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Front Cover: Female northern viper (F61) observed during the long-term monitoring study of Hodges & Seabrook (see articles on pages 6, 13 and 19) shown resting on a corrugated-iron refuge. F61 was born in 2008 and between then and her death by predation in November 2015 she was observed 71 times. There is evidence she reproduced in both 2013 and 2015. (photo C. Seabrook)

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REVIEW ARTICLE **The Herpetological Bulletin 137, 2016: 1-5**

Batrachochytrium salamandrivorans as a threat to British amphibians

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ABSTRACT - Globally, amphibian populations of many species are in critical decline. One major driver of amphibian decline is the emergence of chytridiomycosis. In addition to the initially known causative agent of this disease *Batrachochytrium dendrobatidis*, a novel amphibian pathogen, *Batrachochytrium salamandrivorans*, has recently been discovered. The potential impact this pathogen could have on wildlife health needs to be urgently addressed and assessed. Using amphibian species of the United Kingdom as a case study the present paper is a review of the state of the amphibian trade, current biosecurity measures, as well as the presence of a wild amphibian species in the UK thought to be susceptible to this disease. The review highlights the urgent need for research and bio-control evaluation, in order to ensure wildlife health security for amphibians in the UK.

INTRODUCTION

We are currently in the midst of a biodiversity crisis, which has been termed by many as the sixth mass extinction (Dunn et al., 2009; Barnosky et al., 2011). Amphibians have been one of the hardest hit groups with major declines in a third of species assessed by the IUCN (Beebee & Griffiths, 2005). Many drivers have been suggested for these declines, however disease is continually cited as one of the greatest factors contributing to this loss (Pounds et al., 2006). Chytridiomycosis is one of the most virulent of amphibian diseases, responsible for the highest number of extinctions of any known disease (Skerratt et al., 2007).

Until 2013, chytridiomycosis was thought to be caused by a single species of *Batrachochytrium dendrobatidis*, (Bd) (Daszak et al., 2001; Martel et al., 2013). However, Bd was not found in certain recent mass declines of amphibians that exhibited chytridiomycosis symptoms. These die-offs were eventually explained by the discovery of a novel species, *Batrachochytrium salamandrivorans* (Bsal) (Martel et al., 2013), which appears to target only the Urodelan group (newts and salamanders). Globalisation and trade have been implicated in the spread of both Bd and Bsal. The rate of the spread of Bd, and the geographically isolated nature of the Bsal outbreaks may have only been made possible by anthropogenic means (Martel et al., 2014). Given Britain's limited bio-control of amphibian imports and the mass mortalities that chytridiomycosis can cause, the import of amphibians could present serious epidemiological risks to UK amphibian species. This review focuses on elucidating the potential risks posed by Bsal to British amphibians by assessing the risks of wildlife trade to amphibian epidemiology, reviewing bio-control of amphibian imports to the UK and analyzing threats of Bsal to UK wildlife.

RISKS OF THE AMPHIBIAN TRADE

Scale of amphibian trade

The number of live animals traded each year globally is extremely large with almost 38 million live amphibians, birds, mammals and reptiles legally imported to the US alone every year (Marano et al., 2007). However, accurately quantifying the extent of the trade in animals, including amphibians, is near impossible given the multiple scales of trading from local level transactions to mass international commerce (Karesh et al., 2005). The situation is further complicated by both the illegal wildlife trade (Karesh et al., 2005) and ease of e-commerce (Kikillus et al., 2012). This extensive trade in live animals has been implicated in both amplification and spread of epizootic diseases in amphibians (Fèvre et al., 2006), with serious potential implications for British wildlife (Peel et al., 2012).

Disease amplification through the amphibian trade

The issue of disease amplification was addressed by Karesh et al. (2005) who highlighted how controlling disease at the interface between buyers and sellers is critical, suggesting that markets and shops could be hotspots for spreading and amplifying disease (Karesh et al., 2005; Hartung, 2003). Research into Bd in amphibians supports this assertion, as co-housing studies have shown that common toads (*Bufo bufo*), can cause infection among cohoused animals with increased presence increasing the intensity of Bd infection (Fisher & Garner, 2007; Schmeller et al., 2011). In addition, there are risks associated with long term environmental disease contamination in an area where other frogs may be kept (Peel et al., 2012; Johnson & Speare, 2003). Bsal has been transmitted between adults co-housed on damp toweling (Martel et al., 2013), which highlights how like Bd, Bsal potentially could be spread within the amphibian trade, as amphibians are often co-housed for transport (Peel et al., 2012). However, to determine if co-housing affects intensity of Bsal infection further research is needed.

Links between the amphibian trade and epizootic outbreaks.

These concerns are particularly pressing given the links between disease in the amphibian trade and epizootic outbreaks in wild amphibian populations, as there are multiple examples of how an infection spread by anthropogenic activity, like pet trade, can threaten populations of native species (Schloegel et al., 2010). One example is the Alpine newt, (*Ichthyosaura alpestris*) which has been identified as a spreader of Bd after introduction to the UK (Arntzen et al 2009), and more recently New Zealand (Arntzen et al. 2016). In New Zealand over 70% of individuals caught from the introduced population and screened for disease were found to be infected with Bd, (Artnzen et al. 2016) presenting a serious threat to the survival of native amphibian species.

Bsal in the amphibian trade

Bsal also has links with the international amphibian trade. Bsal was discovered in the Netherlands, following dramatic mortality of the native fire salamanders (*Salamandra salamandra*) (Martel et al., 2014). 96% of the Netherlands fire salamander population was lost between 2010 and 2013 (Spitzen et al., 2013), and subsequent ex-situ conservation efforts were compromised by 49% mortality of the captive population (Martel et al., 2013). After initial screening for known amphibian pathogens including Bd (Spitzen et al., 2013) returned negative results, further investigation by Martel et al., (2013) identified the presence of Bsal in fire salamander specimens from the 2010 mortality, and demonstrated that it causes mortality under lab conditions. Discovery that Bsal DNA showed 100% identity to samples found in Thailand, Vietnam and Japan, and the absence of overt disease in other parts of Europe, suggests that the pathogen was previously limited to East Asia (Martel et al., 2014). However, a recent large scale study to assess Bsal in Chinese amphibians showed no incidence of this disease, yet despite the lack of disease, infection may be widespread. This is because it is difficult to tell the level of infection from the Zhu et al., (2014) work as the long-term formalin storage of museum specimens used could have interfered the genetic analysis, and therefore detection of infection in carriers. Additionally, as noted by the authors many samples were taken in locations which exceeded the thermal tolerance of Bsal, so may not reflect disease prevalence at higher elevation. Therefore, despite disease not being found in the Zhu et al. (2014) study, it remains probable that Bsal recently emerged from Eastern Asia. Therefore, given the distance between Eastern Asia and the Netherlands, and other European countries like Germany (Pinto et al., 2015), it is likely that Bsal was spread by anthropogenic means, probably via the pet trade (Martel et al., 2014). Importantly Eastern Asia is one of the regions with the highest levels of indigenous live newt exports, the scale of which was demonstrated in a recent report which estimated that 2.3 million individuals of *Cynops orientalis* (native to China) were traded in the United States between 2001 and 2009, 44.1% of which were estimated to be wild caught (Herrel & Meijden, 2014). Further to this Bsal was recently discovered in three Vietnamese crocodile newts (*Tylototriton vietnamensis*) traded in Europe, two of which were imported into Europe in 2010 (Martel et al., 2014). All of this indicates that Bsal can probably spread from the amphibian trade to wild populations of urodela with catastrophic effects, and there is evidence to suggest that there is still prevalence of Bsal in the European and international amphibian trade. The seriousness of the risks posed by Bsal has been further highlighted by the alarming recent spread of this disease through Belgium and Germany (Spitzen-van der Sluijs et al., 2016). The US Fish and Wildlife Service has responded to the threat of Bsal by banning of the importation of 201 species of salamander (Injurious Wildlife Species; Listing Salamanders Due to Risk of Salamander Chytrid Fungus, 2016), while the Bern Convention and Council of Europe have issued strong recommendations for disease screening and trade restrictions (Council of Europe, 2015). However, there is still a pressing need for similarly progressive steps to be taken to ensure biosecurity of amphibians in Britain.

BIOCONTROL OF AMPHIBIANS IN THE UK

The current state of biocontrol of amphibians in the UK

In 2006, 131 599 amphibians from outside the EU were transported into the UK through the Heathrow Animal Reception Area as imports for the UK amphibian trade (Peel et al., 2012). However, numbers are likely to be far higher as this figure does not include amphibians transported via Manchester or Gatwick, those transported as pets, or those from within the EU (Peel et al., 2012).

In Britain, the importation of live amphibians is regulated by the International Animal Health division of the Department for Environment, Food and Rural Affairs (DEFRA) (Fisher & Garner, 2007). DEFRA is guided by the European Commission's General Animal Health and Welfare commission, as well as the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) (Fisher & Garner, 2007). Despite strict regulation to prevent disease in livestock (Waage & Mumford, 2008) there is limited legislation in place to counter the spread of disease in amphibian populations. Animals entering from outside the EU may be subject to veterinary examination (Department for Environment, Food & Rural Affairs and Animal and Plant Health Agency, 2015); yet PCR-based screening needed to detect disease in carriers is not required (Peel et al., 2012). Within the EU, imports of live animals are allowed with a certificate of health (McGrann & Wiseman, 2001). However, widespread e-commerce, makes this an area which is challenging to regulate (Lincoln et al., 2012; Kikillus et al., 2012).

UK legislation is in place to mitigate spread of disease once animals have been imported. Importers who notice disease in the animals must report them to local authorities which may require slaughter of the animals without compensation under Council Directive 90/425/EEC. Despite this ruling it is possible that lack of compensation may act as a disincentive to report disease.

Recognition of risks resulting from current state of amphibian biocontrol in the UK

The potential risks caused by lack of strict controls on the amphibian trade have been recognized by the British Veterinary Association and the British Veterinary Zoological Society which issued a joint statement calling for an EU ban on the trade in wild caught amphibians and reptiles (British Veterinary Association, 2003). In addition to this, a large NGO coalition which include members from the UK have written to the European Commission demanding that Europe takes similar steps as the US in banning imports of salamander into Europe (Steenwegen & Funcken, 2016). DEFRA is aware of these concerns as highlighted by the funding of research into amphibian disease (Martel et al., 2014). However, no significant regulatory action appears to have been taken on the research findings. These concerns become even more pressing given the recent finding of Bsal in three species of imported urodele acquired from a UK breeder (Cunningham et al. 2015), which has led to calls for amphibian breeders, keepers and veterinarians to ensure that recommended biosecurity measures are strictly followed (Cunningham et al. 2015).

RISKS OF BSAL TO BRITISH WILDLIFE

Potential vulnerability of British newts

Britain has three native species of Urodelaes (Arnold, 1995): the palmate newt (*Lissotriton helveticus*), the common newt (*Lissotriton vulgaris*), and the great crested newt (*Triturus cristatus*). Martel et al. (2014) carried out a suite of infection studies with Bsal which showed the great crested newt to be very susceptible to this disease, with mortality in all animals experimentally infected after 25-57 days (Martel et al., 2014). Interestingly the palmate newt was found to be resistant to the disease, showing no mortality on infection. The common newt was not tested. However, there are currently no published studies on the passage of Bsal from a resistant individual to a susceptible individual, or any data on how long after infection the resistant species will be infectious to susceptible species. Furthermore, it must be noted that these studies have been carried out under laboratory conditions using small numbers of captive bred individuals and consequently there may be differences in the field.

Potential for emergence and spread of Bsal in the UK

There are also serious gaps in knowledge as to how this pathogen spreads environmentally. Bd has shown potential for transmission via zoospores in water as well as by direct contact (Johnson & Speare, 2003) and potentially even wind-blown during rainstorms (Kolby et al., 2015). The finding that Bsal zoospores are highly motile may suggest transfer by water, while infection by co-housing could suggest transmission by direct contact or contact to a contaminated environment (Martel et al., 2013). However, these speculations need to be addressed in order for an accurate assessment of likely disease spread in Britain, especially given the finding that of Bsal is can grow at 5˚C with an optimum 10-15˚C (Martel et al., 2013). March to August is reported to be the six-month period with the highest great crested newt activity in the UK (Arnold, 1995), the majority of which is confined to England and Wales (Arnold, 1995). Between 1910-2016, the average maximum and minimum temperature recorded for March to August in England was 15.9˚C -7.2˚C, and for Wales, 14.7-6.7˚C (MET office, 2016). This overlap of UK temperatures during peak periods of salamander activity, and temperatures compatible with Bsal growth raises serious concerns about the great crested salamander, with the possibility that Bsal spread is unlikely to be limited by temperature across much of the UK range of the great crested newt.

In addition to addressing questions regarding the environmental spread of this disease, there are also questions regarding the risks of infected captive animals or infected material from captive animals coming in contact with the environment. There are currently unknown levels of disease in captive amphibians in the UK, as well as unknown rates of escape or release, making it difficult to quantify risks (Peel et al., 2012). However, examples like the introduction of North American bullfrogs to the UK, and with them chytridiomycosis (Cunningham et al., 2005; Fisher & Garner, 2007) show that there is precedent for escape (Manchester & Bullock, 2000).

CONCLUDING REMARKS

In conclusion, Bsal can cause mortality in urodeles, including the great crested newt native to Britain, and is able to survive British temperatures (Martel et al., 2013). In addition to these two risk factors, Bsal has been found in the European pet trade, and furthermore has had devastating effects on several wildlife species in Europe (Martel et al, 2014.). There are still gaps in data that need to be addressed before risk can be accurately quantified, like risk of environmental contamination with Bsal, levels of amphibian escape in Britain, and the potential of resistant individuals to act as carriers. However, while current lack of data prevents complete analysis of what risk Bsal poses to Britain, examples of mass mortalities in the Netherlands (Martel et al., 2013) should act as a warning, emphasising the need for both more research into this disease, as well as re-evaluation and enhancement of the bio-controls in place for amphibian disease in both the UK and the EU.

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RESEARCH ARTICLE

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Use of artificial refuges by the northern viper *Vipera berus* - 1. Seasonal and life stage variations on chalk downland

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ABSTRACT -During eight years of a continuing long-term study of *Vipera berus* in a chalk downland nature reserve, records were made of encounters with all life stages at artificial refuges and in the open. The refuges were paired sheets of galvanised corrugated-iron ('tins') and roofing felt, deployed at the rate of 2.7 to 4.2 pairs/ha. Distinctive seasonal patterns of encounters are reported for each life stage and provide insights into viper behaviour. For adults, encounters in the open and at refuges both contribute significantly to records while for immature stages records depended largely on their use of refuges as they were rarely encountered in the open. All life stages were encountered at tins more frequently than felts. Gross encounter rates have been refined by reference to observations on 483 individuals recognised by their head-scale patterns. The numbers of different individual adult males and adult females observed in the open were broadly similar, as were the numbers under tins, but the frequency with which these individuals were resighted was greater in the open for males and greater under tins for females. At least in the case of gravid females, refuge use may be a substitute for mosaic basking. Under the conditions of this study, roughly 70% of individual adult vipers used refuges at least once, usually much more frequently, whilst for immatures the proportion remains uncertain. These observations could be used to improve the planning and interpretation of long-term monitoring studies.

INTRODUCTION

Long-term monitoring of reptiles, and other wildlife, is essential for the collection of evidence on important conservation issues such as habitat management (Fuller et al., 2016) and response to climate change. In temperate climates, artificial refuges can contribute to long-term monitoring of reptiles. They offer both warmth and concealment (Joppa et al., 2009) and consequently are locations where reptiles accumulate and can be counted. The artificial refuges used in the UK are frequently rectangles of galvanised corrugated-iron ('tins') or roofing felt (Froglife, 1999; Reading, 1997) although both in the UK and elsewhere refuges may sometimes be made of other materials and have included *inter alia* Onduline, wood, carpet tiles, and fibrocement.

The northern viper (*Vipera berus*) is of conservation concern and in recent times a range reduction of about 39% has been suggested (Gleed-Owen, 2013). These vipers, especially adults, attain their preferred body temperatures by basking in more open areas either a short distance from vegetation cover or in the dappled sunlight of partial vegetation cover, the latter is referred to as 'mosaic' basking (Gaywood & Spellerberg, 1996). Nevertheless, vipers may also be encountered under artificial refuges that may assist in maintaining body temperature and in providing cover. To date there are no specific recommendations on how to undertake long-term refuge monitoring for *V. berus* or how to interpret the data generated, although there is excellent general advice on both survey technique (Froglife, 1999) and preparing and deploying tin refuges (Langham, 2011). Furthermore, a quantitative survey method with refuge tins, tested for reptiles on lowland heaths, has been proposed but is based on scant data in the case of *V. berus* (Reading, 1997) and is not necessarily intended for long-term monitoring. In the UK, long-term monitoring studies of reptile populations are rare but in a recent 9-year study of *Natrix natrix*, refuge tins were deployed at the rate of 2/ha. These refuges were repositioned annually both to prevent any 'bedding in' effect and also to ensure that if the snakes had learnt where the refuges were located then this factor would remain constant between years (Sewell et al., 2015).

As part of an on-going long-term monitoring programme of *V. berus* at a chalk downland reserve in Kent (UK), we deployed both tin and felt refuges and recorded our observations of different viper life stages at both refuges and in the open for a period of eight years. These observations may help in the future with the interpretation of monitoring data from both our own continuing study and those of others.

MATERIALS AND METHODS

Study site and refuges

The study site is a chalk downland nature reserve at about 51°N, 0°E. The reserve is managed by Kent Wildlife Trust and comprises three distinct open areas totalling 11.1ha of grassland with margins and islands of low scrub, generally sloping at 10° to 15° with aspects to the south, south west or west. The reserve is bordered by open farmland, housing, woodland and for a short stretch by a road, and apparently offers little prospect of connection with other viper populations.

Refuges consisted of galvanised corrugated-iron sheets (0.5mm thick and 0.5g/cm2), referred to as tins, and roofing felt (Garage felt, green slate finish, Homebase, #242805, 2mm thick and 0.3g/cm2). They were both cut to the same dimensions (50cm by 65cm) and pairs, comprising one of each type, were placed in sunny but inconspicuous locations backed by vegetation cover. Tins were camouflaged by spraying their upper surface with brown paint (Espresso, satin finish, Rust-oleum). At the start of the study there were 31 pairs but more were added over time so that the final total was 47 pairs (Table 1); refuge density consequently increased from 2.7 to 4.2 pairs/ha.

Making viper observations

Monitoring was initiated in 2004 and is still on-going although the data presented here are from 2008 to 2015. Observations were made by two or three surveyors during visits to the whole site in the reptile active period from February to October. The number of annual visits varied from 59 to 77 (Table 1) and for each visit the survey followed a standard route between refuge locations. Vipers were recorded at refuge locations and along the standard route in areas with more or less cover. The study involved no animal handling in order to minimise disturbance and stress. Observations were made morning and afternoon on days when weather conditions were not excessively wet or windy. To facilitate individual recognition, photographs were taken of head-scale patterns (Benson, 1999) either in the open at a distance (2-4m), using a long focal length lens, or much closer when individuals were under refuges. The scale patterns were coded and then entered into a database. There were 483 different head-scale records across all life stages, and during the eight year period some individuals contributed data at some or all of the different life stages. Head-scale patterns were found to be stable apart from a single exception (Hodges & Seabrook, 2014). Adult recruitment tables suggested that the density of adults varied from 3 to 5 adult vipers/ha in the study period; it is intended to describe these tables in more detail in future reports. Different life stages were recorded and recognised by their size and the following features - juveniles were individuals that had hibernated only once or had never hibernated (neonates); sub-adult males had completed two or three hibernations and sub-adult females had completed two to four hibernations; adult males were recognised by having completed at least 4 hibernations and in the case of females at least five hibernations. The life stages were known from their size, colour pattern and from individual records kept across years, but are likely subject to occasional inaccuracy. The gender of adults and sub-adults was determined by colouration and body proportions (Smith, 1951; Beebee & Griffiths, 2000) but there was no attempt to distinguish the gender of juveniles.

Statistical analysis

In the initial analysis and interpretation of this study, viper observations are treated as simply ‘encounters’ with no assumptions about the number of individual vipers that contributed the data, subsequent analysis was of data derived from the sightings of ‘known individuals’. Where relevant, whole data sets for all life stages was assessed for underlying heterogeneity using the Kruskal-Wallis one way analysis of variance, and where such heterogeneity was detected pairwise *post hoc* testing of individual life stages for statistically significant differences was undertaken using the Mann Whitney U test (Siegal, 1956). Differences were treated as statistically significant when the probability of them occurring by chance was 5% or less ($p \leq 0.05$). Where data was highly skewed they have been presented as box and whisker plots. The statistical significance of simple linear correlation coefficients (r) were determined from standard tables (Bailey, 1966).

RESULTS

Annual and seasonal encounters rates with vipers at refuges or in the open

During eight years the total number of viper encounters were as follows, with the number of ‘known’ viper sightings in parenthesis: adult males 1071 (853); adult females 1077 (937); sub-adults 725 (521); and juveniles 582 (574). The proportion of vipers encountered at refuges remained above 70% during the first three years of study after which it became more erratic varying from 42% to 74% (Table 1). The total numbers of vipers encountered annually at refuges correlated poorly with either the number of refuge pairs deployed ($r = 0.27$, $n = 6$, $p>0.1$) or with the number of visits made ($r = 0.09$, $n = 6$, $p>0.1$). This suggests that, within the range of refuges densities and visit rates used in this study, other factors were more important in determining viper encounter rates. It was noticeable that for the first six years of study the numbers of vipers in the open per visit increased steadily and only since 2014 did it decline (Table 1). Again, the total annual encounter rate in the open correlated poorly with number of annual visits ($r = 0.215$, $n = 6$, $p>0.1$).

Table 1. The numbers of *V. berus* encounters in the period 2008 to 2015 and the proportion at refuges (both tins and felts)

Year	No. of site visits	No. of refuge pairs	At refuges	In the open	In the open/ visit	Total count	% at refuges
2008	76	31	343	86	1.13	429	80.0%
2009	69	32	314	83	1.20	397	79.1%
2010	65	33	255	108	1.66	363	70.3%
2011	64	35	200	147	2.30	347	57.6%
2012	65	40	458	159	2.45	617	74.2%
2013	59	41	251	191	3.24	442	56.8%
2014	79	46	296	235	2.97	531	55.7%
2015	77	47	139	190	2.47	329	42.3%

The mean monthly encounter rates for each life stage followed a distinctive pattern. In spring time (February - April) the number of adult encounters at refuges was low (Fig. 1), then rose in the summer (May - August) and fell in the autumn (Sept - Oct) as vipers submerged for hibernation.

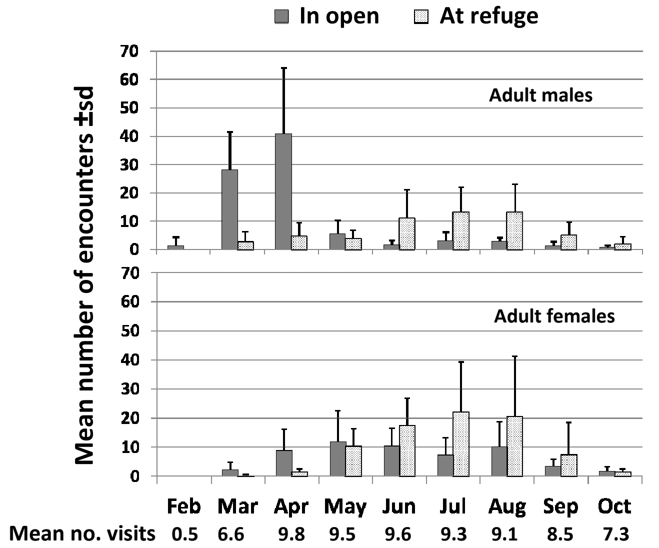

Figure 1. Mean monthly number of encounters (±sd) with adult male and adult female *V. berus* under refuges (tins and felts) or in the open, 2008 to 2015, also showing the mean number of monthly visits made to the site, (n = 8)

The highest encounter rates with adult males were recorded when they were basking openly ('lying out') or searching for mates in March/April. Of these records on average only about 5% were from refuges, while in the period May to October on average 78% of adult male encounters were at refuges. Unlike adult males, relatively few adult females were encountered in the spring (Fig. 1) and likewise these were mostly in the open with only 8% at refuges. From May onwards, the number of refuge encounters with females, especially those that were gravid, rose steeply so that in this period on average 63% of encounters were at refuges.

Throughout the year, encounters of sub-adults and juveniles were mostly at refuges (Fig. 2). In spring encounter rates were low but these rose steeply from May to July. Sub-adult encounters at refuges started to decline from August onwards, at least a month earlier than either adults or juveniles. In the case of juveniles, encounter rates were augmented by the arrival of neonates, when adult females gave birth in August and September, so that decline was not obvious until October (Fig.2).

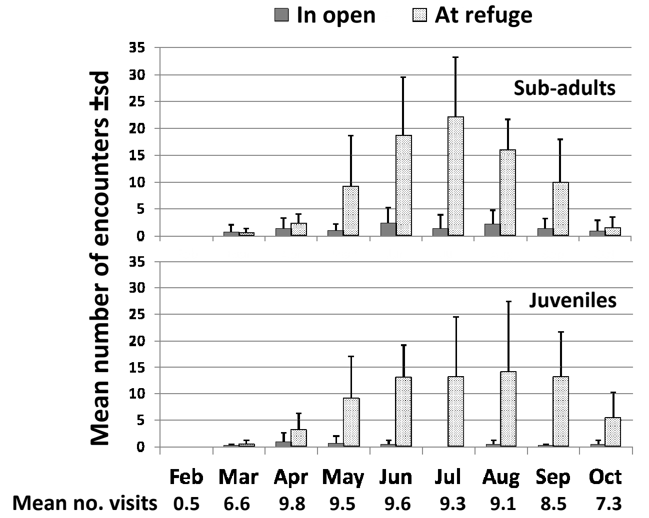

Figure 2. Mean monthly number of encounters (±sd) with sub-adults and juvenile *V. berus* under refuges (tins and felts) or in the open, 2008 to 2015, also showing the mean number of monthly visits made to the site, (n = 8)

Number and proportion of viper encounters at refuges or in the open

Over the eight years of study, the mean annual total (±sd) of adult encounters at refuges (tins and felts combined) amounted to 132.6±66.7, sub-adult 79.3±29.9, and juvenile 69.9±31.2. There was significant heterogeneity between the variables of the data set (Kruskal Wallis, H = 6.5, df = 2, $p<0.05$, N = 24) and pairwise comparisons showed that the adult encounter rate was significantly greater than that for sub-adults or juveniles (Mann Whitney, $p<0.05$), but that sub-adults and juveniles did not differ significantly.

The proportions of each viper life stage encountered at refuges, rather than in the open, were statistically heterogeneous (Kruskal Wallis, H = 24.63, df = 4, $p<0.001$, N = 40). At least 80% all encounters with immatures stages (sub-adult and juvenile) were from refuges (Fig. 3) and these proportions were significantly greater than those for either adult males or adult females. The percentage of adult females seen at refuges was significantly greater than for adult males (Fig. 3).

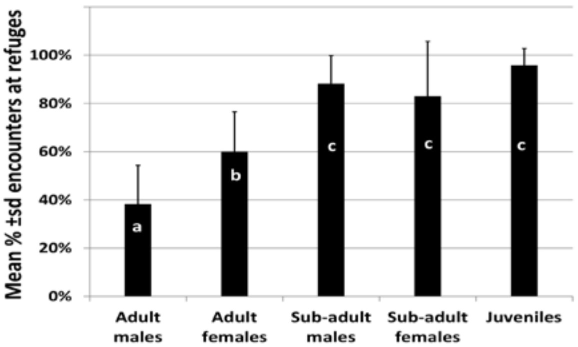

Figure 3. Mean % (±sd) of annual *V. berus* encounters at refuges, rather than in the open, 2008 - 2015. Values with no letter in common are significantly different (Mann Whitney, $p<0.05$), n = 8.

The proportion of all viper encounters at tins, rather than felts, rose quite steeply from 2008 to 2009 and thereafter more or less levelled off (Fig. 4). There was evidence of heterogeneity between years (Kruskal Wallis, H = 19.8, df = 7, $p<0.01$, N = 40) and in the first year, and possibly second year, tins appeared to be less favoured by vipers than the years that followed (Fig. 4).

Observation rates of known individual vipers at refuges and in the open

Over eight years of study, approximately equal numbers of individual adult males (87) and females (85) were detected, suggesting an equal sex ratio, and about 70% of these known adult males and females were observed at least once under a refuge (Table 2). The known immatures stages were more likely to have been observed using a refuge as 81-89% of sub-adults and 98% of juveniles were recorded using tins and/or felts at least once (Table 2). For all life stages, more individuals were recorded using only tins than either only felts or a combination of both refuge types (Table 2).

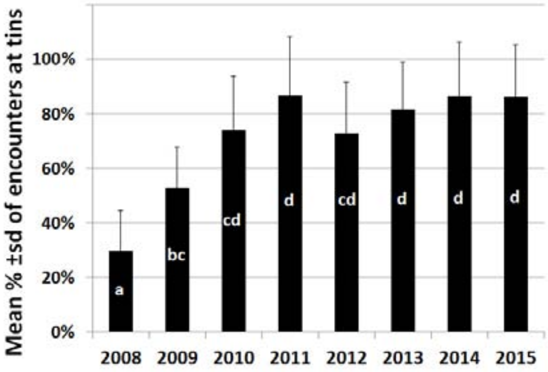

Figure 4. The mean % (±sd) of all life stages of *V. berus* encounters at tins, rather than felts, 2008 - 2015. Values with no letter in common are significantly different (Mann Whitney, p<0.05), n = 5.

Table 2. Proportions of individual known *V. berus* at each life stage observed at least once under corrugated iron (tin), roofing felt (felt) refuges, or both, from 2008-2015 (NB due to the length of the study some individuals were recorded in more than one life stage)

Life stage	Total no. indvs	% of known individuals found at			% of indvs found	
		Tins only	Both tins & felts	Felt only	At refuges	Only in the open
Adult male	87	40.0%	23.2%	6.3%	69.5%	30.5%
Adult female	85	43.5%	25.9%	11.8%	70.6%	29.4%
Sub-adult male	60	54.1%	24.6%	11.5%	88.5%	11.5%
Sub-adult female	76	49.3%	28.0%	4.0%	81.3%	18.7%
Juvenile	202	62.1%	19.4%	16.5%	98.1%	1.9%

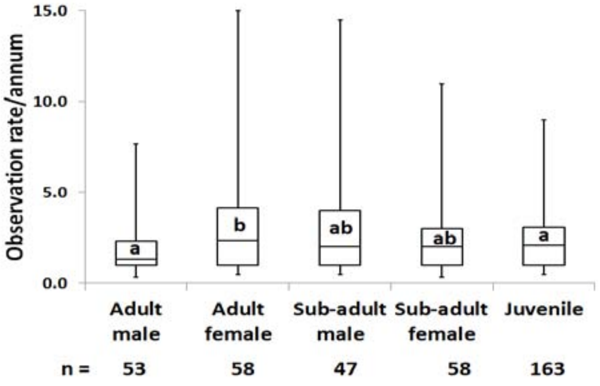

Figure 5. Observation rates/annum of known *V. berus* of each life stage at tin refuges over a period of 8 years. Shown are minimum, first quartile, median, third quartile and maximum rates; boxes with no letter in common are statistically significantly different (Mann Whitney, p<0.05), n = number of individual vipers.

For the eight years of study, the mean annual observation rates of individual vipers at tins, felts or in the open were significantly heterogeneous (Kruskal Wallis, H = 962.2, df = 14, P<0.001, N = 745). At tins, adult females were observed significantly more frequently than adult males or juveniles although did not differ from the sub-adults (Fig. 5). Although median values were similar, statistically significant differences was associated with wider value ranges.

The rate of use of felts by juveniles was significantly greater than for adult males but otherwise there were no significant differences between other life stages (Fig. 6).

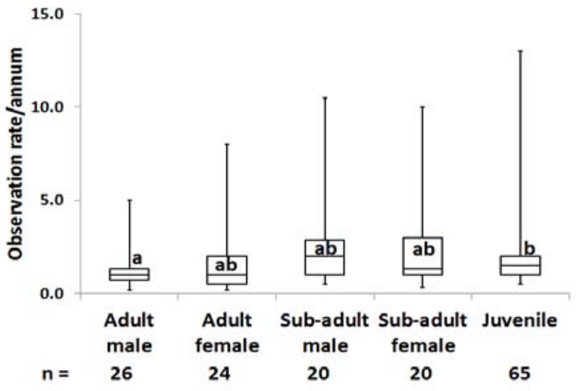

Figure 6. Observation rates/annum of known *V. berus* of each life stage at felt refuges over a period of 8 years. Legend as for Fig. 5.

In the open, adult males were observed significantly more frequently than the other life stages (Fig. 7). Adult females observation rates were significantly greater than those of the sub-adults, which in turn were significantly greater than juveniles (Fig. 7).

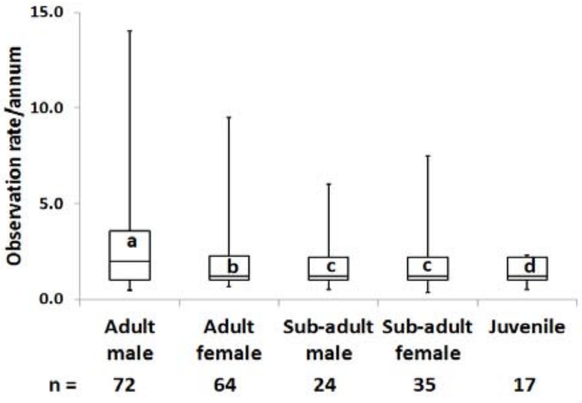

Figure 7. Observation rates/annum of known V. *berus* of each life stage in the open over a period of 8 years. Legend as for Fig. 5.

DISCUSSION

During eight years, there were more encounters with adult vipers than with sub-adults or with juveniles. Adults were also much more frequently encountered in the open than other life stages. In total there were similar numbers of different individual adult males and adult females observed in the open and under tins, but the frequency of resighting these individuals was greater in the open for males and greater under tins for females. All life stages were more commonly observed under tins than under felts.

There were differences between monthly patterns of adult viper encounters in the chalk downland reserve, at both refuges and in the open. Such differences had been expected from the descriptions of adder behaviour by earlier authors, albeit for different habitats. The early spring (Feb –April) abundance of adult males basking in the open (Fig. 1) followed by moulting and searching for females has been well documented previously, as has the emergence of the bulk of adult females at least two or three weeks after the males (Neumeyer, 1987; Prestt, 1971; Viitaten, 1967, *inter alia*). Adult females sun themselves in the open but are more difficult to detect as they keep closer to vegetation, are much less active, and less conspicuously coloured. In early spring, both adult male and female vipers were observed to make very limited use of refuges, instead most individuals warmed themselves by open basking. The surge in spring time observations in the open, particularly of adult males, is the basis of a viper monitoring project 'Make the adder count' (ARG UK) in the UK. Subsequently, in the period May to August, adult males and non-reproductive adult females become difficult to observe as they are foraging (Prestt, 1971; Viitaten, 1967), and in the current study their appearance at refuges was usually confined to times when they are either digesting food or in pre-moult. Adult female encounters rose from May onwards due to gravid females basking frequently either in the open or under refuges. Then in the autumn (September - October) encounter rates for both adult males and females declined as the vipers submerged for hibernation. The total number of encounters with adult males (1071) and adult females (1077) during the study, and different individual known males (87) and females (85), were more or less equal. *Viper berus* is known to have a 1:1 sex ratio (Volsøe, 1944; Prestt, 1971) which was confirmed in the current study (Table 2). However, as females normally breed on a biennial cycle (Prestt, 1971), on average only half the adult female population is likely to reproduce in any given year. In contrast, all adult males may reproduce annually. Potentially, this gives twice as many males lying out in spring as gravid females basking later in the year. Despite this, the total encounter rates for adult males and females are similar. This seems to result from fewer females basking for longer, four months (May to August), matched by a greater number of males that bask openly for only for two months (March to April). Further, given an equal sex ratio and a long period (eight years) there was sufficient time to encounter broadly similar numbers of different individual adult males and females.

Greater usage rate of tins by adult females, especially those that are gravid, could be interpreted as females using refuges instead of mosaic basking. If that interpretation is correct then if there had been no artificial refuges it is possible that the observation rate of adult females in the open would have been similar or even greater than that of adult males and this would be worth further investigation on a site without refuges. Nevertheless, there seems to be variation between females in willingness to use refuges. For example, a gravid female that in 2014 was at least 13 years old was observed 34 times close to a tin but under it only once. In contrast, a younger gravid female, in its 6th year in 2015, was observed close by the same tin four times but found under it a total of 15 times. It is noteworthy that the older female was already an adult before any artificial refuges were laid in the reserve whereas the younger female had a history of refuge use from when first observed as a sub-adult in 2011, albeit not the tin mentioned here. This suggests that some degree of learned behaviour may be important in determining refuge use rather than 'knowldege' of where tins are located since both individuals 'knew' and encountered the same tin.

In the literature there are only a few references to the encounter patterns with immature vipers (Prestt, 1971; Viitaten, 1967). As expected, open basking was relatively rare so that encounters with immature stages were largely dependent on refuge use; even more so for juveniles than sub-adults (Figs. 2 & 7). Avoidance of open basking is common among small snakes, probably so that they remain concealed from avian predators (Webb & Whiting, 2005). Sub-adults emerged from hibernation at roughly the same time as adult females (Neumeyer, 1987; Prestt, 1971; Viitaten, 1967) and reached a peak in July (Fig. 2) and then declined progressively in August and September; they were more or less submerged for hibernation in October (Fig. 2). The autumn decline of juveniles appeared much less pronounced (Fig. 2), but would have followed the same pattern as sub-adults if it had not been that new born vipers augmented observation rates in August and September.

It is tempting to refer to monthly variations in encounter rates (Figs 1 & 2) as 'activity patterns' but many individuals under refuges were either in a condition of pre-moult, digesting prey, promoting embryonic development, or warming themselves prior to foraging. Consequently, the observed patterns are a reflection of changes in seasonal behaviour more than of activity *per se*. The proportion of all viper observations attributable to refuges averaged 65%; roughly similar results were obtained in a study of refuge monitoring on dry lowland heath which yielded a value close to 50% but from a rather small sample size (Reading, 1997). At other sites, however, use of refuges by *V. berus* may vary drastically between years or they may be altogether shunned (Griffiths et al., 2006; Walter & Wolters, 1997). It is not clear what might cause such extreme variation. Possible explanations are that in the case of small populations chance factors may come into play and/or where the study site has relatively newly placed refuges results could be low as there had not been sufficient time for refuge use to become part of the vipers' behavioural repertoire. In the case of the current study, refuge use may have been favoured as refuge positions had been established for several years and, being in a habitat that was largely open, warm sheltered locations that facilitate mosaic basking may have been at a greater premium than in other more sheltered habitats.

More individuals used tins than felts; this preference for tins has been reported previously (Griffiths, et al. 2006; Rijksen, 2008). There was little evidence of differences between life stages in their preference for felts except that the rate of use by juveniles was significantly greater than by adult males. Felts are applied closely to the ground and so the animals using them would be warmed by direct contact with the felt; consequently they are particularly favoured by thigmotherms such as the slow worm *Anguis fragilis* (Spellerberg, 1976). *V. berus* is known to thermoregulate largely by moving to preferred locations and changing posture (Spellerberg, 1976); this would be easier under tins due to the additional space created by the corrugations. However, the use of felts by some vipers suggests that at least occasionally thermoregulation may be achieved by thigmothermy; this has been observed previously in a minority of cases but not specifically in relation to felts (Gaywood, 1990). During the first three years of the study there was a progressive increase in the use of tins and corresponding fall in the use of felts (Fig. 4). This coincided with the gradual loss of vegetation beneath the felts, so that in due course the ground beneath them was largely bare. It seems likely that this change, ‘bedding in’, may have made the felts less suitable for the vipers, as the felts would become more closely applied to the ground beneath. If refuges had been relocated annually then the proportion of viper records from felts could have been higher; however it cannot be concluded that the total number of records at refuges would have been greater since as felts became less favourable it is likely that there was a simple displacement of vipers from felts to the closely located tins with which they were paired. The relatively small contribution of felts suggests that their omission from programmes to monitor *V. berus* would have limited impact on the data gathered, although this is likely to be untrue of other species such as *A. fragilis*, which can be monitored simultaneously.

It was of some interest that the observation rate of vipers in the open increased for the first six years of the study (Table 1). An ability to see vipers basking/mosaic basking is a skill and some of the increase, probably most of that in the first two or three years, could be attributed to increasing surveyor skill and knowledge of the habitat. However, this is certainly not the only factor. The quality of springtime weather will affect the rate at which vipers can arrive at reproductive condition (Herczeg, et al. 2007); periods when sunshine is weaker will require more protracted basking, resulting in higher encounter rates. It is perhaps no coincidence that the year with the highest rate of observations in the open, 2013, was a year with particularly poor springtime weather. Another factor of importance is the number of individuals that would enter reproductive condition. Observation of an island population of *V. berus* in Sweden has shown that if food becomes limiting then adult males and females emerging from hibernation in the following year fail to engage in reproductive activity and instead proceeded directly with foraging (Andrén, 1982). Consequently, they were less frequently encountered in the open. Given the effects of climate and food supply, natural variation in encounter rates of adults in the open is to be expected and is not just a function of population size.

This study gives some indication of the proportion of the total viper population that actually uses refuges. Over the eight years of study at our site, the recruitment of individual adult vipers to our database became increasingly confined to newly matured individuals. Consequently, apart from this segment of the adult population it is assumed that most adult vipers are known (equivalent to mark recapture data where most adults are resightings/recaptures). In that case, from our observations of known vipers at refuges (Table 2) it can be concluded that about 70% of adults have used refuges on at least one occasion. Currently, it is not possible to estimate the proportion for immatures using refuges since there is much less confidence about the proportion of the population that is known to us. The relatively low encounter rate of immatures might be interpreted as reluctance to use refuges, an inability to find refuges, or simply that there are fewer immatures within the population than might be expected. Low numbers may be a reasonable explanation. In our study we recorded more individual juveniles (202) than sub-adults (136) but a relatively high number of individual adults (172). This pattern is similar to that described for other *V. berus* populations (Phelps, 2007), where there was very high mortality in the first three years of life (92%) but where adults were relatively long-lived, with significant numbers at least 25 years old; furthermore it was reported that recruitment was less than expected as females often bred less regularly than the typical biennial cycle (Phelps, 2007).

In the case of adult *V. berus*, observations in the open and at refuges are complimentary in providing encounter data and the use of refuges reveals the summer peaks of females and sub-adults and the arrival of neonates in the autumn. Vipers can be photographed in the open and even more easily at refuges to enable individual recognition by head-scale patterns. Individual recognition can help to resolve the wide annual variations in encounter rates, indicating whether this variation is due to the same vipers being encountered more often or the presence of a greater number of vipers. Any changes in numbers may give clues to the health and sustainability of *V. berus* populations due to factors such as climate, food supply, and habitat management methods. It is intended that the current long-term study will in due course report on population fluctuations and their possible causes. In relation to further development of monitoring, knowledge about how vipers use refuges and the extent of any thermal advantages they confer could improve the interpretation of data, could suggest improvements in refuge design and deployment, and could also indicate what conservation benefits the refuges themselves might offer. We have made a start on investigating these issues, which are documented in two further reports (Hodges & Seabrook, 2016 a&b).

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RESEARCH ARTICLE **The Herpetological Bulletin 137, 2016: 13-18**

Use of artificial refuges by the northern viper *Vipera berus* - 2. Thermal ecology

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ABSTRACT - The body-surface temperatures (T_b) of northern vipers (*Vipera berus*), under galvanised corrugated-iron refuges ('tins') or basking in the open, were investigated in a chalk downland reserve. Corresponding operative temperatures (T_e) were demonstrated by physical models. Although viper T_b under tins reached 34.4°C, once T_e had reached 32.9°C no viper T_b exceeded this temperature; 32.9°C appears to be a practical estimate of the vipers' upper thermal set point ($T_{set\ upper}$). In March/April, there was little or no difference in T_e between locations in the shade and under tins, resulting in no incentive for vipers to use tins. At this time, males basking openly had body temperatures that averaged 8.6°C warmer than the T_e under tins. The heating rate of vipers below tins averaged 0.08°C±0.03°/min, much lower than the observed rates for basking in the open reported previously. The relatively poor thermal performance of tins suggests that design improvements could deliver better results.

INTRODUCTION

Artificial refuges are a valuable asset in reptile monitoring programmes and may also have conservation value where there is a trade-off between predator avoidance and the needs of thermoregulation (Lelièvre et al., 2010). The provision of artificial refuges has also been shown to be an effective approach to the recolonisation of degraded sites (Croak et al., 2010). Consequently, a better understanding of refuge use by reptiles offers potential advantages for both monitoring and conservation management.

As a component of a long-term monitoring programme for the northern viper (*Vipera berus*) on chalk downland in Kent (UK), we deployed both tin and felt refuges (Hodges & Seabrook, 2016a). When the vipers attempt to attain optimal body temperatures (T_b) they may bask openly in sunshine and/or use refuges; in this chalk downland reserve vipers were much more commonly found under tins than felt refuges (Hodges & Seabrook, 2016b). Gross control of body temperature may be achieved by shuttling between shade and sunshine, or by careful positioning between the two when partially concealed by vegetation ('mosaic basking'). Fine control is achieved by changing both body orientation to the sun and body posture; *V. berus* has been described as a posturing heliotherm (Spellerberg, 1976). The upper thermal set point ($T_{set\ upper}$) of *V. berus*, effectively the body temperature that is optimal for current physiological requirements, has been determined in a thermal gradient and ranges from 31.7°-33.8°C (Herczeg et al., 2007); data from other authors is consistent with this (Saint Girons, 1975, 1978; Spellerberg, 1976; Vanner, 1990; Gaywood, 1990; Gaywood & Spellerberg, 1995). Vipers at spring emergence, during digestion of meals, or pre-moult spend longer periods basking and control their T_b within a narrow range, this is referred to as K-thermoregulation. Alternatively, vipers engaging in extensive foraging enter cooler areas resulting in a more variable T_b, referred to as r-thermoregulation. It is suggested that there is a continuous range between r- and K-thermoregulation determined by physiological state (Vanner, 1990). Physiological state is also important as slightly higher temperatures were reported from vipers digesting large meals (Saint Girons, 1978) and on average gravid females selected higher temperatures (Lourdais et al., 2013). Life stage may also affect the selected body temperature although to date almost all studies of *V. berus* thermal ecology have focused on adults. However, when juvenile *V. berus* were presented with a temperature gradient in springtime their selected temperature was on average only 28°C, which was 5°C cooler than adults (Herczeg et al., 2007).

The circumstances under which *V. berus* selects, remains under, and leaves tins are poorly known; this limits the interpretation of monitoring data. Furthermore, the potential role of refuges in the conservation management of *V. berus* appears not to have been considered in any detail. Consequently, in 2014 we initiated investigations into the use of tins in relation to the thermal ecology of *V. berus*. These included measurement of viper body temperatures (T_b) and of physical models that estimate the operative temperatures (T_e) of associated microhabitats.

MATERIALS AND METHODS

Study site and refuges

Details of the study site and refuges are presented in Hodges& Seabrook (2016a). In brief, the investigation was part of a long-term monitoring study on a chalk downland nature reserve at about 51°N, 0°E, with a total open area of 11.1ha. The study was confined to 2014 and included observation of vipers under 46 pairs of refuges of galvanised corrugated-iron sheets (‘tins’) and roofing felt, deployed at a density of about 4 pairs/ha. The ground below refuges lacked vegetation.

The site was visited 79 times for many hours from March to October. Observations were made morning and afternoon on days when weather conditions were not excessively wet or windy. A standard route was followed between refuge locations. Photographs were taken of viper head-scale patterns; these were coded then entered into a database to facilitate individual recognition (Benson, 1999). Adult recruitment tables suggested that on average there were about 4-5 adult vipers/ha in 2014. Life stages were defined as before (Hodges& Seabrook, 2016a) and observations disaggregated by gender except for juveniles. The study involved no animal handling in order to minimise disturbance and stress.

Physical models

Operative temperatures (T_e) were estimated using physical models (Peterson et al., 1993; Shine & Kearney, 2001). These consisted of copper pipe (ID 20mm, wall 1mm thick, length 150mm) flattened so that about 40% of surface was in contact with the substrate beneath, sprayed with grey paint (Surface primer, matt, Rust-oleum), and sealed at either end with silicon sealant and fixed into the ground towards each end with an overlapping strand of wire. The models indicated the temperatures of microhabitats available to vipers and served as null models for quantifying the extent of thermoregulation. Twenty one tins had models beneath them. In addition, at five widely spaced refuge positions each tin had two other closely located models, one exposed to direct sunlight and one in the permanent shade of taller vegetation.

Temperature measurement

Infrared thermometer guns (Foxnovo DT8380) were used to collect surface temperatures of vipers, physical models and tin refuges. These thermometers measure in the range -50˚C to +380˚C, have a distance to spot ratio of 8:1, and a resolution of 0.1˚C. Different units, when measuring the same surface temperature, gave reading that varied by <0.3˚C. A clear plastic tube, 1.8 cm long and 1.8 cm wide, was fixed to the front of each thermometer to act as spacer. To make measurements, the spacer was brought to almost touch the upper surface at the middle of a viper, or in the case of small specimens the centre of the coiled body, ensuring that only the animal was included within the measurement. The recorded temperature was of the dorsal body surface and may be different from internal or ventral surface temperatures. In nearly all cases, measurements were made of vipers that were individually distinguishable using their head-scale patterns.

Calibrations were prepared for the various subjects of temperature measurement. A digital thermometer (6802II) with thermocouples was calibrated against a laboratory certified calibration thermometer at temperatures ranging from 0˚C to 50˚C. The thermocouples were placed inside the cadaver of an adult male viper and inside a physical model. These were located on a brewing heat mat. As temperatures rose readings were taken at regular intervals with the IR thermometer from 10˚ to 40˚C and calibration curves prepared for the model and the snake. A calibration was also constructed for corrugated iron refuges by taping thermocouples to the under surface of a refuge, exposing it to sun light, and taking IR thermometer readings as the refuge warmed from 15˚ to 55˚C.

Where possible, temperature measurements were taken from vipers both under and away from tins. Occasionally, multiple temperature measurements of the same viper beneath tins were made on the same day and when these were made on consistently sunny days it was possible to use the measures to estimate the rate at which vipers were warming up under the tins. All times of temperature measurement are quoted as Greenwich Mean Time.

Statistical analysis

Differences between viper life stages in numbers above and below T_e were evaluated for statistical significance using χ^2 tests (Siegal, 1956). The statistical significance of simple linear correlation coefficients (r) was determined from standard tables (Bailey, 1966). Differences were treated as statistically significant when the probability of them occurring by chance was 5% or less ($p \leq 0.05$).

RESULTS

Operative temperatures (T_e)

In March, the models located in direct sunlight were warmer than those in the shade or under tins by an average of about 7˚C (Fig. 1), the models in the shade were about 0.8˚C warmer than those under tins (Fig. 1). As shade temperatures and those under tins were similar, there can have been little or no incentive for vipers to rest under tins. This is confirmed by measurements in March of 11 males basking in sunshine. When compared with the temperatures of models under the closest tins, the viper body temperatures (T_b) were on average 8.6˚± 5.6˚C warmer than the models. Likewise the models in sunlight were warmer than those under tins by an average of 6.7˚ ± 5.2˚C.

From May to August temperature records from models under tins were higher than those in shade by 6.0˚ to 7.5˚C (Fig. 1). In the autumn, the temperatures under tins began to decline and the difference from models in the shade started to narrow in September to about 3.0˚C and then in October to only 2˚C (Fig. 1).

Taking one of these five refuge locations as an example, it can be seen that, as expected, the model exposed to direct sunshine was generally warmer than the model on the ground beneath the tin (Fig. 2) which in turn was generally warmer than the model in the shade.

Viper body temperatures (T_b)

The T_b of roughly similar numbers of known individual vipers of each life stage were recorded (7-10 individuals, Table 1); however adult females, especially those that were gravid, were measured more frequently resulting in about 50% more recordings (63) than average (40). Juveniles were seen less frequently with about 50% fewer records (21) than average.

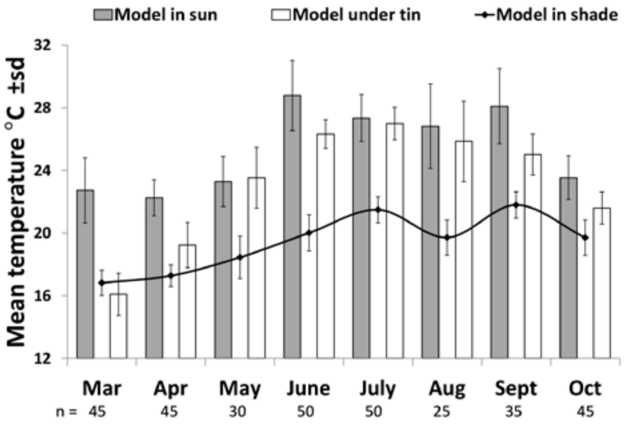

Figure 1. Mean (±SD) monthly temperatures ˚C of variously located physical models at five locations on chalk downland in 2014 in the period 09.00h to 12.00h (n = number of observations per bar)

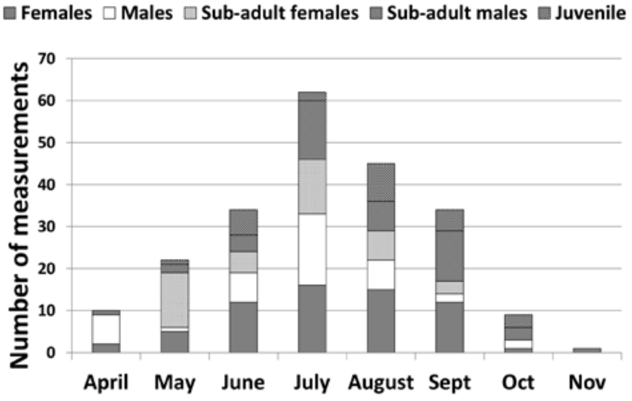

Figure 3. The frequency distribution of body-surface temperature measurements of different life stages according to months of the year in 2014.

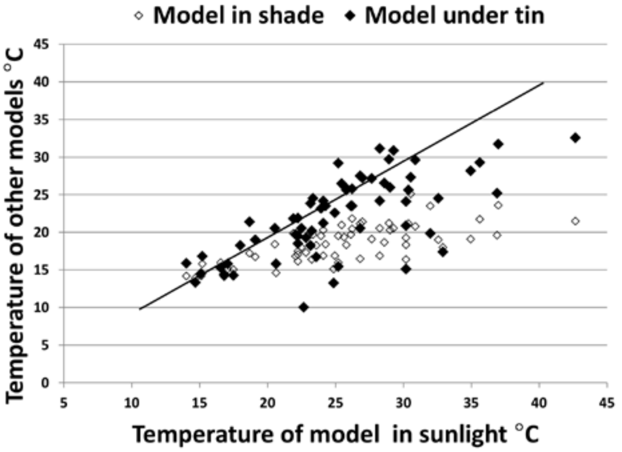

Figure 2. Relationship between the temperatures of the physical model in sunshine and the corresponding temperatures of models under tins or shaded by vegetation. Records are from March to October and made between 08.00h and 17.00h (N = 64). Above the solid line other models are warmer than the model in sunshine.

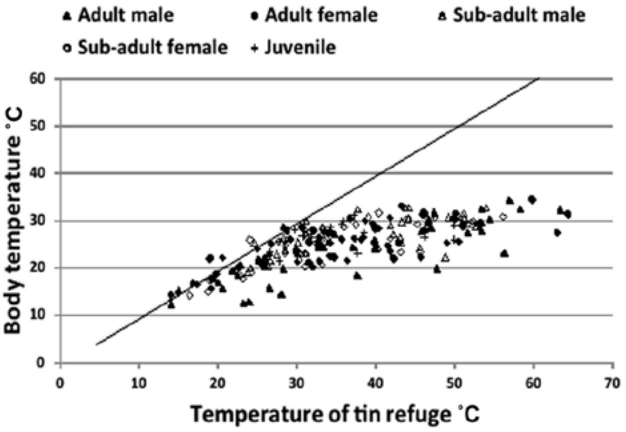

Figure 4. Relationship between the temperature of tin refuges and the body-surface temperatures of *V. berus* (T_b) of all life stage found beneath tin refuges (N = 211). Above the solid line, viper T_b was warmer than the corresponding refuge tin.

Table 1. Number and identity of individual vipers from each life stage for which temperature measurements were made while they were under tins in 2014 and the total numbers of temperature records for each life stage (n).

Male	n	Female	n	Sub-adult male	n	Sub-adult female	n	Juv	n
M39	23	F50	16	SM?	1	SF?	1	J162	2
M52	7	F53	14	SM33	7	SF40	2	J174	3
M53	2	F59	3	SM38	16	SF41	1	J176	2
M69	2	F60	2	SM43	6	SF54	17	J179	2
M85	2	F61	4	SM44	8	SF65	3	J191	1
M87	5	F64	1	SM45	5	SF69	5	J192	1
M88	1	F81	13	SM51	1	SF71	1	J194	4
		F84	1			SF73	2	J197	5
		F92	9			SF75	8	J?	1
						SF79	1		
7	**42**	**9**	**63**	**7**	**44**	**10**	**41**	**9**	**21**

The temperature measurements for each life stage were distributed over the period April to October (Fig. 3); there was just a single observation in November.

When in full sunshine, the tins reached quite high operational temperatures; up to 65˚C. However, no viper life stages below these refuges were observed with body temperatures (T_b) more than 34.4˚C (Fig. 4).

Viper T_b frequently exceeded T_e beneath the tin (Fig. 5) and was recorded as high as 34.4˚C (Fig. 4). However, when models under tins reached a T_e of 32.9˚C or above all corresponding viper temperatures were at or below T_e (Fig. 5). This gives an approximation of $T_{set\ upper}$. The T_e under tins did not exceed 38˚C and only 8% of measures exceeded 32.9˚C.

For both adult and sub-adult males the majority of T_b measures were below T_e (Table 3), adult and sub-adult females were more evenly distributed between hotter and colder, while juveniles were on average mostly hotter than

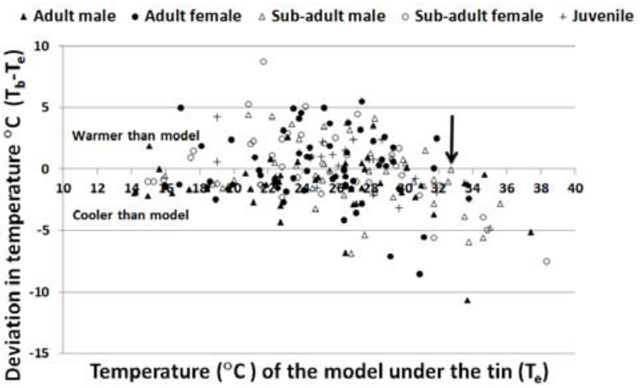

Figure 5. Deviation of *V. berus* body-surface temperature (T_b) from the temperature of the model on bare ground beneath the tin refuge (T_e). The arrow indicates the maximum T_e value for which there was an equally high viper T_b, to the right of the arrow all T_b values are lower than T_e.

Table 3. Numbers of records of body temperature (T_b) of each life stage under tin refuges that were warmer or cooler than the corresponding physical model (T_e), values with no letter in common are significantly different (p<0.05)

	Males		Females		Juveniles
	Adult	Sub-adult	Adult	Sub-adult	
n=	42	44	63	41	21
Warmer than model	10	16	29	26	12
Cooler than model	32	28	34	15	9
% warmer	23.8% a	36.4% abc	46.0% bc	51.0% c	57.9% c

T_e (Table 3). There was significant heterogeneity between life stages in the numbers of individuals that were warmer or cooler than the model under the tin (χ^2 = 15.83, df = 4, p<0.01). Pairwise comparisons suggest statistically significant differences between adult males and females, sub-adult males and females, while only adult males were significantly different from juveniles (Table 3).

On most days, individual vipers had T_b measured only once. Occasionally, a viper would remain under the tin for an extended period and this gave the opportunity for a sequence of measurements that allowed daily fluctuations to be observed. The best case of this was a sub-adult female on the 1st July (Fig. 6). Initially, at 08.00h, the refuge was in partial shade. At this time the tin and model under the tin were just a little warmer than the viper, while the model exposed to direct sunlight was already some 6˚C warmer (Fig. 6). In the following 25 minutes the tin became exposed to full sunshine so that its temperature rose by about 15˚C, making it about 5˚C hotter than the model in sunshine. Below the tin this resulted in an increase of 3˚C of both the viper and model. There followed a sunny period of 65 minutes that heated the tin to about 52˚C, making it about 15˚C hotter than the model in sunshine. Below the tin the viper and model both reached about 30˚C. Later in the day (16.00h) the temperature of both the tin and model in sunshine fell quite sharply. However, below the tin there was a lag in the temperature fall of model and viper. Interestingly, the lag in temperature decline of the viper was greater than the model giving the viper a 1˚C temperature advantage at 16.00h and, 55 minutes later, an advantage of just over 2˚C, presumably due to active thermoregulation by the viper and/or slightly greater thermal inertia. The observed temperature of the model placed in the shade of vegetation was very stable over the whole period of observation, fluctuating close to 20˚C (Fig. 6).

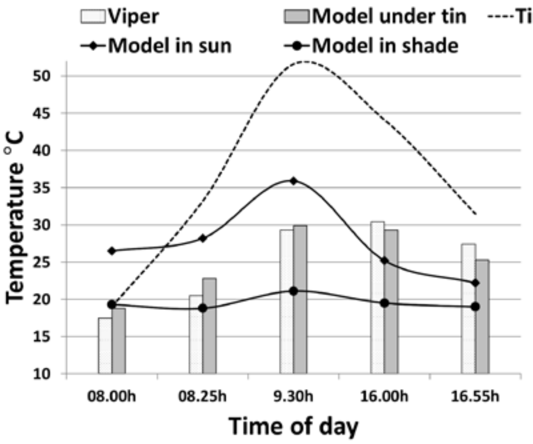

Figure 6. Body surface temperatures of a sub-adult female measured on five occasions on 1st July with corresponding temperatures of the tin under which it rested and physical models in the sun, in the shade or beneath the tin (note x-axis scale is not even)

From April to September, there were 18 occasions when the same vipers had more than one body temperature measurement on the same sunny day (adult males - 5, adult females - 3, sub-adult females - 8, sub-adult males 1, juvenile - 1). The temperature readings were on average 70±34 minutes apart and from this data the estimated heating rates ranged from 0.05˚C to 0.14˚C/min with an average of 0.08˚C±0.03˚/min.

DISCUSSION

The quickest way for vipers to warm themselves would appear to be to bask directly in sunlight rather than resting under tin refuges. This was demonstrated very clearly by temperatures of adult males in the open in March 2014 that were 8.6˚±5.6˚C warmer than the models under tins. Typically, males are very rarely found under refuges in the spring time prior to their first moult. At this time of year the operational temperature (T_e) indicated by physical models under tins was little different from that in the shade of vegetation suggesting that males are unlikely to select positions under tins as they would obtain little or no thermal advantage (Fig. 1). The months in which tin use by vipers are likely to be most favourable are when the models under tins are consistently warmer than in the shade, in this case May through to September (i.e. those months where the error bars of the models in the shade and under the tin show no overlap in Fig. 1). However, it seems likely that even under warm conditions the heating rates of vipers under tins are much lower than those in direct sunlight. In the summer, heating rates of vipers in the open of 0.48° - 0.58°C/min have been recorded (Vanner, 1990) which are about x7 greater than the average of 0.08°C/min (range 0.05° to 0.14°C/min) observed in the current study. This is understandable as tins would be expected to reflect a significant proportion of solar radiation and radiate the remainder as much to the air above as to the ground below the tin; in effect the tins act as screens against rapid temperature rise.

When in full sunshine, tins were heated to as much as 65°C and model beneath tins to 38°C. However, no viper life stages below these refuges were observed with body temperature (T_b) exceeding 34.4°C (Fig. 4). This implies active thermoregulation by moving to the coolest location below the tin, adopting postures and orientations that limit heat uptake and then, if necessary, leaving the tins for cooler places. The maximum T_b of 34.4°C is a little higher than the expected upper thermal set point ($T_{set\ upper}$). It seems that vipers sometimes overshoot the preferred maximum and Gaywood (1990) recorded a maximum T_b of 40.4°C. However, in this study at higher T_e the viper T_b became more constrained until T_e 32.9°C when all viper T_b measurements were lower than T_e (Fig. 5). It would appear that 32.9°C is a convenient and practical estimate of $T_{set\ upper}$ that fits within the range already suggested for this parameter, 31.7°-33.8°C (Herczeg et al., 2007). It may be more than a coincidence that observations on *V. berus* during mosaic basking, in thermally unlimiting conditions, returned an average T_b of 32.8±3.4°C (Gaywood, 1990). The use of a refuge, such as a tin, that warms in the sunshine might reasonably be considered to be a variant of the more natural mosaic basking in providing warmth and cover simultaneously.

The majority of males, both adult and sub-adult, under tins were cooler than the T_e indicated by the corresponding model. Adult and sub-adult females were more or less evenly distributed above and below T_e, and juveniles were on average mostly hotter than T_e (Table 3). This suggests that the behaviour of the vipers in relation to refuges may differ, for examples if males are more inclined than other stages to leave refuges once $T_{set\ upper}$ is reached then those males observed would be more likely to be below T_e. Likewise if females are more inclined to remain under refuges when at or approaching $T_{set\ upper}$ then recorded temperature would be inclined to be higher. No evidence was found to support the earlier records of juveniles selecting lower $T_{set\ upper}$ than adults (Herczeg et al., 2007). However the original study referred only to springtime and as the immature stages emerge later from hibernation than adults then the lower temperature preference may well be a reflection of a time of year when the juveniles are still in a physiological transition that the adults have completed.

There is evidence to suggest that the thermal performance of tin refuges is relatively poor in relation to the needs of vipers. The low ground temperature below tins early in the season may account for the rarity of tin use by male vipers that emerge first from hibernation. Also the very slow warming rate of vipers below tins suggests that there are significant advantages in basking in direct sunshine, despite the obvious risks from predation. If the thermal properties of refuge tins were improved then vipers may gain increased benefits from them. This has been investigated by placing insulation mats beneath tins and is reported elsewhere (Hodges & Seabrook, 2016b).

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RESEARCH ARTICLE The Herpetological Bulletin 137, 2016: 19-23

Use of artificial refuges by the northern viper *Vipera berus* - 3. An experimental improvement to the thermal properties of refuges

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ABSTRACT - To increase the use of galvanised corrugated-iron refuges (‘tins’) by northern vipers (*Vipera berus*) in a chalk downland reserve, insulation mats were placed on half of the bare ground area below the tins. During the day time the operative temperatures (T_e) on the mats, demonstrated by physical models, averaged 2-4˚C warmer than bare ground under the same refuges but ranged as much as 10˚C warmer. Vipers were found more frequently under insulated than uninsulated tins and, when under these tins, were more likely to be on the mat than on the bare ground. Adult males on mats in March/early April had body temperatures (T_b) 9.4˚-15.8˚C higher than T_e (which at this time of year is particularly low). At other times adult males, females and juveniles were on average about 1 to 2˚C warmer but, surprisingly, sub-adults showed no temperature difference. Insulation mats, by enabling the vipers to maintain higher T_b, should improve their physiological performance and if they reduce open basking and the incentive to search for warmer positions then they could reduce exposure to avian predators. The inclusion of an insulation mat as a component of an improved refuge design is now under investigation.

INTRODUCTION

Galvanised corrugated-iron refuges (‘tins’) are frequently used for monitoring reptiles in the UK and elsewhere (Reading 1997; Froglife 1999) and their use by the northern vipers (*Vipera berus*) has been described recently (Hodges & Seabrook, 2016a). They have the advantage of providing locations where there is both warmth and cover, perhaps helping vipers remain concealed and closer to their upper thermal set point ($T_{set\ upper}$), estimated as 32.9˚C (Hodges & Seabrook, 2016b). However, previous study has demonstrated some shortcomings in the thermal performance of these refuges (Hodges & Seabrook, 2016b). In particular, the bodies of vipers beneath tins warm much more slowly than in direct sunshine and that early in the reptile active season the low operative temperatures (T_e) below tin refuges would discourage their use. These disadvantages are expected to reduce viper observation rates at refuges and consequently the value of refuges for monitoring. Their value in conservation may also be reduced since the animals may be more inclined to leave refuge cover periodically to bask in the open to achieve preferred body temperatures and so increase the risk of predation.

In an effort to improve the thermal performance of tin refuges for *V. berus*, we undertook an investigation of the potential benefits of placing an insulation mat on the ground below tins in a 7-month study in a chalk downland reserve.

MATERIALS AND METHODS

Study site and refuges

Details of the study site and refuges are presented in Hodges & Seabrook (2016a). In brief, the investigation was part of a long-term monitoring programme at a chalk downland nature reserve at about 51˚N, 0˚E, with a total open area of 11.1ha. Refuges consisted of galvanised corrugated-iron sheets (‘tins’) (0.5mm thick and 0.5g/cm2) cut to 50cm by 65cm, and were camouflaged by spraying their upper surface with brown paint (Espresso, satin finish, Rust-oleum). These were placed in sunny but inconspicuous locations backed by vegetation cover. Observations of vipers were made under 46 tins, deployed at a density of about 4/ha; the ground below refuges lacked vegetation.

The study was undertaken in 2015 when the site was visited 77 times for many hours from March to October. Observations were made morning and afternoon on days when weather conditions were not excessively wet or windy. A standard route was followed between refuge locations. Photographs were taken of viper head-scale patterns; these were coded then entered into a database to facilitate individual recognition (Benson, 1999). Adult recruitment tables constructed for the two sites suggested that there were about 3-4 adult vipers/ha in 2015. Life stages were defined as before (Hodges & Seabrook, 2016a) and observations disaggregated by gender except for juveniles. The study involved no animal handling in order to minimise disturbance and stress.

Physical models and temperature measurement

The use of physical models (Fig. 1) to determine operative temperatures (T_e) and infra-red thermometers (Foxnovo DT8380) to measure viper body surface and model temperatures were the same as described in Hodges & Seabrook (2016b). Where possible, temperature measurements were taken from vipers both under and away from tins. The physical models consisted of copper pipe

(ID 20mm, wall 1mm thick, length 150mm) flattened so that about 40% of surface was in contact with the substrate beneath, sprayed with grey paint (Surface primer, matt, Rust-oleum), sealed at either ends with silicon sealant and fixed into the ground with two overlapping strands of wire. A total of 22 tins had models on the ground beneath them and seven also had models on the insulation mat. These seven tins also had two other closely located models, one exposed to direct sunlight and one in the permanent shade of taller vegetation. The temperatures of all models were recorded routinely using an infra-red thermometer at least five times a month in the period 09.00h to 13.00h. Additionally, temperature measurements of vipers under tins also included measurement of any corresponding models. All times given for temperature measurement are Greenwich Mean Time (GMT).

Figure 1. Adult male *V. berus*, below a tin refuge (removed) and resting on an insulation mat. Photograph taken just after body temperature measured; on the left hand side is a physical model for determination of operational temperature.

Temperature loggers (Gemini TK-4014, TinyTag Talk 2, accuracy ±0.4˚C) were used to compare temperatures under tins (on bare ground and on the insulation mat) and elsewhere in direct sunlight and in shade of vegetation. They were used over the course of a day and set to read at 10 minute intervals. The loggers were placed in aluminium cylinders (10cm by 4cm), painted with the same grey undercoat as the physical models, and flattened on one side to increase contact with the ground. The cylinders were held in place using metal skewers and under the tin positioned beneath one of the convex corrugations but not touching the tin.

Below tin insulation

Insulation mats (Fig. 1) measured 30cm x 50cm and consequently covered one half of the ground beneath a tin, the other half was left as bare ground. The mats were prepared from two layers of bubble foil plastic insulation (Baird double aluminium bubble foil insulation 200g/m2 and RSI = 2.93) held together by parcel tape and sprayed above with brown paint for camouflage (Espresso, satin finish, Rust-oleum). The insulation mats were placed alternately under 23 refuges (referred to as insulated tins), leaving 23 without mats (referred to as uninsulated tins).

In this way two different comparisons can be made. First, the numbers of vipers recorded under insulated tins can be compared with those under uninsulated tins (are refuge positions with mats favoured over those without?). Second, for insulated tins the numbers of vipers resting on mats can be compared with those resting on the bare ground (are mats preferred to the ground?).

Statistical analysis

Differences between numbers of vipers using insulated and uninsulated tins and using or not using mats under insulated tins were tested for statistical significance by the Mann Whitney U test and the heterogeneity of body temperature measures of the different viper life stages was examined using Kruskal-Wallis one way analysis of variance (Siegal, 1956). The statistical significance of simple linear correlation coefficients (r) was determined from standard tables (Bailey, 1966). Differences between the numbers of viper life stages on mats and the on bare ground under insulated tins were evaluated for statistical significance using χ^2 tests (Siegal, 1956). Differences were treated as statistically significant when the probability of them occurring by chance was 5% or less ($p \leq 0.05$).

RESULTS

Observations on the T_e of insulated tins

During each month of the study, temperature measurements from 09.00h to 13.00h showed that models on insulation mats were on average always warmer than those on bare ground (Fig. 2). Models on mats were on average 4.3˚C warmer; their greatest mean temperature advantage was in June (5.7˚C) and least in October (3.3˚C). It was noticeable that in March there was little difference in the temperature between models on bare ground under tins or in the shade of vegetation but thereafter the models on the bare ground became distinctly warmer (Fig. 2).

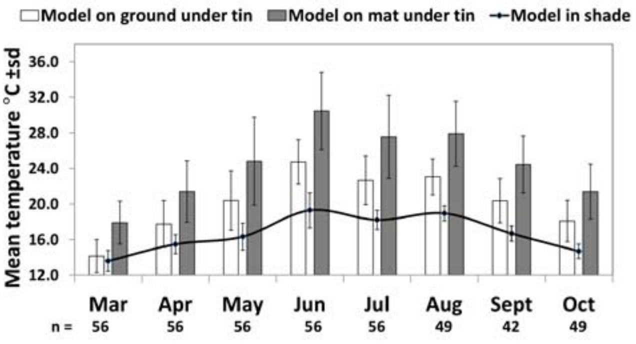

Figure 2. Mean (±sd) monthly temperatures of physical models at seven locations to show differences between operational temperatures under a tin on bare ground or on an insulation mat, or in the shade of vegetation, in the period March to October 2015 (n = number of observations per bar)

As the temperatures of tins increased so the temperature difference between models on the mats and adjacent bare ground became greater (Fig. 3); there was a significant positive correlation between the temperature of the tins and the magnitude of this difference ($r = 0.64$, $df = 54$, $p<0.001$). This relationship was to be expected since rapid rises in temperature of the tin would be expected to have more impact on the model on the mat, which is insulated from the cooling effect of the ground, than on the model on the bare ground.

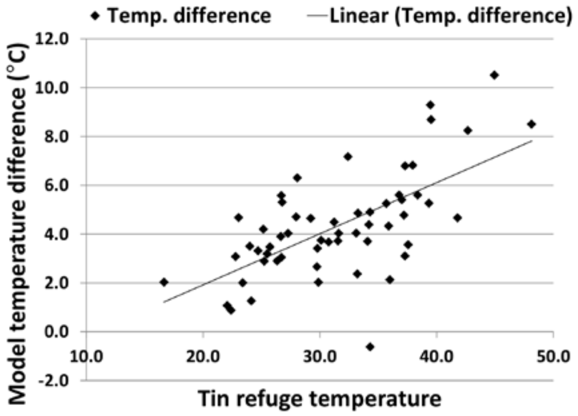

Figure 3. Difference in temperature between the physical models on the mats or adjacent bare ground plotted against the corresponding temperature of tin refuge they were beneath. Assessed 09.00h to 13.00h from March to October (N=56)

For each of the 8 months of the study (March to October), in the period 05.00h to 18.00h the mean temperature differences between mats and the bare ground under the same tins, recorded by loggers, were -0.5°C to just above +4°C (Fig. 4) and from 08.00h to 16.00h were 2°C to 4°C. There were also wide ranging maximum and minimum values (Fig. 4). Negative values, when the insulation mat was actually cooler than the bare ground, occurred from about 17.00h to 09.00h. This appears to result from sharp night time falls in air temperature. In this situation loggers on mats were not buffered by the warmer ground below and so registered lower temperatures.

One possible disadvantage of the insulation mats is that they could heat to temperatures above upper thermal set point ($T_{set\ upper}$) of the vipers (in this case taken as 32.9°C), thus forcing them to seek cooler locations. Inspection of the temperature records from the loggers shows this to be the case for periods of one to nearly five hours in five of the eight months (Table 1). However, only once was $T_{set\ upper}$ exceeded on the adjacent bare ground below the tin and this was for only 2 hours, yielding a very low degrees*minutes product (Table 1). Consequently, it would be expected that vipers frequently have the opportunity to move from the mat onto the bare ground below the tin to enable them to remain within their preferred temperature range.

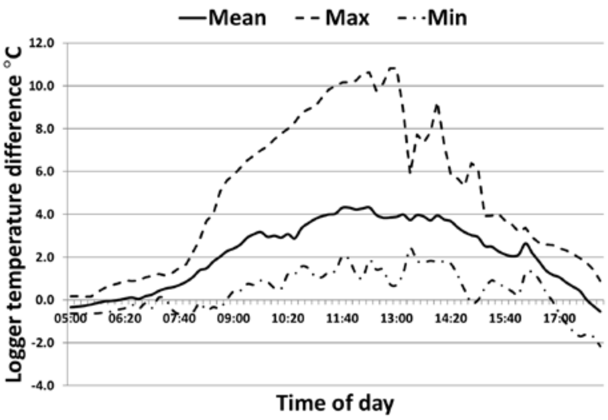

Figure 4. Mean and range of differences between temperature records of loggers under tins placed on mats or on the ground, recording at 10 minute intervals one day each month from March to October

Table 1. Number of minutes, temperature range, and degree*minutes, above viper $T_{set\ upper}$ (32.9°C) recorded over one day (05.00h to 18.00h) from March to October by temperature loggers placed beneath tin refuges either on an insulation mat or on bare ground.

Month	On mat			On bare ground		
	Mins above	Degree range above	Degrees* mins. above	Mins above	Degree range above	Degrees* mins. above
March	0	0	0	0	0	0
April	290	0.17 - 4.29	613	120	0.03 - 0.91	61.12
May	10	0.22	2	0	0	0
June	290	0.4 - 4.46	731	0	0	0
July	60	0.48 - 4.36	169	0	0	0
August	0	0	0	0	0	0
Sept	170	0.21 - 4.4	471	0	0	0
Oct	0	0	0	0	0	0

Observations of vipers under insulated and uninsulated tins

There were significantly more vipers (Mann Whitney U = 3, n = 7, p = 0.009) recorded at insulated than uninsulated tins (Table 2, a v. b). Vipers were observed at a total of 12 locations under insulated tins and 9 locations under uninsulated tins (i.e. 21 out of the 46 refuge locations were in use by vipers). When vipers were found under insulated tins, twice as many were resting on the insulation mat as on the bare ground (Table 2, c v. d), a statistically significant difference (Mann Whitney U = 10.5, n = 7, p = 0.049).

The majority of observations of adults and juveniles were at insulated tins while the sub-adults (comprising mostly males) were evenly divided between the two (Table

Table 2. Number of *V. berus* under insulated and uninsulated tins, and for insulated tins the numbers of observations of vipers on or off the insulation mats.

Month	No. viper records			
	Under insulated tins (a)	Under insulated tins (b)	Under insulated tins on mat (c)	off mat (d)
March	3	2	3	0
April	12	7	9	3
May	20	5	14	6
June	18	8	10	8
July	11	8	6	4
August	16	6	12	4
Sept	8	2	4	4
Totals	**88**	**38**	**58**	**29**

3). There was significant heterogeneity between the numerical values for the numbers of vipers using the mat or using the ground below insulated tins ($\chi^2 = 25.88$, df = 3, $p<0.001$). Significantly greater numbers of adults were observed on mats than immatures, while adults themselves differed with significantly more males than adult females, while the sub-adults and juveniles were not significantly different from each other.

Table 3. Numbers of records of vipers, by life stage, under insulated tins or uninsulated tins and numbers resting on the insulation mat (not including repeated observations on the same day). Values not followed by the same letter are statistically significant (χ^2 $p<0.05$).

	Adult males	Adult females	Sub-adults	Juveniles
Uninsulated tins	1	11	22	4
Insulated tins	21	31	22	16
% at insulated tins	95.5%	73.8%	50.0%	77.8%
- of which on mats	21	23	9	5
- of which on ground	0	8	13	11
% on insulation mats	100% a	74.2% b	40.9% c	35.7% c

The T_b of all viper life stages was on average warmer than T_e, whether under insulated or uninsulated tins (Table 4). There were apparent differences in viper T_b between insulated and uninsulated tins; adult males had an average 4˚C advantage under insulated tins, adult females 1.8˚C, juveniles 1˚C but sub-adults were on average 0.7˚C cooler (Table 4). The small number of observations under uninsulated tins precludes statistical comparisons with T_b under insulated tins. However, when taken together, the life stages under insulated tins did not show any statistical heterogeneity so further statistical testing was not justified (Kruskal Wallis, H = 1.4, df = 3, p = 0.7). In the case of the adult males the strong advantage under insulted tins resulted from eight high readings (9.4° to 15.8˚C of difference) in late March early April. If these eight readings for males are removed from the estimate then the mean difference falls to +1.2˚C±3.0˚, comparable to adult males under uninsulated tins. The large differences in March and April are explained by the particularly low T_e at this time when only males were active, and when only insulated tins were selected. At low T_e the potential difference between T_e and $T_{set\ upper}$ is large. However, as the season progressed other life stages started to emerge and use tins but the prevailing T_e rose so narrowing the range from T_e to $T_{set\ upper}$, and reducing the size of any potential differences.

Table 4. Mean (±sd) and median temperature deviation ˚C of *V. berus* body temperature (T_b) from models on bare ground under tins (T_e), for uninsulated and uninsulated tins (n = number of temperature measurements including some repeat measures on the same day).

		Adult males	Adult females	Sub-adults[1]	Juveniles
Uninsulated tins	Mean±sd	+1.4±1.6	+0.4±2.5	+2.2±3.1	+1.1±2.4
	Median	-	-0.4	+0.76	+0.5
	n	2	15	21	3
Insulated tins	Mean±sd	+5.4±6.7	+2.2±3.7	+1.5±3.3	+2.1±3.6
	Median	+3.6	+2.5	+0.92	+2.04
	n	24	15	26	16

[1] Sub-adult females - 2 under uninsulated tins, 4 under insulated tins

DISCUSSION

The potential for low ground temperatures to limit refuge use suggested that an insulating layer placed between the refuge and ground could offer a thermally more beneficial environment (Hodges and Seabrook, 2016b). More vipers were recorded at insulted tins, which had insulation mats covering half the area below them, than under uninsulated tins. Furthermore, under insulated tins greater numbers of vipers were observed resting on the insulation mat than on the adjacent bare ground. Adult vipers particularly favoured insulated tins, sub-adults were equally divided between insulated and uninsulated tins, while juveniles, although more frequent under insulated tins, were at insufficient numbers to draw firm conclusions. Presumably the higher temperatures offered by the insulation mat either attracted the vipers, especially the adults, to move under the insulated tins and/or resulted in them remaining under these tins for longer.

The adult males emerge from hibernation early in the year and observation in March/early April showed individuals with body temperature (T_b) 9.4˚-15.8˚C warmer than the operational temperature (T_e) demonstrated by models on bare ground. Later in the year when T_e was higher, adult males, females and juveniles were on average about 1 to 2˚C warmer when under insulated tins but, surprisingly, sub-adults showed no temperature difference. These values provide some circumstantial evidence of the thermal benefit of insulation mats for males, females and juveniles. The absence of any observed benefits for sub-adults is perplexing but may be explained by the fact that under insulated tins they were less often seen on mats than the other stages (Table 4). We do not know if this should be interpreted as them using the mats less frequently or that they heat up more quickly and so spend less time on mats. Unfortunately, in 2015 there were insufficient multiple temperature measurements of the same viper on the same day to draw any conclusions about the heating rate of vipers on insulation mats. However, simple logic would suggest it should be at a higher rate than for those vipers resting on the ground.

It might have been predicted that the thermal advantages of the insulation mat would largely be restricted to times when ground temperatures were low and there was plenty of sunshine, i.e. during the early months of the year.

Surprisingly, the insulated tins actually contributed greater numbers of viper observations throughout the period March to September. With the exception of September, under insulated tins the numbers of vipers on mats were greater than on bare ground (Table 2). This suggests that the climatic conditions in 2015 favoured the use of the insulation mat for most of the year. Nevertheless, temperature loggers placed on insulation mats did exceed the viper $T_{\text{set upper}}$ quite frequently and for extended periods. However, bare ground adjacent to the mat beneath the tin rarely exceeded $T_{\text{set upper}}$ and so vipers may have moved onto cooler ground to remain within their preferred temperature range. This is supported by observations in 2014 when models on the ground under tins were never observed to exceed a T_e of 38˚C and only 8% exceeded an upper set point of 32.9˚C (Hodges & Seabrook, 2016b). However, it is possible that insulated refuges might be less useful in years with particularly hot summers or in exceptionally dull years, or months, when there would be little difference between resting on the ground or on a mat.

It seems likely that insulation mats enable vipers to achieve body temperatures closer to $T_{\text{set upper}}$ more quickly and for longer periods. This would optimise the rate of physiological processes and may also encourage vipers (especially adults) to remain under for longer periods, not least because the closer vipers are to $T_{\text{set upper}}$ the lower will be the incentive for them to leave the refuge to find a warmer position. This could help to reduce predation especially from birds. Both slow worms and common lizards were also frequently seen resting on the mats and it is possible they receive similar benefits. It seems unlikely that the use of insulation mats would find a place in short-term presence/absence refuge surveys, but for long-term surveys linked to conservation they may offer benefits for both recording and to the vipers themselves. Further investigations are in progress to include an insulation mat as a component of a refuge design that offers a wide range of thermal options so that vipers, and possibly other species, can gain maximum benefit from refuge use.

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RESEARCH ARTICLE

The Herpetological Bulletin 137, 2016: 24-27

Successful reproduction in *Paramesotriton chinensis* after more than a decade of reproductive inactivity, with observation of parental care

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ABSTRACT - Amphibians may be held in captivity without successful reproduction, due to the absence of environmental cues, for many years. However, there are few data concerning the potential for amphibians to recover reproductive capacity after such hiatuses or their impact on fecundity and viability. In one male and two female *Paramesotrition chinensis* newts, I report successful reproduction including previously unreported oviposition site guarding behaviour, but very low rates of viability, over two breeding seasons after more than a decade of aseasonality and reproductive inactivity, following the introduction of appropriate seasonal environmental variation. These findings suggest that reproduction can be achieved in animals that have not reproduced for many years, but that possibly age-related fertility issues may compromise the degree of reproductive recovery.

INTRODUCTION

Many amphibians breed annually in response to regular circannual environmental cues, which may be important in driving hormonal cycles needed to produce gametes and secondary sexual characters over long periods or in triggering hormonal cascades leading to spawning in the short term (Duellman and Trueb, 1986). In order to breed amphibians in captivity, most species require annual environmental cycling relevant to their geographic and microhabitat origin as well as exposure to suitable mating and spawning conditions at the appropriate time in the year. In many species, so-called ‘reproductive triggers’ may be difficult to identify or to replicate and, in these cases, individuals that are otherwise healthy may never reproduce. For this reason, among others, a number of ex situ conservation breeding programmes have failed to breed target species despite holding founder animals for many years (Gagliardo et al., 2008; Michaels et al., 2014; Tapley et al., 2015). There are few data, however, on the impact of long reproductive hiatuses on the reproductive capacity of captive amphibians. Although individuals of many amphibian taxa may live for years or even decades (e.g. Bowler, 1975; Wagner et al., 2011; also see Max-Planck-Gesellschaft, 2002), it is possible that populations of non-breeding adults may be doomed to extinction well before animals themselves senesce due to compromised reproductive ability. This paper describes success, albeit limited, in recovering reproductive capacity in Chinese warty newts (*Paramesotriton chinensis*) after more than a decade of near aseaonality and no reproduction.

METHODS

One male and two female *P. chinensis* were acquired from a private collection in August 2014. *P. chinensis* is a large, robust newt species (see Fig. 1) with a wide distribution in China, where it inhabits stream pools and surrounding broadleaf forest at medium altitude (Gu et al., 2004). Although it is listed as Least Concern by the IUCN (Gu et al., 2004), it is heavily exploited for the pet trade (Rowley et al., 2016) and this combined with habitat loss and degradation, as well as its very slow rate of maturation, means that its population is rapidly declining. In many respects, this species is similar in terms of husbandry and biology to other more range-restricted *Paramesotriton* (Pasmans et al., 2014).

Figure 1. Dorsal aspect of and juvenile *P. chinensis*, the result of the 2014-2015 breeding period, and portrait of an adult female (inset top) and of eggs laid in clusters between leaves of *M. pteropus* (inset, bottom).

The newts had originally been purchased in 2003 from the pet trade, originating in the wild, probably in the vicinity of the animal trading hub of Shanghai, China. However, over the subsequent 11 years the newts were maintained under quasi-aseasonal conditions (i.e. conditions were not completely stable, but the changes throughout the year were very minor – see below) and did not reproduce. During this period the animals were maintained in soft tap water (alkalinity <20 mg/L, pH c. 6.5) at between approximately 14 and 22 °C and with an invariant 12:12 photoperiod.

In the author's collection, newts were housed in a 120x30x30 cm (LxWxH) aquarium lit by T8 fluorescent lamps ('Freshwater lamp', Arcadia), filled to a depth of 28 cm with a 8:2 mix (pH c. 7.5, alkalinity c. 35 mg/L) of reverse osmosis and London tap-water treated with chelating water conditioner (sodium thiosulphate; API) and furnished with stacks of terracotta roof tiles to provide shelter. Potted *Vallisneria* sp. and loose Java fern (*Microsorum pteropus*) were also provided. Life support systems included an external canister filter (EF-2, All Pond Solutions) and an aquarium chiller (DC 750, Deltec). A gentle current replicating the stream-pool habitat of this species was created using an internal pump (Eheim Compact Pump 1000) to drive water through the life support system, but refugia from water flow were available. *P. chinensis* requires several months of temperatures consistently below 10 °C followed by a slow increase in temperature during spring time (reflecting annual variation in temperature in their natural range; World Weather Online, 2016) in order to successfully breed, and this may be facilitated by varying photoperiod according to natural patterns (Pasmans et al., 2014; H. Jansens pers. comm.). Therefore, water temperature and photoperiod were varied to mimic natural seasonality (Fig. 2). Partial water changes of between 10 and 20% were performed weekly during summer, autumn and spring, and every 2-3 weeks during winter. Food, comprising aquatic invertebrates (Chironimidae, *Gammarus pulex*, *Asellus aquaticus*, *Lumbricus terrestris*) and pieces of defrosted freshwater fish (*Oncorhyncus mykiss*; *Rutilus rutilus*) and prawn (*Paenaeus monodon*), was offered weekly, but food intake and activity decreased during the coolest months with animals barely moving and accepting a meal once every 2-3 weeks.

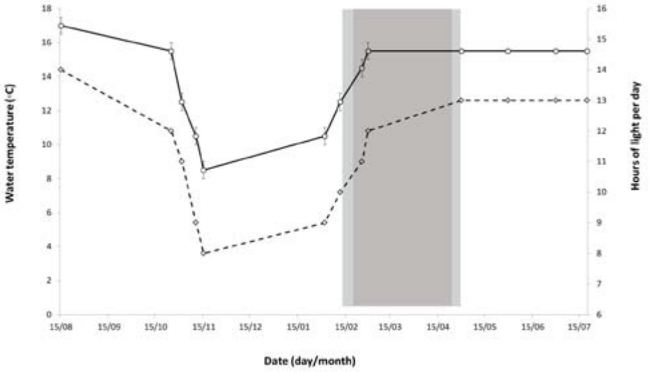

Figure 2. Annual variation in water temperature (continuous line) and photoperiod (broken line) used to house Paramesotriton chinensis; parameters remained constant between 15/04 and 15/08. Error bars represent max-min variation in temperature around the setpoint of the chiller. Oviposition period shown in light (2014-15 season) and dark (2015-16 season) grey blocks.

Eggs produced in the 2014-15 breeding period were removed from the aquarium in batches soon after laying and maintained under identical water parameters at 16-18 °C in separate aquaria to avoid predation by the adults. In 2015-16, when no juveniles were to be reared, eggs were removed from the aquarium on discovery and destroyed by freezing.

RESULTS

In late October in both 2014 and 2015, females began to display pseudo-oviposition behaviour. The cloacae of the females became more triangular and elongated in profile. At the same time, the iridescent blue stripe on the tail of the male intensified and his cloaca became more noticeably swollen, with papillae visible inside the lips. Active courtship (similar to that described in *P. caudopunctatus* by Sparreboom, 1983; Sparreboom, 1986) occurred between early November and early February (2014-15 reproductive period) and between late October and February (2015-16 reproductive period). One of the two females successfully reproduced and laid eggs in 2014-15 and both females reproduced in 2015-16. In 2014-15, the oviposition period lasted from 11/02/2015 to 30/4/2015 (79 days) and in 2015-16 from 19/02/2016 to 25/04/2016 (66 days); see Fig. 2. Between 0 and 7 eggs were produced per day and laying rate was highest at 15-16 °C. Oviposition started at a temperature of 10-11 °C (2014-2015) and 12-13 °C (2015-2016). Almost all eggs were laid in rows between *M. pteropus* leaves (see Fig. 1) in areas of moderate water flow. *M. pteropus* was selected over all other plants in the enclosure, which had different leaf forms. Breeding females became slightly defensive of favoured nesting sites with a 'boundary' of around 5-10 cm radius of the site, attacking the other female if approached and driving her away from the eggs. Defensive behaviour was less vigorous against the male, but he was also kept from approaching oviposition sites.

In 2014-15, the breeding female produced a total of 111 eggs. However, viability to hatching was 11.7% with only thirteen eggs developing. Of these, four larvae were deformed and died shortly after hatching; the remaining 9 larvae (i.e. 8.1%) developed normally under similar environmental conditions as the adults at 16-18°C on a diet of *Panagrellus*, *Enchytraeus*, *Chironomus* and other aquatic invertebrates. In these animals, the larval phase lasted approximately 120 days. In 2015-16, a total of 114 eggs were produced by both females, but the proportion contribution of each animal could not be determined. Again, the vast majority (c. 102/114 or 89.5%) of eggs were infertile; however, no larvae were raised in 2016 due to housing capacity limitations.

DISCUSSION

Amphibians in captivity, including those held in some conservation breeding facilities, may undergo long periods of reproductive inactivity due to the absence of appropriate environmental stimuli. Amphibians may be long lived, especially in captivity, but the impact of reproductive hiatuses on the reproductive capacity of animals, and therefore the viability of such populations beyond the lifespan of founder adults, is largely unknown. The longitudinal studies required to investigate such phenomena may reduce the availability of relevant data as individual animals must be followed for long periods of time. The observations reported here show that reproduction can be triggered in at least this species of newt after a very long reproductive hiatus. Michaels et al. (2015) showed that reproductive activity could be triggered in *Xenopus longipes* after seven years of reproductive inactivity in the absence of appropriate environmental cues, but the present findings are the first data concerning a non-reproductive period of more than a decade and the first concerning a tailed amphibian.

Although reproduction was immediately triggered in one female after appropriate environmental cycling, the other female required two years of environmental cycling to regain reproductive function, presumably due to hormonal cascades requiring substantial environmental input to re-initiate. Moreover, the viability of the eggs that were deposited in both years was low, suggesting that recovery of full reproductive capacity may be problematic. The age of the animals in question is unknown, but the newts were adult with mature colouration (dorsally a putty green-grey and ventrally a faded yellow-orange, rather than the black, orange and yellow dorsal and vibrant orange ventral colouration seen in young adults) when purchased in 2003 and take at least 10 years to reach full adult size and coloration (H. Jansens, pers. comm.). Therefore these animals were likely at least 20 years old, if not older, approaching the maximum lifespan of around 30 years (H. Jansens, pers. comm.). Given that eggs produced by both females had low hatch rates and even lower rates of viability beyond hatching when offspring were reared, the most parsimonious explanation is that the fertility of the male animal was waning. There are, however, no data available to provide insight into either age or aseasonality on fertility or gametogenesis in the long term in amphibians. The clutch of eggs produced in the 2014-2015 breeding period was very large for this species (H. Jansens, pers. comm.; also see comparison with total number of eggs from two animals in the 2015-2016 reproductive period), which may be the result of accumulated fat reserves after having not invested in ova for a long period. Although these individuals were alive and in good body condition following the long non-reproductive period, these data should not be used to suggest that withholding seasonality from newts when reproduction is not required is appropriate.

Without seasonally cycled animals for comparison, and thorough internal investigation it is unclear whether the reproductive hiatus has had any negative impact on the health or longevity potential of these long-lived animals.

It is also significant that moderate oviposition site guarding, possibly a form of parental care, was elicited in females and that this behaviour survived reproductive hiatus. Such behaviour has been reported from *P. caudopunctatus* (Sparreboom, 1983; Rafaelli, 1989), but has not been observed in this species or any other congener. Reproductive function may therefore be recovered in amphibians after long non-reproductive periods in captivity, but the viability of populations comprising aging animals may be compromised by declining fertility. In the context of maintaining viable breeding populations of amphibians in captivity, for conservation, research or other purposes, this highlights the importance of developing appropriate breeding protocols for amphibians as early as possible after a population is established in captivity. Reference to climatic variation to which a species is exposed in nature may, as in this case, facilitate the provision of stimuli necessary to induce reproduction (Michaels et al., 2014).

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Husbandry of the axolotl *(Ambystoma maculatum)*: successful treatment of a case of constipation

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Between 5th and 8th January 2015 we recorded the birth of 81 axolotl (*Ambystoma maculatum*) larvae at the Science Museum of Camerino University. They were maintained at 17˚C and fed on brine shrimps. A number of these animals were subsequently dispersed to other collections, and in September 2015 one of these, now 8 cm in total length, was returned to the Museum with foreign body constipation symptoms. At first glance, the subject showed an abnormal lower limbs posture, not relaxed in a normal orthogonal position to the body but with parallel poise. There was also swelling in the region of the cloaca. The patient was then transferred to the Veterinary Teaching Hospital of Camerino University for a body X-ray examination (Fig. 1, taken on 7th October 2015) which showed the presence of many round-to-oval, smooth, radiopaque foreign bodies at the level of the stomach and intestine, with some of these little stones enlarging and blocking the cloaca. Olive oil was then administered orally with a catheter normally used for human babies and a few stones in the cloaca were extracted with tweezers. After one day, an olive oil enema, performed with the same catheter, resulted in a reduction in the number of these foreign bodies. After a further week, another radiograph was taken, showing the reduction in number of the foreign bodies (Fig. 2). The animal eventually made a complete recovery. We are unaware of any literature relating to problems of this kind and hope that this experience might be of use to others who might be faced with problems due to constipation in small amphibians.

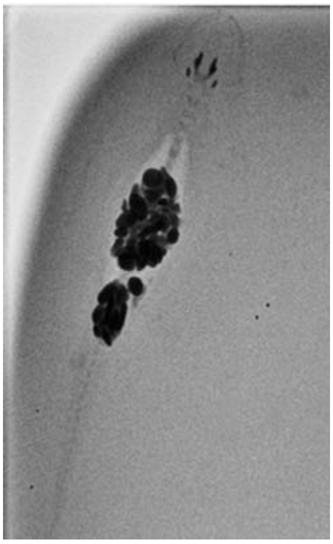

Figure 1. First radiograph of the young axolotl showing almost total occlusion of the stomach and a severe intestinal constipation.

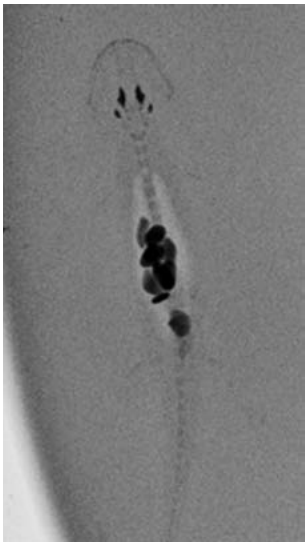

Figure 2. Radiograph taken after the first treatment. The clinical picture of the subject is much improved, with about 75% of the grit ejected.

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Movements, mating and parturition in a female aspic viper, *Vipera aspis*, in western France

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INTRODUCTION

The aspic viper, *Vipera aspis*, is a small venomous viviparous snake of the western Palearctic region. It is heliothermic, basking in the sun to regulate body temperature. A capital breeder, females accumulate large amounts of energy before reproduction resulting in low reproductive frequency. Most females in western France apparently only reproduce once during their lifetime (Bonnet et al., 2002). Compared to non-pregnant females, pregnant females increase basking time substantially, display higher thermal preferenda and thermoregulate more precisely around thermal set points. Body temperature ranges of female *V. aspis* are within the highest level of thermal precision reported in squamates (Angilletta 2009; Lorioux et al., 2013). The advantages of attaining thermal optima on the developing embryos are larger fitter offspring and fewer stillborn. However, this strategy compels pregnant females to bask in more open locations compared to non-pregnant females, which increases predation risk (Lourdais et al., 2004) especially from predatory birds (Naulleau et al., 1997: Lourdais et al., 2013).

Most research on *V. aspis* in western France has been undertaken in Department 44 (Loire–Atlantique; 47°03’ N, 02°00’ W) in a habitat consisting of a mosaic of meadows and regenerating scrubland (e.g. Bonnet *et al.,* 1999; Bonnet et al., 2002; Lourdais et al., 2014). However, behaviour and reproductive strategy may differ in other regions and habitats (e.g. Luiselli & Zuffi, 2002; Altweg et al., 2005) and hence additional observations are of interest. This note describes movements in a female *V. aspis* during 2014 and 2015 in western France, but in a fragmented landscape in Department 85 (Vendee; 46°27`N;1°53`W) approximately 80km to the north of the Department 44 study locality, when mating and parturition were observed.

METHODS

Observations were made around a hibernaculum initially discovered during the spring of 2013. This consisted of a series of disused drainage pipes almost completely covered by soil situated in a hedgerow where the entrances were covered with an understory of bramble (*Rubus fruticosus*) and canopy of European ash (*Fraxinus excelsior*). The surrounding terrain was mainly agricultural land, small urban areas and patches of woodland, usually connected by extensive hedgerows. Several species of reptile have been sighted annually around the hibernaculum since the spring and autumn of 2013 (see Meek, 2013; 2014). Surveying was by VES (visual encounter survey; McDiarmid et al., 2011) on most days during the active season (March – November) when the weather was favourable. Surveying was along the hedgerows to the north, north-east, west and south of the hedgerow where the hibernaculum was located and also a 10 - 15m strip of surrounding farmland (Fig. 1). In an attempt to minimize disturbance and disruption of normal activity during each visit, the hedgerows were surveyed only once during morning and once in the afternoon. When a sighting was made, observation time was limited to around 10 seconds maximum.

The female *V. aspis* had initially been identified from photographs at the hibernaculum during March 2013 and was readily identifiable thereafter. She was observed in the spring and autumn of 2014 through to the spring of 2016, but there were no sightings (or of any other viper) near the den during the summer. Estimated total length was 450 - 470 mm.

OBSERVATIONS AND DISCUSSION

Between 12 March and 22 April 2014 10 sightings were recorded around the hibernaculum but no reproductive activity was observed. The first sighting in 2015 was on the morning of 4 April when basking was observed near the main entrance to the hibernaculum in close proximity to two other individuals. A further 5 sightings were made in the main hedgerow including basking at 09.50h (CET) on the morning of 12 April near the entrance to the hibernaculum. Later the same day at 18.10h, in the same hedgerow, mating was observed with one of the other two vipers. Both individuals were resting motionless 200mm above the ground on a fallen log in dappled sunlight inside the hedgerow (log surface temp = 32.9C, air temp = 22.0C). The last spring sighting of the female was at 09.45h on the morning of 17 April basking in the main hedgerow close to the mating location.

The first autumn 2016 sighting was of basking in open or in semi-exposed locations alongside the western hedgerow on 4 September at 09.40h (Fig. 1). Sightings were daily each subsequent morning at the same locality (±2 metres) and at 09.50h on the morning of 9 September parturition was observed. Time from mating to parturition

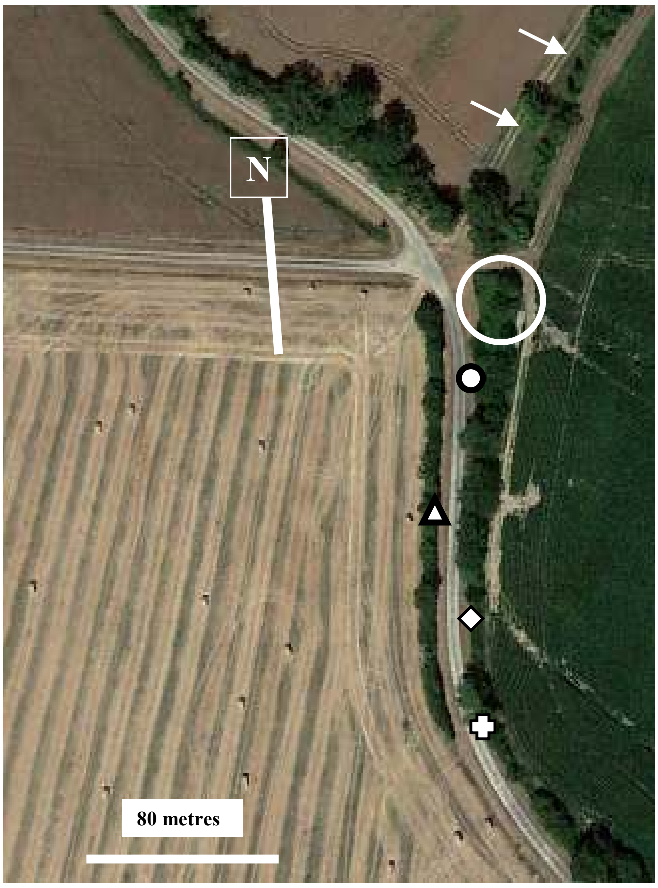

Figure 1. Aerial view (Google Earth) of the study area showing main locations of sightings. Large circle indicates area around the hibernaculum where post hibernation basking was observed, small circle = location of mating, triangle = site of parturition, cross = post parturition locations and diamond = sightings during spring 2016. Arrows along the northwest hedgerow indicate previous sightings of basking pregnant female *V. aspis*. See text for further details.

was thus 151 days, which is in good agreement for *V. aspis* in France (e.g. Bonnet et al., 2001) and Italy (Luiselli & Zuffi, 2002). Estimated number of offspring was a minimum of 6 but extensive plant cover at this time did not facilitate an accurate count. The snake was next sighted in the main hedgerow on 17 September at 17.35h, indicating a road crossing (Fig. 1). In total 10 autumn observations were made during 2015 the last on 9 October. The first sighting in 2016 was 21 April when basking was observed at a regularly used basking site. This indicated survival through the winter period after parturition. The first 2016 sighting was later than in previous years, but the spring was un-seasonally cold with frequent north winds, overcast days and cold nights. Only 3 observations were made, the last on 27 April.

The absence of summer sightings at the hibernaculum (including of other vipers) suggests migration to a summer home range. The hedgerows represent narrow stretches of linear habitat situated in an area of agricultural landscape with presumably limited resources. However, hedgerows function as movement pathways between prime habitat (Saint Girons, 1996) and in the study locality lead to woodland 200m to the north east and south circled around a farm 360m from the hibernaculum. Returning to the area around the hibernaculum in early in autumn for parturition has been observed twice previously in other females (see Fig.1) and this behaviour may be adaptive. For instance, the population dynamics of *V. aspis* in some areas of Europe is driven by juvenile survival during the first winter when mortality may be higher than other age classes. It has been suggested that this is due to young individuals being less experienced in finding suitable winter quarters than adults (Altweg et al., 2005). Neonates born in the vicinity of frequently used and tried hibernacula in autumn may have enhanced chances of survival during the first winter. This would also support the notion of female *V. aspis* as 'dedicated mothers' (Lorioux et al., 2013).

It is recognized that there are limitations attached to the present observations, in particular the absence of summer movements, which could be achieved by radio tracking. Body temperature measurements would give insight into thermoregulatory precision but necessitate disturbance through measurement and hence potentially disrupt natural behaviour. It has been assumed that all vipers had left the area immediate to the hibernaculum during early summer but this could be attributed to sampling error, although would require repeated patterns in sampling error year on year. Additionally, foraging whip snakes (*Hierophis viridiflavus*) are frequently seen in the hedgerow during summer, remaining in the locality for periods up to 10 days at a time. Although *H. viridiflavus* is a larger species, for an experienced observer *V. aspis* is no more difficult to detect in a hedgerow.

The observations have value in that they indicate increased basking exposure during pregnancy with the associated increase in predation risk (e.g. Lima & Dill, 1990; Saint Girons, 1994). Non-reproductive females and males usually employed mosaic basking in dappled sunlight and selected basking locations deeper into dense vegetation (bramble for example), which substantially reduces predation risk, especially from raptors (e.g. Saint Girons, 1994). In general the observations agree with data from female *V. aspis* studied in Department 44 (e.g. Bonnet et al., 1999; Bonnet et al., 2002; Lourdais et al., 2014) indicating that thermoregulation, predator avoidance and reproductive behaviour in *V. aspis* are intrinsically linked.

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SHORT NOTE

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Melanism in the pond slider (*Trachemys scripta*) in northern Indiana, USA

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INTRODUCTION

Melanism in turtles is generally rare among species, but can be extremely common in some species and subspecies. Pond Sliders (*Trachemys scripta*) show ontogenetic melanism with larger, and presumably older, individuals often showing melanism of the plastron and other body parts (Lovich et al., 1990).

Previous studies of melanism in *T. scripta* have found that the proportion of melanistic individuals can vary among populations. For example, some populations from Oklahoma show 100% melanism for both adult males and females, whereas other populations from that state show percentages of melanistic adults ranging from 44% to 75% (McCoy, 1967; Hays & McBee, 2009). In southeastern Missouri, 81% of adult males were melanistic (Glorioso et al., 2010), and in southern Illinois, approximately 18.7% of adult male *T. scripta* were melanistic (Reehl et al., 2006). Some authors have argued the observed variation in the proportion of melanistic *T. scripta* does not appear to be related to geographic parameters, such as latitude (see McCoy, 1967), but Cahn (1937) suggested that melanism was more frequent in *T. scripta* from southern Illinois than those from northern Illinois. However, relatively few populations have been examined to quantify melanism, especially northern populations. Here we report on melanism in a population of *T. scripta* at the northeastern extreme of the range of the species in the Wabash River drainage in Indiana (see Seidel & Ernst, 2006 for distribution). This population is believed to be natural, although supplementation by pet trade releases over many decades cannot be ruled out.

MATERIALS AND METHODS

As part of a larger investigation of the turtle community, we captured *T. scripta* elegans from Dewart Lake, Kosciusko Co., Indiana, USA (41°22'03.31"N, 46°18.22"W) nearly annually for 35 years (1979 – 2014) using fyke nets, wire traps, basking traps, and by hand (see Smith et al., 2006 for details about turtle sampling). Turtles were individually marked using notches in the carapace. In addition, we noted melanistic males and females. We used straightline carapace length (CL) of melanistic individuals to estimate the size at the first observation of melanism in the individual (i.e., Stage 2, Tucker et al., 1995).

RESULTS AND DISCUSSION

We examined a total of 161 individual *T. scripta*. Of these 161 turtles only 18 (11.2%) were melanistic. In males 12 of 86 (14.0%) were melanistic, and in females 6 of 75 (8.0%) were melanistic. The observed incidence of melanism did not differ between males and females ($\chi^2_1 = 1.43$, $P = 0.23$). The proportion of melanistic individuals in our population (11.2%) was lower than any other previously reported value (see above). It is interesting to note that the two lowest proportions of melanistic sliders were in the two most northern populations (Indiana [this study] and Illinois [Reehl et al., 2006]). This is also consistent with Cahn's (1937) observation that melanistic *T. scripta* were more frequent in southern Illinois than in northern Illinois. These observations suggest that there may indeed be an inverse correlation between the proportion of melanistic *T. scripta* and latitude.

The smallest melanistic male *T. scripta* we captured in our northern Indiana population was 190 mm CL (mean CL ± SE of melanistic males = 212.8 ± 4.8 mm), and the smallest melanistic female was 220 mm (mean CL ± SE of melanistic females = 237.7 ± 7.8 mm). The size threshold for melanism ranges from 114 to 156 mm plastron length (PL; estimated CL = 122 to 168 mm) within Oklahoma (Hays and McBee, 2009), and from 100-140 mm PL (estimated CL = 107 to 150 mm) in populations from Texas, South Carolina, and Alabama (Cagle, 1950; Lovich et al., 1990; Garstka et al., 1991). The smallest melanistic male sliders in southeast Missouri (Glorioso et al., 2010) and in Illinois (Cahn, 1937) were 172 mm CL. Thus, for our population in northern Indiana, the size at which melanism was expressed was larger than all previous observations, which may reflect trends in maximum body size variation across the range, which have not been analyzed.

Taken together, our observations suggest that our understanding of geographical variation in melanism in at least *T. scripta* needs further evaluation. More data from northern populations in North America, as well as from introduced populations in other regions of the world are needed, especially to test the latitudinal variation hypothesis, and to evaluate potential environmental causes of that variation (e.g., Etchberger et al., 1993; Rowe et al., 2006, 2009).

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SHORT NOTE

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An extraordinary large prey observed in the diet composition of *Natrix tessellata* (Squamata: Colubridae)

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INTRODUCTION

Dice snakes are a group of semiaquatic snakes that occur in Europe, Asia, and north Africa (Mebert, 2011a). The Dice snake, *Natrix tessellata*, is highly aquatic European species being predominantly piscivorous and diurnal. It employs both sit-and-wait and active foraging for hunting (Luiselli et al., 2007; Capula et. al., 2011; Mebert and Trapp, 2011). To a lesser extent, *N. tessellata* is known to feed on amphibians (mainly frogs and their tadpoles), gastropods, insects, reptiles, birds and small mammals (Gruschwitz et al., 1999; Luiselli et al., 2007; Bakiev et al., 2011; Göcmen et al., 2011). Ponec (1978) photographically documented ophiophagy and even cannibalism. The diet composition of *N. tessellata* is thus one of the best documented among snakes of the Western Palaearctic (see Gruschwitz et al., 1999; Mebert, 2011a; Šukalo et al., 2014; Weiperth et al., 2014 and references therein). In contrast to diet composition, very little is known about the quality and maximum sizes of food of *N. tessellata*.

METHODS

Field search to record *N. tessellata* was conducted either visually by walking through the Sušanka reservoirs (49.79725°N, 18.43720°E, 252 m a.s.l.; Czech Republic) from end of March to the beginning of October (from 2009 until the present). The locality is visited approximately five times per month each year. The Sušanka reservoir is a system of seven open water ponds which were used for sediment deposit and retreatment of water from the bituminous coal mines. The surface of the study area is circa 22.5 ha (1500×170 m). An estimated number of *N. tessellata* at the locality is >100 individuals. Snakes were measured using a tape measure (0-200 cm) and weighed with a Pesola spring balance (0 to 500 g = 5 g).

RESULTS AND DISCUSSION

During surveying we made three records of extraordinarily large prey of *N. tessellata* (see details in Vlček et al., 2010; 2011). On May 27th, 2016 we found a dead specimen of *N. tessellata* [female, total length (TL) = 101 cm; Fig. 1A] with a dead roach (*Rutilus rutilus*) in its mouth (Fig. 1B). The fish was hooked in the oesophagus of the snake, which apparently resulted in suffocation. Due to the high degree of decomposition of the fish body, we only measured the skeleton. The length of the fish was 23.5 cm (Fig. 1C). Another large prey was recorded on April 22nd, 2016 when

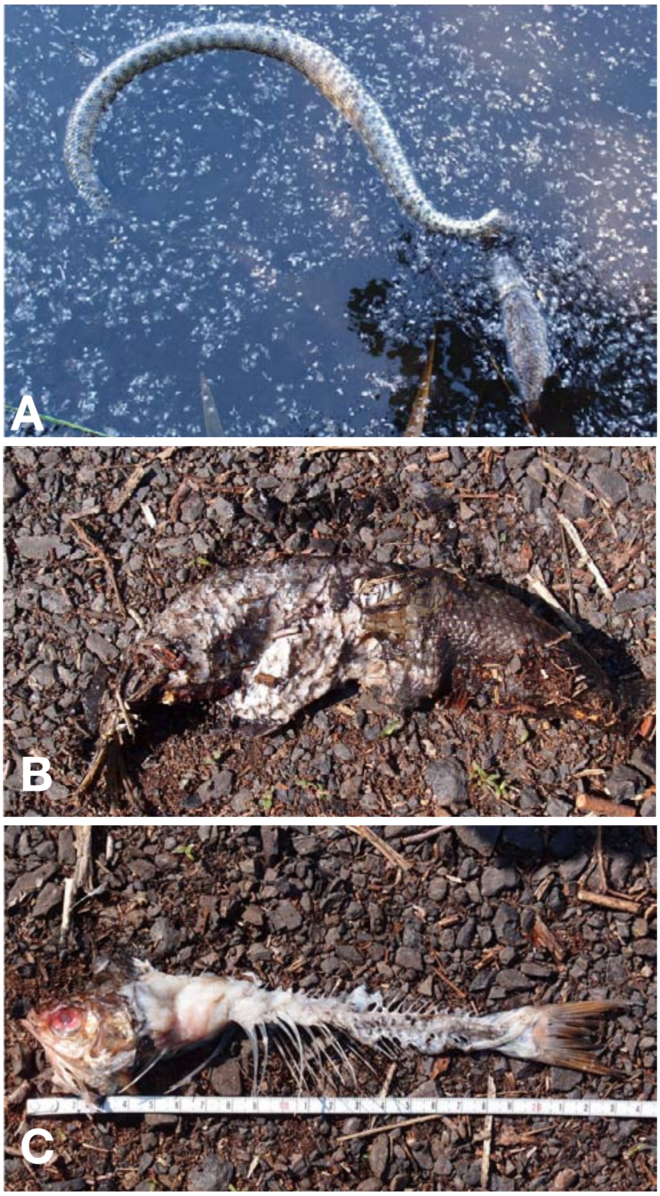

Figure 1. **(A)** Dead female *Natrix tessellata* with caught *Rutilus rutilus*; **(B)** prey removed; **(C)** length of the fish skeleton.

Figure 2. (A) Female *N. tessellata* feeding on *Carasius auratus*; **(B)** the size of caught fish; **(C)** female *N. tessellata* with regurgitated specimens of *R. rutilus*; **(D)** the size of regurgitated fish.

a ~100 cm long female *N. tessellata* consumed a goldfish (*Carassius auratus*) which was 20 cm long and weighed 117 g (Fig. 2A, B). However, after several minutes, this prey was regurgitated and abandoned. Finally, on May 7th 2015 we observed a female *N. tessellata* with a length of 90.4 cm and a weight of 271 g. This female subsequently regurgitated four specimens of *R. rutilus*. Their total weight was 34 g with individual sizes ranging from 8 to 12 cm (Fig. 2C, D).

Laňka (1978) presented records of an adult specimen TL = 84 cm that consumed a 16 cm long *Rutilus rutilus* and a 17 cm long undetermined species of Pleuronectiformes. Probably the largest sized prey for *N. tessellata* was recorded by Kabisch (1966) with a 95 cm long female having consumed an individual *Gaidropsarus mediterraneus* (a coastal marine ground fish), which was 21.5 cm long and weighted 160 g. The largest prey items recorded by Göcmen et al. (2011) were a pike (*Esox lucius*, approx. TL 230 mm), a lizard (*Lacerta trilineata*, approx. TL 200 mm) and a mouse (*Dryomys nitedula*, approx. TL 320 mm), which were found in three large female *N. tessellata* (TL= 88.70; 85.80; 91.50 mm respectively). An explanation for such extraordinarly large prey is in the larger size of female *N. tessellata*. Females are larger than males and reach adult size at 80-130 cm, whereas males are usually <100 cm (Mebert, 2011b). Hence, females can consume larger prey, which provides more energy especially during vitellogenesis and when they are gravid (Gruschwitz et al., 1999; Luiselli et al., 2007; Göcmen et al., 2011).

Related observation are rarely published despite their informative value regarding the feeding biology of this species. Mebert & Pölzer (2011) described a case in which the bullhead *Cottus gobio*, a bottom dwelling fish, killed an adult female *N. tessellata* after it locked its jaws around the snout of the snake in some type of defensive reflex. Our observations suggest that (i) female *N. tessellata* could have increased food requirements when gravid, (ii) they are not immune from miscalculating the size and defensive capacity of certain prey with an occasional fatal consequences.

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Dark coloration in Sardinian grass snakes (*Natrix natrix cetti*)

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The grass snake (*Natrix natrix*) is a widespread snake that includes several subspecies distributed in different areas of Europe and North Africa (Kindler et al, 2013). Sardinia Island hosts the most differentiated subspecies from the *N. natrix* ssp. complex, *N. n. cetti* (Capula et al, 1994; Corti et al, 2010). It is distributed across the island, even if the majority of detections have been made in the southern and eastern part of Sardinia (Corti et al, 2010; Salvi and Bombi, 2010). Such discrepancy is probably a result of the limited number of studies performed on this subspecies (Lanza, 1986; Stefani, 1983; Capula et al., 1994). However, no authors have reproted abnormal colouration, even if darker colourations are known within *Natrix* species and subspecies (Gvozdenović and Schweiger, 2014; Jandzík, 2004; Mollov, 2012).

In two occasions (May 2011 and October 2015) we found 3 individuals of *N. n. cetti* on Seven Brothers Mount. We photographed each snake and then they were measured using the program ImageJ. The first snake was abundistic and its total length was 109.48 cm (Fig. 1a). The second showed a particular bluish colouration but the darker pigmentation was regular; total length 91.94 cm (Fig. 1b). The third snake was melanotic (Zuffi, 2008; Fig. 2a) and showed an uncommon shortened tail, perhaps a result of injury with only 21 subcaudal scales (Fig. 2b). The total length was 65.52 cm, with SVL 62.08 cm and tail length 3.54 cm. One way to recognise gender in this subspecies is subcaudal scale count: in *N. natrix cetti* the average number for males is 59, while the average number for females is 50 (Corti et al, 2010). In this individual the subcaudal scales were too few so we were unable to recognize the sex. However, it was likely an adult and since SVL > 60 cm a female (Capula et al, 1994; Corti et al, 2010). *N. n. cetti* showed a marked polymorphism in coloration within the same population. All these unusual colorations involved an increase of dark pigmentation: two snakes had expanded black pigmentation (Fig 1a and 2a), while in the other the ash ground colour was darker, tending to blue (Fig. 1b). In this subspecies the ground colour is usually more prevalent than black pigmentation (Stefani, 1983) so our observations are unusual for this subspecies.

Figure 1. Two individuals of *N. n. cetti* with unusual coloration: first snake has abundistic pattern (A), while the second has a general bluish bottom colour (B).

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Figure 2. Melanotic individual of *N. n. cetti* (A); photograph showing shortened tail (B).

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NATURAL HISTORY NOTE

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Boa constrictor (Reptilia, Serpentes, Boidae): opportunistic predation on *Diopsittaca nobilis* (Aves, Psittacidae).

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Boa constrictor is widely distributed in Central and South America, inhabiting forested and open regions (Henderson et al, 1995). It is an ambush predator (Greene, 1997), and selection of ambush site may be of crucial importance for the foraging success (Shine & Xin, 2002). Here we report two predation events by a *B. constrictor* held in captivity in an outdoor enclosure at Instituto Butantan, São Paulo, Brazil.

On 6 August 2015, at 09:06 hrs, a *B. constrictor* was observed capturing an adult *Diopsittaca nobilis*, Psittacidae (red-shouldered macaw). The snake, born in the Biological Museum of Instituto Butantan (SVL 1480mm; VTL 150mm; mass 3530g) in 2007, was regularly fed on rodents. The enclosure is open to the public for visits and altogether houses 20 *B. constrictor*, although the predation attempts were observed in a single snake. The enclosure has a ground area of 386.22m^2 but has no overhead cover. The snakes are confined by a 1.8m high wall (Fig. 1). The enclosure is provided with shelters, a pond (length 3.7m, width 1.0m) and also flowing fresh water. Vegetation consists of large palm trees shrubs and underbrush. The presence of shrubs and trees allows free vertical movement of the snakes and thus they are able to select places of refuge, rest and ambush. Frequent pruning of branches next to the enclosure wall minimises escapes.

The Boa had been seen resting on a palm tree (*Caryota urens*), at a height of 11m, hidden among foliage and hence was difficult to detect. When a *D. nobilis* flew into the enclosure and perched close by, the snake seized it by constriction, suffocated it and, in a few minutes, started swallowing the prey headfirst. The process took exactly 101 minutes for full ingestion (Fig. 2). The subject remained at the same location for 15 days following the ingestion event. On 13 November 2015, the same snake, identified by body pattern, in the same palm tree at a similar height to the previous event captured a second adult *D. nobilis*. The snake seized the bird at 08:08 hrs, constricted it, killed it by suffocation and started ingesting headfirst but appeared to be having difficulty swallowing the prey. At 10:09 hrs, the head was only partly ingested and at 10:20 hrs, little more than 2 hours after capture, feeding was abandoned and the prey left among the palm leaves. On the same day, at 16:40hrs, the dead bird was sighted in the same place. On the morning of 16 November 2015, the prey was found on the enclosure floor and biometric measurements were taken; exposed culmen 34.6mm; head 48.3mm; wing chord 185mm; total length 331mm.

Observations of predation on adult psittacids by *B. contrictor* are unusual, probably due to their size, flight ability and intense flock vigilance (Begotti & Filho, 2011). After the predation events part of the flock remained relatively close to the event, emitting vocal alarm signals for a few minutes. The *B. contrictor* may have used the same palm tree because it is frequented by birds, and offers effective camouflage and shade from the sun, including for a large snake.

Figure 1. Partial view of the snake enclosure at Instituto Butantan.

Figure 2. *B. constrictor* beginning ingestion of *Diopsittaca nobilis*.

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Bird mobbing of *Boa constrictor* in lowland tropical rainforest of Costa Rica

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Mobbing is well documented in birds – particularly in songbirds (Passeriformes) – as a predator deterrent behaviour that also communicates the presence of predators to others. Most information on avian mobbing of potential predators comes from studies involving north temperate species, with the targets typically being raptors. Here, we document mobbing of a *Boa constrictor* by a Neotropical bird assemblage, noting the composition and number of individuals in this mobbing group. The diet of *B. constrictor* consists of a variety of vertebrates, which can include poultry as well as wild birds (documented prey items range from smaller songbirds like Blue-gray Tanager (*Thraupis episcopus*) and antbird (Thamnophilidae) [Greene, 1983] up to Chestnut-fronted Macaw (*Ara severus*) [Begotti and Filho, 2012]).

We were alerted on 04 March 2014 by naturalists about the presence of a small (ca. 1 m long, including tail) *B. constrictor* that was coiled up on a branch ca. 3 m above the ground beneath a leaf cluster of a *Miconia impetiolaris* (Melastomataceae). This location was next to a heavily used concrete pathway through a maintained lawn with clusters of shrubs, located adjacent to the dining hall on the property of the La Selva Biological Station (Organization for Tropical Studies), Heredia Province, Costa Rica. The following day, the snake had relocated about 15 m away, and was coiled up ca. 4 m above the ground in an isolated *Trophis racemosa* (Moraceae) above the lawn. At this time, it was being actively mobbed by a substantial group of birds. Most individuals were giving chip notes or other calls rather than singing. No birds made physical contact with the snake during our observations. The *B. constrictor* did not attempt to retreat from the birds, nor strike out at them as prey, during our observation time.

The 25 individual birds mobbing the snake were: *Phaethornis striigularis* (Stripe-throated Hermit) – 1; *Amazilia tzacatl* (Rufous-tailed Hummingbird) – 2; *Trogon massena* (Slaty-tailed Trogon) – 1 male, 1 female; *Pitangus sulphuratus* (Great Kiskadee) – 2; *Megarynnchus pitangua* (Boat-billed Flycatcher) – 1; *Myiozetetes granadensis* (Gray-capped Flycatcher) – 1; *Campylorhynchus zonatus* (Band-backed Wren) – 2; *Turdus grayi* (Clay-colored Thrush) – 1; *Setophaga pensylvanica* (Chestnut-sided Warbler) – 1; *Ramphocelus passerinii* (Passerini's Tanager) – 5; *Cyanerpes cyaneus* (Red-legged Honeycreeper) – 1; *Chlorophanes spiza* (Green Honeycreeper) – 2; *Saltator maximus* (Buff-throated Saltator) – 1; *Piranga rubra* (Summer Tanager) – 1 female; *Icterus galbula* (Baltimore Oriole) – 1; *Euphonia luteicapilla* (Yellow-crowned Euphonia) – 1.

Hummingbirds and songbirds have been noted mobbing snakes elsewhere in the Neotropics (e.g., in Ecuador by Matheus et al., 1996), but the presence of a trogon in this mobbing group was unexpected. Three of these species – *S. pensylvanica*, *P. rubra*, and *I. galbula* – are Neotropical migrants that breed in forests of North America and winter in Central America, and would only encounter *B. constrictor* during the five to six months of their annual cycle spent in the tropics. Snakes are known to be primary nest predators (Robinson et al., 2005), but their role in predation on adult birds is not well studied.

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Rana temporaria: Leucism in combination with retarded development

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There are several forms of pigmentary lacks known for amphibians (Thomas et al., 2002). Leucistic animals possess a completely white body but pigmented eyes. In the latter named aspect they differ from albinistic individuals who additionally lack eye pigmentation resulting in red eye colour (Twelbeck & Forman, 1983; Klemz & Kühnel, 1986; Thomas et al., 2002). Albinistic and leucistic tadpoles and adults are reported for various anuran taxa. Also white spawn is reported. It seems to be produced by white females but the tadpoles colour out rapidly if the spawn is fertilised by a normally coloured male (Smallcombe, 1949; Klemz & Kühnel, 1986). *Rana temporaria* (Linnaeus, 1758) is common in Europe except for Portugal and Iceland. Depending on the temperature it needs two to four months from egg deposition to metamorphosis (Golbach et al., 2014).

A leucistic tadpole of *R. temporaria* (collection number: ZFMK 97380, see Fig. 1) in the Gosner stage 36-37 (Gosner, 1960) was communicated to us in February 2015 by Andreas Niechciol. He had found it in an artificial small water body at Vennhausen near Düsseldorf (51° 13’ N 6° 52’ O, vgl. Thissen, 2015). Andreas Niechciol observed the leucistic tadpole spending the winter in this water body. The artificial small pond also housed numerous normally coloured tadpoles. The leucistic tadpole did not metamorphose like its conspecifics. Hibernation of tadpoles is known from *Pelobates fuscus* (Nyström et al., 2002), *Alytes obstetricans* (Thiesmeier, 1992) and some other species, but not from *R. temporaria*. In spring 2015 it was taken to an aquarium in the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany, but died because of an excessive heat period in Bonn in that time after a few days and was subsequently transferred to 70% ethanol and deposited in the herpetological collection of the museum.

Figure 1. Leucistic tadpole of *R. temporaria* (collection number: ZFMK 97380). Photo: A. Reich (2015).

In contrast to normal *R. temporaria* tadpoles our specimen was of a whitish-golden coloration, lacking body pigments. The eyes of the tadpole were normally coloured with a whitish-greyish iris and black pupil. The body shape of the tadpole was oval. It had an elongated body with a rounded snout. The tadpole measured about 3.9 cm in total length, 1.3 cm in body length and 0.7 cm in body width. Our tadpole therefore was of normal size for *R. temporaria* but exceeded the time usually needed for development and hence presumably hibernated in the larval stage. Such a developmental delay in combination with a lack of pigment has never been reported to the best of our knowledge.

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We want to address special thanks to Andreas Niechciol for committing the leucistic tadpole to Museum Koenig. Furthermore, we want to thank Wolfgang Böhme for supervising this project and providing additional literature and his expertise.

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NATURAL HISTORY NOTE The Herpetological Bulletin 137, 2016: 43

New occurrence record of the Algerian ribbed newt *Pleurodeles nebulosus* (Guichenot, 1850) in Algeria

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The taxonomic status of the Algero-Tunisian *Pleurodeles* was reanalysed and classified in two genetically and morphologically distinct forms; *Pleurodeles poireti* restricted to the population of the Edough Peninsula (northeastern Algeria) and *Pleurodeles nebulosus* representing all other populations in northern Algeria and Tunisia (Carranza & Wade, 2004; Merabet et al, 2016). In Algeria *P. nebulosus* is present in humid, sub-humid and semi-arid Mediterranean areas (Mateo et al., 2013). Several authors have suggested that it is present from the wilaya of Mascara in the west through to Tunisia in the east (Fig.1). However its occurrence within this range is very limited due to fragmented habitat and little is known about the connectivity of the different known populations (e.g. Mateo et al., 2013).

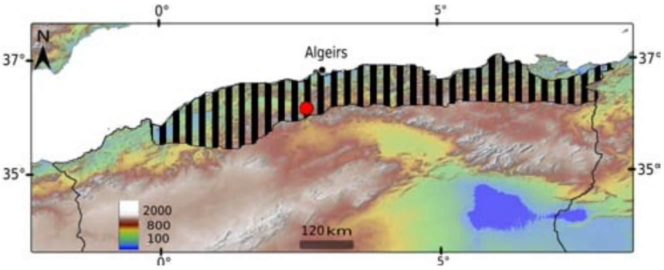

Figure 1. Distribution map of *P. nebulosus* (Guichenot, 1850) in Algeria. Shaded area, supposed range according to Veith et al. (2004). Red dot represents the new site discovered.

During field work in northern Algeria (22 May 2014), we discovered the species in one new area of occurrence in the lake Dhaya of Tamezguida (36.3677532 N, 2.6929010 E) in the wilaya of Medea (Fig. 1). This new record represents the first report of species occurrence in this region. The site is a large pond (Fig.2A) situated in an oak forest at 1185 m above sea level. We found large numbers of larvae at the first stages of development with a total length not exceeding 18 mm (we got larvae in each dip net shot) but only one adult (male) was found under a stone at 10 meters from the water body (Fig.2B).

The reproduction site of *P. nebulosus* in Tamezguida is situated in forest habitat within the National park of Chrea. According to national park officers, the site is subject to fire, overgrazing and anthropogenic pressure such as hiking. This discovery may increase our understanding about the distribution patterns of *P. nebulosus* in Algeria. Such new sites are especially important in drawing conclusions about the connectivity with other known or potentially occupied sites for this species and thus better react to the threats affecting its existence.

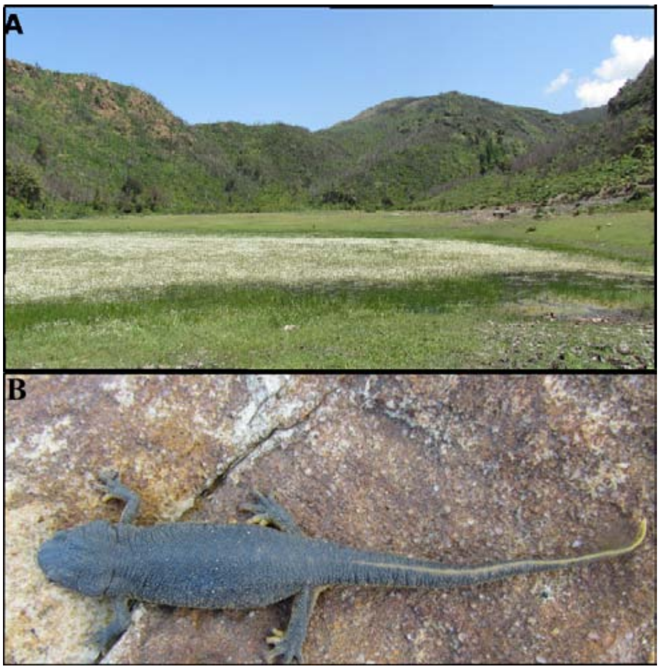

Figure 2. A) The pond where larvae of *P. nebulosus* were found. **B**) An adult specimen of *P. nebulosus* found in the lake Dhaya in Tamezguida.

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NATURAL HISTORY NOTE **The Herpetological Bulletin 137, 2016: 44-45**

The first record of melanistic *Trachemys scripta* from an introduced population of Slovakia

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Trachemys scripta (Thunberg in Schoepff, 1792) is a freshwater turtle that occurs in the Mississippi River Valley, USA (Ernst & Lovich, 2009) and adjacent northeastern Mexico (Legler and Vogt, 2013). Due to the brightly colored shell of juveniles, the species has been introduced via the pet trade worldwide, including Europe (Franke & Telecky, 2001; Ernst & Lovich, 2009). These turtles are named for their characteristic red postorbital stripes on either side of their heads. In adults, the head, neck, legs, and tail of the slider are often olive to brown in colour and striped with cream to yellow coloured ribbons that vary geographically. The carapace is brown to olive with yellow markings. The plastron has distinct markings that range from blotches to intricate patterns. Larger male slider turtles become melanistic as they get larger. Melanism is well known from the native range. The skin, carapace, and scutes ontogenetically become darker to brown and dark gray or green due to increased melanin deposition in the integument and shell. The red stripe behind each eye fades or disappears (Cagle, 1950; McCoy, 1966, Lovich et al., 1990). Melanism might be a non-adaptive by product of hormonal changes (Lovich et al., 1990) although several exclusive hypotheses have been proposed to explain the potential adaptive function of melanism in *T. scripta* (thermoregulation, crypsis, sex recognition or sexual selection; Lovich et al., 1990; Stone et al., 2015).

During a study of the distribution of introduced turtles in Slovakia, we recorded on 12th and 13th August 2016 (around 14:00 hrs of local time) a basking adult male of *T. scripta* (Fig. 1A) with black colouration of the head and

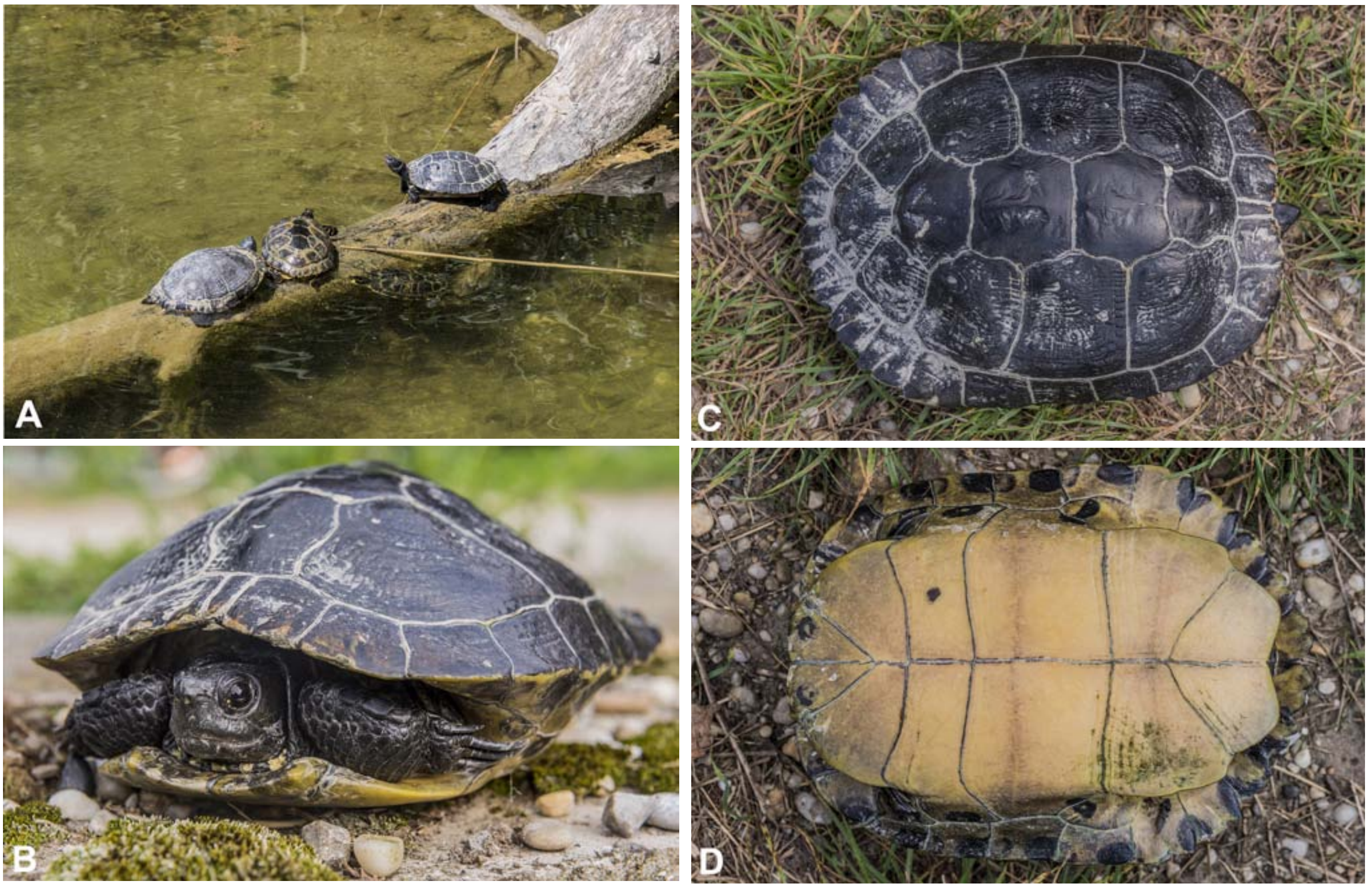

Figure 1. Melanistic individual of *T. s. scripta* from Slovakia. A – the individual (at the top right) together with other individuals of the species on the locality; B – detail from the front side; C – carapace; D – plastron.

body. The individual was captured on the 13th and deposited in the breeding collections of the Department of Zoology, Comenius University in Bratislava (the tissue sample number 4583). The individual had dark dorsal parts of the carapace, head and legs (Figs. 1B, C). Ventral and marginal parts of the carapace, plastron and neck were dark yellow (Figs. 1B, D). Black dots occured around the ventral parts of the carapace. Red postorbital stripes were not visible. Body measurements were: weight 1179 g, plastron length/width 185/125 mm, carapace length/width 195/145 mm, carapace height 85 mm. The locality of the observation is an artificial canal (former oxbow of the Danube River), called "Chorvatské rameno", located in the southern suburban area of the Bratislava (48.10006°N, 17.12931°E, 134 m a. s. l.). There is an introduced population of two subspecies of *T. scripta* (*T. s. elegans*, *T. s. scripta*; Fig. 1A) that are both represented by tens of individuals. The melanistic individual is a *T. s. scripta*. According to our preliminary results, nominotypic subspecies prevails slightly at the location. The present observation represents the first record of a melanistic individual of *T. scripta* in Slovakia. To the best of our knowledge, no melanistic individuals of the species are reported from populations in surrounding countries.

While males of this species become darker as they become larger, females develop limited, slightly darker melanism (McCoy, 1966; Lovich et al., 1990). Melanism in males does not develop until they are 6–11 years old despite attainment of sexual age during their third year (Lovich et al., 1990). Some males remain melanistic for their entire life (Gibbons & Semlitsch, 1982). The male we observed is an older individual and showed signs of age-dependent melanism. Determining how long this individual lived in the locality it is impossible. However, pet turtles are often released when they begin losing the juvenile colouration and size. As melanism in *T. scripta* probably develops with growth it can be concluded that the male had been present for several years. This locality is warm and sunny, well known for the occurrence of these turtles for at least 25 years, providing ideal conditions. Observations elsewhere in Europe indicate that these turtles are able to adapt to specific conditions prevailing on localities outside areas of the United States.

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Marsupial Frogs: Gastrotheca and Allied Genera

William E. Duellman with Osteology by Linda Trueb

Publisher: John Hopkins University Press, 2015, 408pp, ISBN 978-1-4214-1675-5. Hardcover.

When I looked through the Contents pages of this book I was concerned that the material would be so dense as to make it impenetrable to the average reader. I was pleased to find that this was not in fact the case. The book is divided into 13 chapters and an Index. The first eight chapters deal with descriptions of the marsupial frogs in terms of diverse subjects including, Phylogeny and Classification, Osteology, Morphology (both external and internal), Reproductive Biology, and Biogeography.

The first chapter introduces the scope of the book and sets out its goals and methodology. Duellman starts by introducing the Hemphractid marsupial frogs, a unique family of frogs, the females of which carry their eggs in pouches on their backs. In spite of studying over 5000 specimens, the author is very transparent about the limitations of the information contained within the present work, and his expectations that the taxonomy of family will change in the future. Chapters two and three deal with the phylogeny of the Hemiphractids in terms of the their relationship within the Anurans and of the family itself, respectively. Both chapters cover historical and contemporary views of the subject, and without going into too much detail the story is complicated. The current belief is that the Hemiphractid marsupial frogs are a subfamily of the Hylidae and consist of six genera and 106 species, although Duellman concedes that the overall placement within the phylogeny of the anurans is still unresolved.

The next three chapters deal with a variety of morphological characteristics of the Hemiphractids. Chapter four is written by one of the leading authorities of anuran osteology, Linda Trueb, and covers in great detail the cranial structure of the group. Trueb's amazing illustrations help to guide the reader through the descriptions contained within the chapter. The next chapter covers the topic of external morphology in the same detail. It starts with a huge amount of information on the characteristics of adult frogs, from concise definitions of the measurements used to descriptions of the variety of morphological variation found within the marsupial frogs (including the state of the canthus rostralis, finger and toe lengths, webbing, and opening of the brood pouch, and so the list goes on). The format is then repeated for tadpoles. The final chapter deals with further internal characteristics including chromosomes, throat musculature, and vocalisations complete with oscillograms of the various calls for species where recordings exist.

Probably the most interesting aspect of the marsupial frogs is their unique reproductive biology. This subject is covered, in the same great detail we come to expect from Duellman, in chapter seven. Of particular interest are the various structures of the brood pouch, which can range from simple open pouch on the back to closed pouches on the back and sides and even inside the body cavity. Other aspects covered by this chapter include embryonic development and respiration, hatching methods, and the possible evolution of this amazing life history. In an attempt to understand the current diversity of marsupial frogs, Duellman goes on to describe the biogeography of the group, starting with the history of South America and finishing with present distributions and habitats.

Chapters nine through 12 comprise the vast majority of the book and describe in some great detail the genera, subgenera, species that make up the Hemiphractidae. All the chapters in this main section follow a similar format and cover details of the descriptions and characteristics of each genera and species in evolutionary order. Each chapter is illustrated with photographic examples of frogs belonging to the group in question and distribution maps. The thirteenth, and final chapter, entitled "In Retrospect" is more personal than the rest of the book and includes the authors opinions on the state of the taxonomical discipline.

The marsupial frogs of the family Hemiphractidae are truly some of the worlds most fascinating amphibians. While we currently know more than ever about them, there is still much more to learn. This book does a great job of achieving its goal of summarising the current knowledge of the family and highlighting where knowledge is currently lacking. The writing style is easy to follow and concise making this book a very compelling read. I very rarely use the following words when writing book reviews, but this book is a must for anyone with an interest in neotropical herpetology or amphibians in general.

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