reflects what one would expect for its size and content and its colour plates do not disappoint. I continue to recommend its essential purchase and/or reading for all research students heading for Middle America. The book is aptly dedicated to the late Peter Stafford, who worked tirelessly on the Belize chapter and regrettably never saw the final content. My only wish is that the book was not available earlier in my career!


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Amphibian and Reptile Conservation & British Herpetological Society
Joint Scientific Meeting 2013

**amphibian and reptile
conservation**





Amphibian and Reptile Biology and Conservation

Sunday 8th December 2013, 0930-1700
*Lecture Hall, Bournemouth Natural Science Society,
39 Christchurch Road, Bournemouth, Dorset, BH1 3NS.*

0930-1000	<i>Arrival, registration and coffee</i>
1000-1005	<i>Welcome: Prof. Richard Griffiths, BHS President</i>
1005-1030	<i>Endocrine disruptors in agricultural landscapes – Dan Pickford (Brunel University)</i>
1030-1055	<i>Pheasants and reptiles – Rory Dimond (University of Worcester)</i>
1055-1120	<i>Common toad habitat suitability – Rosie Salazar (University of Oxford)</i>
1120-1200	<i>Coffee</i>
1205-1230	<i>Chytrid in the trade – Emma Wombwell (Institute of Zoology)</i>
1230-1255	<i>Brazilian reptile diversity – Moacir Tinoco (University of Kent)</i>
1255-1320	<i>A genetic monitoring programme for endangered leaf frogs – Alex Petchy (University of Salford)</i>
1320-1445	<i>Lunch</i>
1450-1515	<i>Heterospecific acoustic attraction in the great crested newt – Neil Madden (University of Salford)</i>
1515-1540	<i>Reptile refugia study – Robert Jones Parry (WT of South and West Wales)</i>
1540-1615	<i>Frogs of the Seychelles – Jim Labisko (University of Kent)</i>
1615-1700	<i>Coffee and departure</i>

EXHIBITORS / VENDORS WELCOME, BY PRIOR ARRANGEMENT ONLY PLEASE
Please see next page for Registration details...