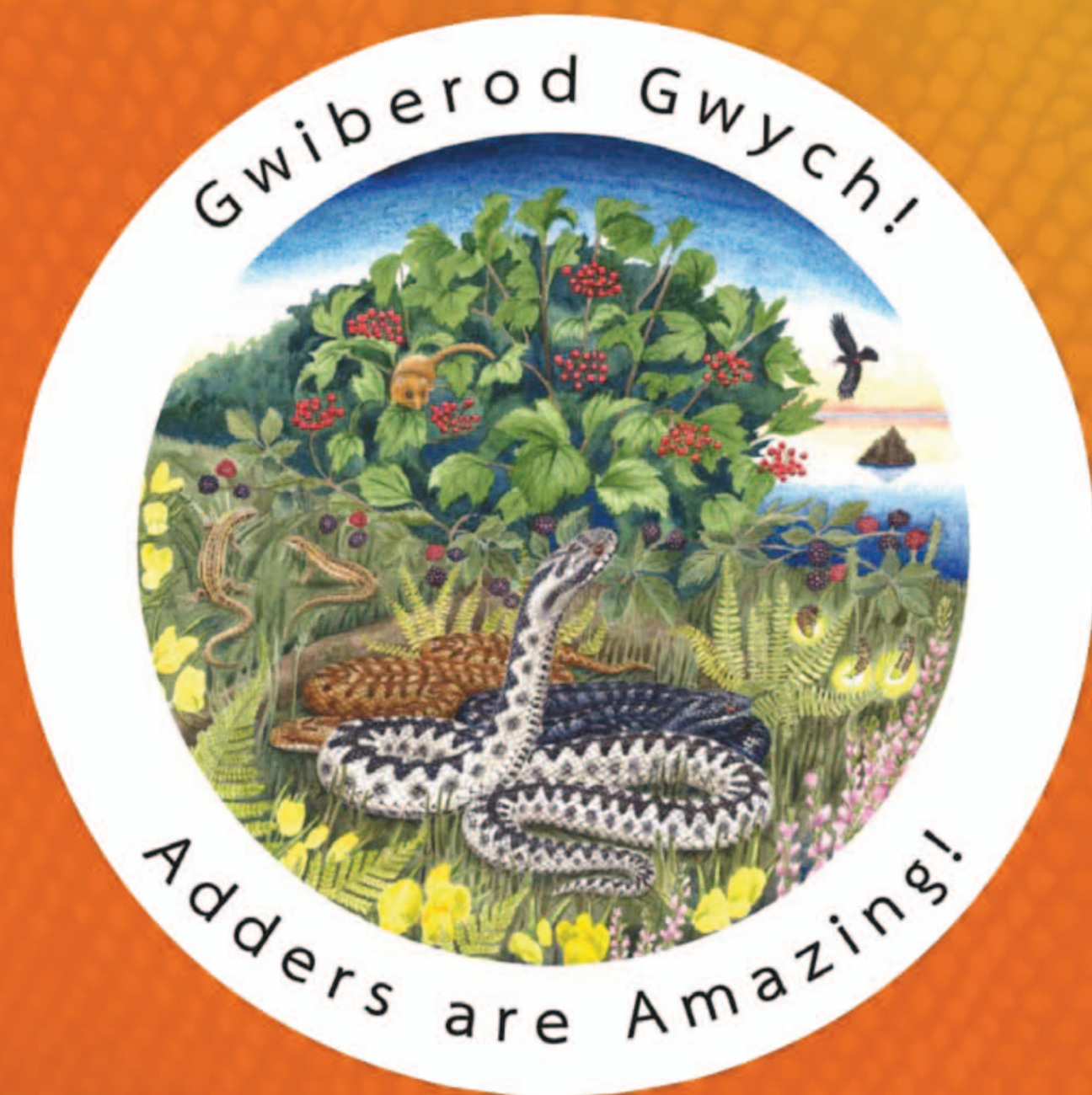




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Front Cover: Artwork created by Mabel Cheung Harris (www.mabelart.com) in celebration of the 'Adders are Amazing!' project with text in both the English and Welsh languages. The image shows flora and fauna typical of coastal lowland heath in Wales with *Vipera berus* as the centre piece. See the article about the Vanishing Viper meeting on page 1.

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The Vanishing Viper: themes from a meeting to consider better conservation of *Vipera berus*

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ABSTRACT - ‘Vanishing Viper 2019’ was organised in the wake of continuing evidence for declines in British populations of the northern viper or adder (*Vipera berus*). The meeting included British participants as well as those from other European countries, whose insights suggest that the drivers of adder declines in mainland Europe are similar to those in Britain. Consequently, we stand to benefit from their knowledge and experience. It was concluded that if adder declines continue at the present pace then Britain may be left with a few large, flourishing populations, but that many of the smaller populations will be lost as appears to have happened in Belgium, France, Germany and The Netherlands. The meeting considered a range of conservation actions including improvements to habitat management, the creation of adder ‘ARKs’, population restoration by genetic or demographic enhancements, community level promotion and outreach, and opportunities for new policy-driven environmental schemes. It is recommended to create a national Adder Working Group to help crystallise and prioritise the main issues and a larger, more informal Adder Network to link and co-ordinate adder researchers and land managers to ensure a free flow of ideas, information and advice. Our chances of success in these ventures will be all the greater if we continue to share experience with our European neighbours.

BACKGROUND

In June 2019, herpetologists and land-managers met at Bangor University in North Wales to consider the conservation of the northern viper or adder (*Vipera berus*). ‘Vanishing Viper 2019: A European approach to developing an adder conservation strategy’ had 110 participants from Great Britain and elsewhere in Europe (Belgium, France, Germany, Sweden, Switzerland and The Netherlands). The meeting was introduced by a message of support recorded by local celebrity naturalist, Iolo Williams, and by a parade through the auditorium of ‘Gwiber’ the giant paper adder created by school children in Wales as part of the ‘Adders are Amazing!’ Project (Fig. 1). For the full meeting agenda see ARG UK (2019).



Figure 1. ‘Gwiber’, a Chinese-lantern adder created by school children for the ‘Adders are Amazing!’ project in Wales, parading through the meeting auditorium

The rationale for VV 2019 stems from long-held concerns that British adder populations have been declining over many years. In November 2011, the Kent Reptile and Amphibian Group (KRAG) organised the first national adder-focused meeting in Kent. With over 100 delegates, the meeting passed a unanimous motion stating that “The adder is in more urgent need of new conservation efforts than any other reptile or amphibian species in Britain”, and a subsequent statistical study by Gleed-Owen & Langham (2012) suggests that declines of this species may have been by as much as 39 % since the 1980s.

In 2016, a second national meeting ‘Vanishing Viper: Priorities for adder conservation’ was organised by the Reptile and Amphibian Group of Somerset (RAGS) with ARG UK and Amphibian and Reptile Conservation (ARC). VV 2016 brought together 150 conservationists who reviewed the evidence of researchers, landowners and statutory bodies to clarify the options for adder conservation. A consensus from all the issues raised was achieved at the meeting using a mind-mapping process, which was refined through a Delphi style analysis to provide a logical starting point to develop a shared understanding of the conservation concerns. The analysis was included in the first session of VV 2019. This report on VV 2019 gives an overview of the meeting and consolidates the subsequent discussions and e-mail exchanges with the intention of stimulating greater efforts for adder conservation.

MEETING SESSIONS

1. What are the concerns?

Richard Griffiths (DICE, U. of Kent) presented the findings

of the 'VV 2016' mind mapping exercise. Thirty eight conservation issues were identified as important, and these were consolidated into eight themes (Fig. 2). The vast majority of conservation issues identified were ranked as 'important', but none were regarded as being tackled well. The biggest gap between 'importance' and 'delivery' relates to habitat management (Fig. 2). The impression from the analysis was that there is no 'silver bullet' that will halt adder declines, but instead improvements in delivery are needed across a range of key issues where there is room for renewed focus and innovation.

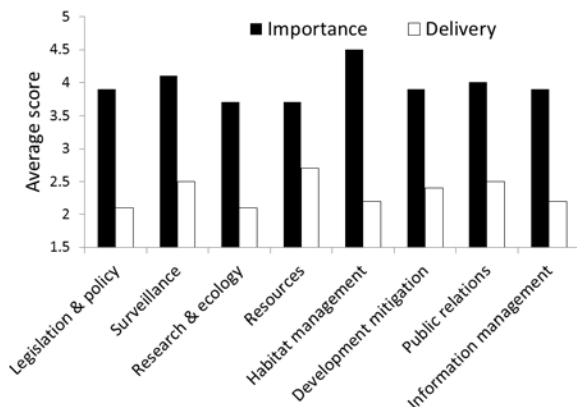


Figure 2. Summary of the importance and delivery of eight key conservation themes based on 'expert' consensus at 'Vanishing Viper 2016'. Importance: 1= 'unimportant'; 5= 'very important' ; Delivery: 1= 'not done'; 5= 'done very well'

Concerns about poor habitat management and increasing predation were highlighted by Nigel Hand (Central Ecology). Over the last 50 years, there have been serious declines in adder populations in the English counties of Herefordshire, Worcestershire and Gloucestershire and extirpation in the counties of Warwickshire, Oxfordshire and Nottinghamshire. These declines are often associated with land use changes, including large-scale mechanised forestry operations. Telemetry has been used to understand adder movements, barriers to dispersal, linkages, and the effects these may have on populations. This has highlighted the secretive, underground habits of the adders, even during the summer months, making them vulnerable to injury from the use of heavy machinery. If prior to large-scale management land managers were more aware of the landscape features essential to adder survival, such as hibernacula, important foraging and birthing areas, and corridors of connectivity, then this damage could be limited. To provide this information across large tracts of land would require considerable effort, but could be achieved if there was a focus on selected sites. This is the rationale for establishing 'ARK' sites for adders. Also highlighted were the clear impacts of predators such as wild boar, pheasants, corvids and raptors; fire (wild fire and planned burns on adder sites); recent landscape-scale rewetting projects; and, the unknown potential of accidental exotic snake releases to transmit novel pathogens.

Inbreeding is another potential cause for population declines and has been demonstrated in a small isolated

adder population in Sweden (Madsen et al., 1999). In this context, Sarah Ball (Institute of Zoology) reported on the genetics of adders in the West Midlands where, in the small populations sampled, a high proportion of individuals were closely related. Nevertheless, the study populations showed no detectable loss of heterozygosity, which is in line with published levels for mainland Europe, even for small isolated populations with a high risk of decline. This suggests that reliance on standard indicators of inbreeding and diversity may be masking demographic factors that make small adder populations vulnerable to extinction. Consequently, risks of extirpation probably relate more to the small size of populations *per se* than to inbreeding.

2. Managing adder sites sympathetically

Presentations in this session gave an opportunity to learn from the experiences and practices of land managers concerned, at least in part, with adder conservation as well as managing many other, sometimes conflicting, commitments.

Tom Simpson (Natural England) used his presentation to address the question 'Do adders have a future in the Wyre forest?' which since the 1970s has suffered a mysterious long-term adder decline. Previously, adders were described as abundant in the Wyre Forest and its surrounds, even under a heavily industrialised landscape (charcoal production, tanning and railway), but the population has now crashed and in 2018 only 39 animals were recorded at 11 locations, down from a peak of 267 animals at 61 locations in 1991. This has prompted an urgent need for action that has included: improving adder habitat by positive management practices (Figs. 3a & b) such as creating open spaces, corridors linking populations, brash piles to create habitat corridors on newly cleared sites, a reduction in burning, and excluding people from sensitive areas (using fences and signs, and by making footpaths unsuitable). The impact of these practices will be monitored.

Next, Geraint Jones of the Pembrokeshire Coast National Park Authority explained that in the National Park the habitat suitable for adders is often squeezed into a narrow strip between intensively farmed land and rocky coastal cliffs. The justification for active management of the latter area has been compelling. During the last 20 years or so, a partnership including landowners and conservation organisations has succeeded in re-introducing positive, proactive management to the coastal zone. Additionally, advice and practical assistance are available from two voluntary umbrella organisations. The Pembrokeshire Grazing Network (PGN) helps to source appropriate livestock for conservation grazing, and the Pembrokeshire Wildfire Group (PWG) provides a lead on wildfire issues and best practice for controlled burning. PWG is a partnership of farmers and graziers, the Mid and West Wales Fire and Rescue Service, and local conservation bodies. It focuses on reducing uncontrolled wildfires and ensures that controlled burns are planned in accordance with relevant regulations. Informed and sensitive implementation of management actions (especially controlled burning) is critically important, and this is being achieved with the help of Pembrokeshire

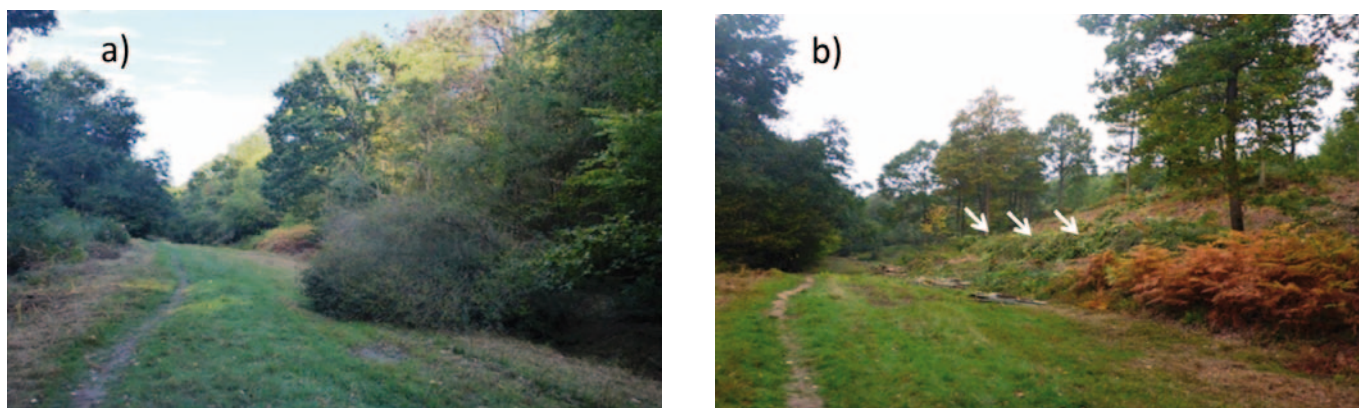


Figure 3. Improvement of a ride and adjoining bank in the Wyre forest **a)** unimproved ride, **b)** the same ride with bank opened up to basking, but with plenty of cover remaining and brash piles made of birch and oak crowns (arrows) placed along the bank to provide protection from people and dogs

Amphibian and Reptile Group through the development of predictive geo-mapping of adders. The geo-mapping identifies likely adder habitats and hibernacula to inform the decisions of land managers and even the fire rescue service, which can now access the maps via mobile terminals.

Finally, Kevin James of the Lincolnshire Wildlife Trust (LWT), presented the example of the management of the Woodhall Spa airfield cluster. As land manager, LWT encounters the widely reported problems of juggling the interests of different species and often conflicting targets, especially when maintaining 'favourable condition' on Sites of Special Scientific Interest (SSSI). Significant financial support for the management of wildlife sites comes from agri-environment schemes. Unfortunately, these schemes are directed at farming rather than conservation, which has resulted in many Wildlife Trusts being unable to enter the schemes, or in some cases having to consider changing their management practices in order to fit into the criteria. There are also difficulties with negative public perceptions such as with essential work such as tree felling, and the negative press coverage of adder bite incidents.

3. The Northern European Perspective

This session provided some fascinating insights into the situation elsewhere in Europe, where the drivers of adder declines appear similar to those in Britain, and where insights from long-term research offer potential solutions to our own problems.

Ina Blanke (Independent consultant) described the situation in Germany where the adder is considered a rare species restricted to the cooler areas. Stable populations still exist in parts of the Baltic Sea coast and at higher altitudes. Elsewhere, they are considered critically endangered due mainly to habitat loss in moorland (raised and lowland bogs), heathland, and the formerly large peatland complexes that are now much reduced due to land reclamation, peat extraction, and drought. The situation continues to deteriorate as there is increasing pressure from traffic and infrastructure development, intensive agriculture, particularly grazing that is supported by current agri-environment schemes, peat extraction which continues under long-term contracts, and climate warming exacerbating dehydration. Other concerns include

predation from exploding populations of wild boar, and site specific interventions such as the removal of purple moor grass that otherwise provides vital cover for adders. On nature reserves, a few remedial measures are being implemented to try and reverse this decline. Habitat is being improved with the creation of forest clearings and re-wetting (formerly disastrous in the way it was carried out, but more sympathetic nowadays). Pond creation is another important measure, since wetlands support prey species such as moor frogs. But the process needs to be ongoing, and more reptile sympathetic conservation measures are urgently needed, to which end a handbook for 'Reptile Friendly Management' will be published soon.

Dirk Bauwens and Katja Claus (U. of Antwerp) presented their 20-year study of an exceptionally large and stable adder population in the Groot Schietveld, military training area in northern Belgium. The habitat comprises a mosaic of lowland heaths, moorland ponds, woodlands and pastures over an area of ca. 1570 ha, but is entirely isolated from other adder populations. Using mark-recapture methods the group has built an extensive data-set of around 7300 records of some 3500 individual adders, from a population that at any one time numbers several thousand snakes. Adult adders undertake seasonal migrations between 'winter' and 'summer' habitats, where they go to forage and build up their fat reserves on the wet flushes, where prey is more plentiful than in the nutrient-poor 'winter' heathlands. Whilst adult adders are most easily observed in the 'winter' habitats, the immature and non-reproducing animals appear to spend most of their time in feeding habitats near, but outside of, the heathlands. The research also highlighted some important features of the adders' life cycle. Overall, the study found no evidence for age-dependent differences in yearly survival rates or between reproducing and non-reproducing females. Most females reproduce for the first time when they are 4 years or older, after this giving birth at 2-3 year intervals. However, on average females produced only 1.3 litters during their entire reproductive lifetime. A key finding is that overall population growth is most sensitive to the survival rates of immature adders. This is an important message, and indicates that attention should be given equally to both the important winter (heathland) habitats, where the adult

snakes are most easily observed near their hibernacula, and the often more neglected moist summer habitats that are so important for the immature adders. Special care is also required to maintain the migration routes between these two habitats.

Rolf van Leeningen (Reptile, Amphibian & Fish Conservation Netherlands – RAVON) indicated that The Netherlands was similar to Belgium with two large unbroken clusters of adder populations restricted to protected areas of heathland and raised bog. Research on the 800 ha Hijckerveld reserve also shows seasonal movement of adders between different habitat types. During the winter they inhabit the heathland areas, but in summer they move to wetter areas with greater prey availability. As elsewhere in Europe, habitat loss is the main threat to adders in The Netherlands, and this is being mitigated by a major project, 'Viper Verbindt' (Viper Connect) in the east of the country which aims to increase, connect and restore habitat for adders, whilst also benefitting other species. In addition, a special volunteer adder working group 'Werkgroep Adderonderzoek Nederland' (WAN), set up by RAVON, plays an important role in collecting research data that is entered into a new snake portal web application (www.snakeportal.org). Data can be stored, analysed, exported into GIS applications, and photos of head scales uploaded and compared for individual recognition. The user manual and internal workings are both available in English. Our second Dutch speaker of the day, Marnix de Zeeuw (Statistics Netherlands) introduced TRIM (TRENds and Indices for Monitoring data). TRIM is a statistical package for evaluating the conservation status of a species by calculating population trends and estimating population numbers. These numbers are based on repeated counts at various sites while counts may be missing for certain sites at certain times. Estimation depends on a model-based imputation method. TRIM has been rebuilt as an R package, RTRIM. RTRIM can work with multiple data sets and is a powerful tool for developing a broader European approach to monitoring and conserving adders. The package is available on the Comprehensive R Archive Network website (CRAN, 2019) and a manual and two articles that discuss the method are available on the Statistics Netherlands website (CBS, 2019).

Gaëtan Rey (Conservatoire d'Espaces Naturels, Nord Pas de Calais) presented the situation in the Haut de France, where adders are critically endangered. Genetic studies in 2010 indicated poor genetic diversity, although interestingly these animals are genetically closer to Belgian populations than the rest of France. Adders are a low priority for conservation and often ignored by site managers, who have little knowledge of their distribution on their sites, and as a consequence animals are frequently harmed by over-grazing and unsympathetic habitat work, particularly mechanical scrub clearance that damages hibernacula. An additional major issue is of persecution, since adders are widely disliked and feared. A number of regional action plans (2012-28), have been set up to try and redress these issues and have included all relevant stakeholders in discussions. The aims of these plans are threefold: to

train volunteers to monitor adder populations to improve knowledge; to conserve existing populations by developing more sympathetic land management practices, e.g. lower impact grazing regimes; and, to improve public perceptions through an outreach campaign. In 2018, a National Viper Working Group was created in France for both *V. berus* and *V. aspis*. This aims to develop a national monitoring programme, more sympathetic management practices, and work with land managers to improve viper protection nationally.

On behalf of Sweden, Thomas Madsen (U. of Wollogong) presented observations from two sites: Smygehuk, and the island of Hallands Väderö, which have been under investigation for nearly 40 years. At Smygehuk in the late 1980s, the small population of adders began a gradual decline and deformed neonates were detected. This suggested the negative impacts of inbreeding. As a potential solution, in 1992 twenty male adders were introduced into the population (Fig. 4). From 1998 onwards the population recovered (Fig. 4). Genetic studies using whole genome sequencing have demonstrated an increased diversity of genes resulting from the introduction (Fig. 5) and more detailed study has suggested that this has resulted in greater heterozygosity in at least the genes involved in innate immune responses to disease. Interestingly, adder numbers fell dramatically again in 2009/2010 but this bottleneck apparently had no lasting impact on either numbers in subsequent years (Fig. 4) or genetic diversity when assessed in 2017 (Fig. 5).

In contrast to the Smygehuk population, the isolated population on the island of Hallands Väderö has maintained high levels of genetic diversity despite the 200 adders on the island being isolated for 6,000 years. This implies that small isolated populations will not necessarily become inbred in the long-term. This is related to the adder breeding system. Adder populations have low fecundity and low vagility and might therefore be expected to be prone to inbreeding, but female adders are adapted to avoid this. One adaptation is that a single female may mate with several males before the eggs are fertilised (polyandry) resulting in litters with higher levels of genetic variation. Furthermore, Thomas Madsen's own insights suggest that the eggs themselves are responsible for sperm selection in a way that maximises heterozygosity. Consequently, only if populations are founded on a very narrow genetic base does this lead to inbreeding, characterised by still births and deformities. Such observations have implications for the sustainability of genetically enhanced populations. Once successfully enhanced, the adder breeding system should then, at least theoretically, be able to maintain genetic diversity without further intervention.

There was a further important observation from Sweden. Adders may be seriously limited by climate variations, a good example being the exceptionally hot dry year of 2018, when two populations in Sweden lost access to both surface water and dew. This led to a reduction in numbers of males by 80 % at one site and 50 % at the other. In addition, survivors had a 20 % reduction in residual body mass indicating that feeding had been disrupted.

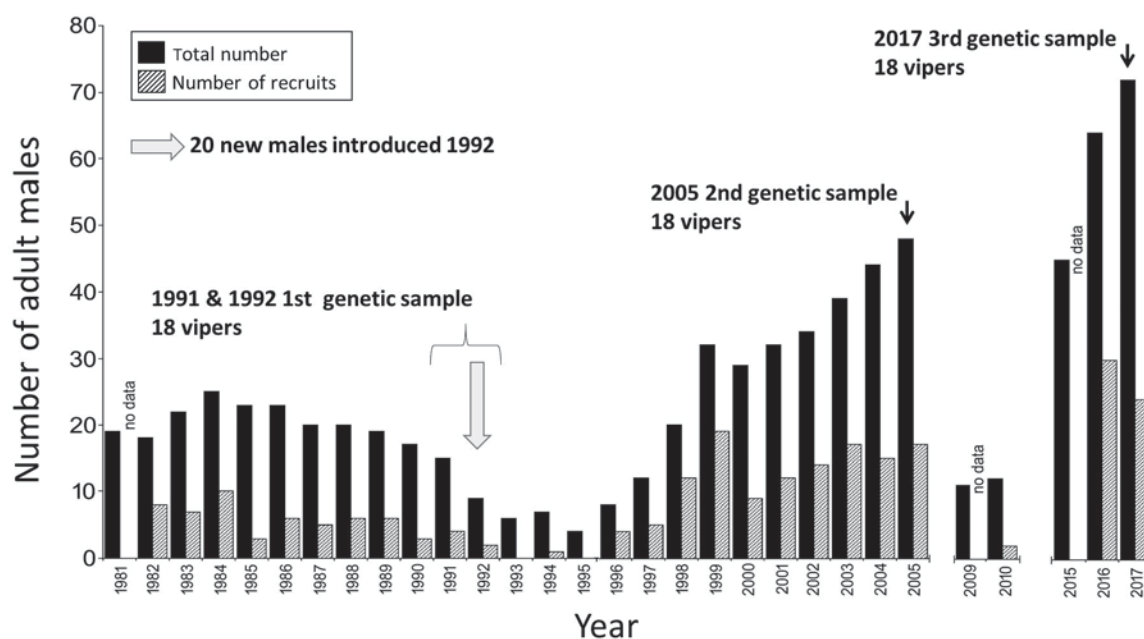


Figure 4. Genetic enhancement of a small *V. berus* population in Smygehuk, after 20 males were introduced in 1992. Population restoration is noticeable from 1998. Samples for genetic analysis were taken in 1991/1992, 2005 and 2017 (see Fig. 5). The introduced males were not included in the counts. In 1995, the surviving 8 introduced males were returned to their natal population.

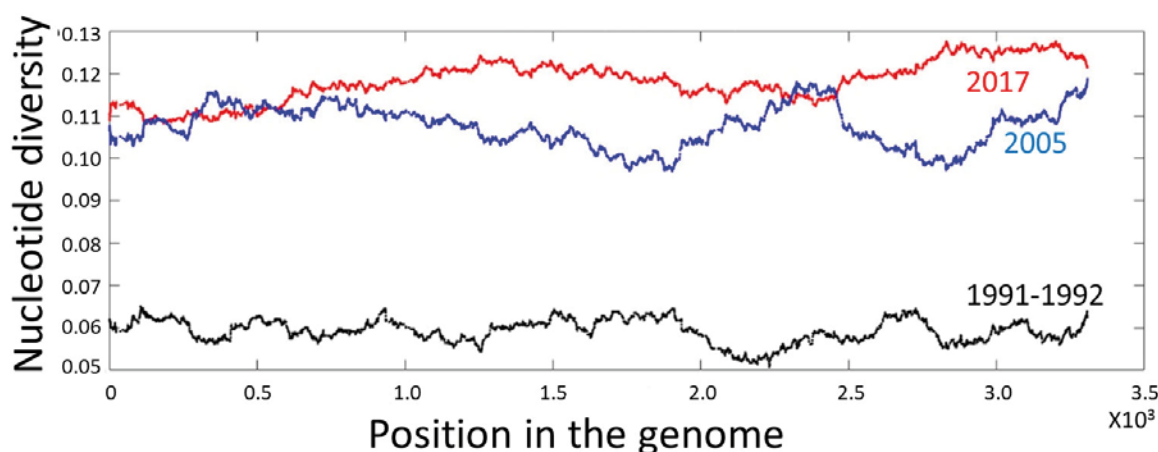


Figure 5. The genetic effect of introducing novel genes into the Smygehuk population of *V. berus*. Smoothed Manhattan plot of nucleotide diversity showing a highly significant increase in genetic diversity from 1991-1992 to 2005 and 2017.

This emphasises the potentially very damaging impact of periods of hot dry weather, which are expected to become more prevalent with future climate change.

4. Engaging volunteers and communities

Improving the public perception of adders is potentially a significant component in any strategy to help protect the species. To the naturalist community, adders are already a wildlife icon along with the red squirrel, otter and hedgehog, but in the wider population there is still a long way to go. This session indicated how we might engage people using the creative arts and how we should deal with the fact that adders are venomous.

The 'Adders are Amazing!' project, which was presented by Sam Langdon (ARG UK), is an excellent example of how a programme of community engagement can improve public perceptions. The project has piloted a range of activities to

change the attitudes of local communities in West Wales by combining scientific engagement with artistic elements such as creating Gwiber the giant paper adder (Fig. 1), creating adder stone trails, and producing a variety of other art works such as the adder quilt (Fig. 6).

The project has now engaged almost 2,000 members of the local community of all age groups with activities in seven local schools, as well as with more mature members of the community, e.g. University of the 3rd Age (U3A), Womens' Institute, and local craft, wildlife and history groups. To measure the impact of the programme, attitudes towards adders were assessed in 263 children aged 5-11, before and three months after each school had received between 0.5-10 hours of input from the ARG UK project leader and a local artist. The intervention showed a significant improvement in attitudes towards adders, more so in older children, even when participants maintained a dislike of these snakes.



Figure 6. Creation of a quilt, coloured with natural dyes extracted from plants collected in the adder's habitat, celebrating the species as part of the 'Adders are Amazing!' project

This change may reduce adder persecution later in life. The position of the adder relative to other species was also assessed after three months intervention and it rose from number six to number three, but still behind the dormouse and curlew.

'Adders are Amazing!' has shown that it is possible to change attitudes without direct experience of adders, and that creative activities and 'fun' types of engagement really do improve perceptions. Nevertheless, those wishing to improve public perception of adders must take into account that the species is venomous. This was considered by Wolfgang Wuster (U. of Bangor) who indicated that to be realistic conservation of adders requires reconciling the needs of an animal in chronic decline with hostile public perception grounded in fear. This fear is often nurtured by sensationalist press coverage when people or dogs are bitten. Addressing this issue requires a rational assessment of the risk posed by the adder to the public in general, but also of the dangers posed when a person is actually bitten. In public health terms, the adder poses a negligible risk. For PR purposes, this can be compared to everyday risks the general public can more readily understand, e.g. a member of the public is 10,000 times more likely to choke to death on a piece of food than to die of an adder bite. However, the risks of an actual bite must not be understated; while the majority are relatively trivial, a significant minority of bites can give rise to serious illness, and a small proportion are life-threatening. It is important for our own credibility not to down play the risks as in some cases (15 % in Sweden) bites can be severe and even life threatening. Adder bites are not like a bee sting, and are also unpredictable so that we never know in advance how serious the effects will be. Ironically, a survey of participants at the meeting showed that those working with adders were the worst culprits for not seeking medical attention, for most their strategy was to go home and 'wait and see' rather than immediately attend an accident and emergency unit as advised.

Reducing negative press coverage requires that the frequency of bites be lowered by public education in bite prevention, and by ensuring that those willingly interacting

with snakes take appropriate precautions. On the one hand, fact-based outreach is needed to avoid the trap of suggesting that adder bites are not serious. On the other hand, couching public education in inclusive language likely to elicit empathy with adders, rather than fear and hostility, may play an important role. One important area is public signage, which may often be expressed in terms that encourage dislike and fear (Fig. 7a) which in fact could be re-imagined in terms that encourage respect and appreciation (Fig. 7b).



Figure 7. Adder warning signage could be improved to make the public more sympathetic towards the species, a) A relatively negative message in actual use, b) The type of message that could evoke a more sympathetic response

Volunteer recording effort plays an important role in assessing the conservation status of the adder. Emma Gardner (U. of Reading) reported on ARG UK's 'Make the Adder Count' survey that records population trends for adders in Britain based on springtime emergence counts. Counting began in 2005 when volunteer surveyors were asked to make three or more spring visits each year to their survey sites, whilst also noting any positive and negative factors that could be affecting the adders. Between 2005 and 2016, 181 surveyors provided information on 260 sites. Of these sites 129 contributed data for three or more years and these were used to derive average population trends over time. The data confirmed that on average, sites with small populations (where surveyors typically record peak counts < 10 individuals, N = 117) declined by 55 % over the 11 year monitoring period. By contrast, sites with large populations (site with mean peak counts > 10 individuals, N = 12) on average showed a 33 % increase over the same period. If these trends are representative of Britain as a whole then within 15–20 years adders will become

restricted to just a few sites with a large population. Public pressure/disturbance was reported as the most frequent negative factor, affecting 48 % of sites, followed by habitat management and habitat fragmentation. Habitat management was reported as having negative impacts almost as frequently as positive impacts, suggesting that many management plans do not adequately consider the requirements of adders, a conclusion in agreement with the mind mapping exercise mentioned earlier.

5. Adder conservation the way forward

The final session considered how data collected by volunteers can be made to meet the needs of conservation planning, and what initiatives are in place now, or will be in the near future, to improve the status of the adder in Britain.

Steve Langham (Surrey Amphibian and Reptile Group - SARG) presented SARG's experience in developing an integrated approach to using volunteer survey and monitoring effort to drive conservation outcomes. SARG have successfully mobilised their volunteers to collect large numbers of amphibian and reptile records across the county but this begs two questions. First, what does this information really tell about conservation status? Second, how do we turn 'dots on maps' into conservation action? Historically, a number of factors have impeded these connections, including the diversity of approaches to data collection, multiple data sources, variable data quality, outdated information, inconsistency in survey effort, and less effort on 'less popular' sites.

To address some of these inconsistencies, SARG have developed a new internet-based software solution for volunteer groups called 'ARGWEB'. This is an integrated system for survey support that fuses data sources including those from effort-based structured surveys and opportunistic recording, and builds heat maps using the Conservation Assessment Model (CASM 4) to show the distribution of different species. This information is also made available to land managers and planning authorities through specific data portals, allowing decision makers access to real time information about their sites that can be used to support sympathetic conservation management. For adders, the in-built simple distribution modelling can provide survey targets, and deduce conservation status up to county level. This includes status trends, which can be an independent check on whether appropriate conservation measures are in place or whether a re-think is required.

The final presentation of the meeting was from Jim Foster (Amphibian and Reptile Conservation). He reviewed the current situation and identified opportunities for the future. Adder declines appear to be driven by a number of factors including: ecological vulnerability due to the preference for mid-successional habitat, modest dispersal, and small populations; inadequate sectoral awareness and prioritisation; and poor public and political understanding and support. A major concern is the increasing pressure on land, and conflict with agri-environment schemes. The current uncertainty surrounding the future of farm subsidies and land management has major implications for the adder.

There is no single answer to this complex issue, and we need a broad-based, systemic approach to solve the problem. This will include changes in policy, informing changes in agri-environment schemes notably the new Environmental Land Management Scheme (ELMS), better conservation practice and improved awareness through outreach programmes like 'Adders are Amazing!'. There is also a need to scale-up local good practice. Some measures are already being put in place with proposed revisions to SSSI selection criteria to include a single-species adder interest feature, protected sites need to be bigger, better and more joined up, and monitoring needs to be more objective and targeted. One issue that has arisen is the conflict between the needs of the adders and other site objectives, including species conservation, and recreational needs. One approach to reconciling these conflicts is through cross taxa projects, as currently demonstrated by the national 'Back from the Brink' programme and the 'Woodland Wildlife Toolkit'.

DISCUSSION OF CONSERVATION THEMES

During and after the meeting many potential components of adder conservation strategies were raised and discussed. Some of these are elaborated below.

More attention to habitat management

In Britain we have tended to focus our habitat conservation effort on hibernacula and springtime adder basking areas, and paid less attention to summer feeding areas. But, research shows that such feeding areas are very significant for the survival of immature animals; a key issue in adder demographics (Bauwens & Claus, 2018). A shift in emphasis is required to ensure these are better preserved and that migration corridors between summer and winter areas are identified and retained by providing adequate cover for movement such as hedgerows and ecotones with complex sward. Climate change, especially summers with long hot dry periods, has been seen to have very negative impacts on adder populations, as reported by Thomas Madsen for 2018 in Sweden. Ina Blanke confirmed that in Germany adders can only exist in otherwise dry areas where there is some standing water. Consequently, retaining moisture in habitats, especially the crucial summer feeding areas, is an essential conservation effort. One approach may be to build more ponds on adder sites, as they have done in Germany, to offer drinking water and to increase the availability of amphibian prey.

More broadly, certain habitats could be designated as ARKs for adder conservation as suggested by Nigel Hand, who is currently working towards the development of ARK sites in the West Midlands. The advantage of such an approach would be that key adder sites are recognised, and management would prioritise adder conservation based on a detailed understanding of the adder populations present. In addition, the populations could be easily assessed for their suitability in providing specimens in support of the genetic or demographic enhancement of other sites.

It was suggested that better defined legislation is needed to ensure that planned burning is on a stronger

environmental footing especially that it should be cyclical (every 7-10 years), there should be effective fire breaks, and a prescribed burning season. It is also clear that the public frequently present a problem and this raised the question of how best they should be engaged, and how access to sensitive areas can be limited. For limiting public access, one suggestion was the use of brush piles to help limit interactions between adders and dogs. They can also be used to channel people away from sensitive areas.

Restoration of populations by genetic or demographic enhancement

The 'Make the Adder Count' survey has highlighted the vulnerability of Britain's small and potentially isolated adder populations (Gardner et al., 2019). These may be at risk because their genetic diversity is too low, resulting in inbreeding depression, and/or because their demographics are unfavourable, i.e. their reproductive output is insufficient to prevent eventual extirpation. The solution to these two problems may not be the same. Greater genetic diversity can be achieved by adding new males to the population as described by Thomas Madsen (Madsen et al., 1999). Males are chosen for this purpose as they have the advantage of breeding every year, unlike females that generally only breed every second year. But if there is a demographic problem then the solution is to raise recruitment by adding more females to the population, although additional males might still be required. Nevertheless, each threatened population will have its own unique issues and may require an individually tailored solution beyond just bolstering the population. What life stage is released and at what time of year will probably depend largely upon what adders are available, and when. However, in the case of older sub-adults and adults the fixed behaviour patterns they develop within their natal habitats may make them a sub-optimal choice for release (Hodges & Seabrook, 2019; Nash et al. 2018).

The genetic or demographic restoration of small isolated populations is potentially a vital component of the conservationist's toolkit. But such practice has not been fully researched and might present various risks including depletion of the donor population, death by disorientation/predation to the individuals translocated, and transmission of disease or parasitic infection to the beneficiary population. Richard Griffiths summed this up as "...we are in the classic conservation dilemma of needing to make some important decisions based on incomplete evidence. Acting too hastily with only limited evidence could mean expensive mistakes; waiting until we have done more research may be too late. As a group we need to decide where on this spectrum we should intervene and how it will be resourced and implemented".

Under what circumstances and how population restorations should be attempted both require urgent consideration. The best way forward is likely to be through carefully controlled pilots that generate a body of evidence justifying enhancement. In the case of genetic enhancements, knowledge of the prior genetic condition of both donor and beneficiary populations should underpin at

least the pilots. An important step to facilitate this would be to develop a genomic Single Nucleotide Polymorphism (SNP) toolkit with which to analyse samples in such a way that the data from different sites are comparable, leading to a robust database on the genetic health of adders. The genomic sequencing effort to develop the SNPs would be a one-off initiative separate from the recurring costs of genetic analysis of study populations. As to the source of adders for enhancements, mitigation projects are a potential option, although the pros and cons of such an approach need exploring in the context of a species management strategy. There are many commercial adder translocations undertaken in mitigation of development and offering translocation sites to ecological consultants based on well-defined conservation objectives is likely to be a considerable improvement on existing practice where there is generally no conservation objective other than avoiding immediate death at the development site. In the long-term, we need to build mitigation/receptor partnerships. This could be managed conveniently by an adder database and this could perhaps become a future function of the ARGWEB being developed by Steve Langham. More difficult is to ensure that these population enhancements are carefully monitored for a period long enough to determine whether restoration is sustainable. This could be limited to a demonstration of increased genetic diversity of immature animals within the first two or three years of enhancement, but demographic enhancements may take much longer requiring successful completion of the life cycle.

Greater legal protection

Concerns were expressed about the persecution of adders in France, although apparently there is no persecution in Sweden, which raises the question about the need to increase legal protection for adders and their habitats in Britain. There was general agreement that this is only worth doing if it is enforced. Smooth snakes and sand lizards are already European Protected Species (EPS), but both have suffered from planned habitat burns in the New Forest, and there has been no prosecution brought forward. Would it be any different for adders? Adders will only be truly safeguarded by a greater public awareness and sympathy and in part this could be generated by investment in projects such as 'Adders are Amazing!' that both engage the public and provide working material for celebrity naturalists and the media. Perhaps in time we can arrive at the happy situation in Sweden!

Making the best of opportunities with environmental schemes

It is believed that the current agro-environmental schemes are in some measure detrimental to wildlife but that the new Environmental Land Management System (ELMS) could offer a better alternative. It will pay farmers for environmental services and benefits, with pricing based on a natural capital valuation approach. The benefits include: improved air, water and soil quality, increased biodiversity, climate change mitigation, cultural benefits and better protection of historic environments. It is important that

conservationists engage with the roll out of ELMs to obtain the best benefits for wildlife and particularly reptiles that must be included in biodiversity indices and/or targets.

LOOKING FORWARD

The meeting presented a wide range of actions for improvements in the conservation status of British adder populations and these should be considered as part of a national adder strategy. They included actions to:

- Create a National Adder Working Group (NAWG) to decide how to move conservation activities forward
- Link and co-ordinate those involved in adder research and land management in a larger informal Adder Network to ensure a free flow of ideas, information and advice between each other and the NAWG
- Set protocols or guidelines for genetic and demographic restoration of small populations, based on evidence from pilot studies, and implement genetic testing in a manner that data from different sites will be comparable
- Establish a suite of 'ARK' sites for focused adder conservation that can lead the way in establishing best practice
- Continue to gather data and monitor adders, to increase the volume of evidence available to pressure policy makers into action with regards to improving legislation and incorporating adders/reptiles in SSSI criteria and ELMS
- Engage with the proposed revisions to SSSI selection criteria to include an adder interest feature, and ensure that adders are better supported in areas where they are scarce or declining
- Achieve greater public sympathy for the species by further support to projects such as 'Adders are Amazing!'
- Investigate and support linking adder conservation actions to those of complementary species, for example butterflies such as the Pearl border fritillary and Duke of Burgundy, other reptiles and invertebrates, mammals, birds and plants
- Explore achievable approaches to more sympathetic management practices, such as finding alternatives to the inappropriate use of heavy machinery on important adder sites, managing bracken more sensitively, establishing the benefits of pond construction for adders, etc.
- Promote management strategies on adder sites that will result in carefully considered grazing timings and stock densities, and best practice for controlled on-site burns, and
- Initiate research into understanding and mitigating the impact of predators, particularly alien species such as wild boar and pheasants.

The problems identified in different countries/regions demonstrated similar themes including the negative impacts of infrastructure development and housing, intensive agriculture, the loss of good quality natural habitats, and climatic change, which are fragmenting populations

and driving adders into smaller pockets. Furthermore, persecution in Britain and France is thought to have systematically reduced populations in some areas. In many countries of mainland Europe the adder is no longer widely distributed and, although large populations can be sustained on protected land, this depends on its continued protection and sympathetic site management. Indeed, it could be argued that across many parts of northern mainland Europe the adder can no longer be described as a 'widespread species'. Based on the evidence presented, it seems likely that this pattern could easily be repeated in Britain, with small and fragmented populations failing to thrive, and negative environmental factors reducing breeding success and juvenile recruitment. Without intervention, the adder could become a 'rare species', flourishing in just a few areas, such as Surrey and coastal Wales and Scotland, but vanishing elsewhere.

However, there is also good news. Collectively, we have a large body of knowledge and experience. There are many people championing the adder, representing a range of different disciplines: volunteers, NGOs, academic researchers, land owners, and statutory bodies, so the species will not slip away unnoticed. If we can gather and implement best practice, support and inform land owners, champion adders with local communities, and continue to gather sufficient evidence to prompt policy makers to include adders in any new wildlife legislation, then we may be able to halt the declines. And the chances of success will be all the stronger if we continue to share experience between ourselves and our European neighbours.

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Vicente's poison frog (*Oophaga vicentei*) in the wild: calling activity, bioacoustics and diet

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ABSTRACT – Calling activity, bioacoustics and the diet of males is reported for three different populations of *Oophaga vicentei* in central Panama. Calling activity was predicted by time of day, season and temperature with peaks between 08:00 h to 10:00 h and was more frequent during the dry season. Only at one location was calling activity positively correlated with air temperature. The temperature was also positively correlated with call repetition rates and negatively with call duration. Within the ten first calls of a call-bout, there was a cline variation in dominant frequency, call duration and silent intervals. The stomach contents of males comprised mainly insects but also a large number of oribatid mites.

INTRODUCTION

Vicente's poison frog *Oophaga vicentei* (Jungfer, Weygoldt & Juraske, 1996) is an arboreal dendrobatid frog endemic to Panama. Since its description, few data on the natural history or ecology of this species have been published. *Oophaga vicentei* is included in appendix II of CITES and is considered as data deficient by the International Union for Conservation of Nature (IUCN SSC Amphibian Specialist Group, 2019).

Acquiring baseline ecological data is important not only to contribute to our understanding of the natural history of a species but also, critically, for establishing and monitoring its conservation status and ranking (Rodrigues et al., 2006). Here we present data from observations in the wild on the calling activity, differences in bioacoustics, and diet in three *O. vicentei* populations along the Pacific and Caribbean slopes in the Santa Fe National Park of central Panama.

MATERIALS AND METHODS

Study site

Field work was conducted within, and in the buffer zone of, the Santa Fe National Park (SFNP) located in the north of Veraguas province in central Panama. Coordinates of the exact study sites are not provided to avoid the threat of illegal collection. The study sites were located on the continental divide (Loma Grande, 750 m a.s.l.), and on the Pacific (Isleta, 460 m a.s.l.) and Caribbean (Alto Ortega, 380 m a.s.l.) slopes of the park. In addition, we provide preliminary data from previous surveys of two other populations from Santa Fe and one from the Caribbean slope in the district of Donoso (Fig. 1). Field data from the first three populations were collected during the end of rainy season 2015 (November), dry season

2016 (January to April) and rainy season 2016 (May to September), so season was coded as either 'dry' or 'rainy' in the analysis. In addition, oscillograms and spectrograms of *O. vicentei* and for most Panamanian species within the genus are shown for further comparison (Supplementary materials, Fig. 1).

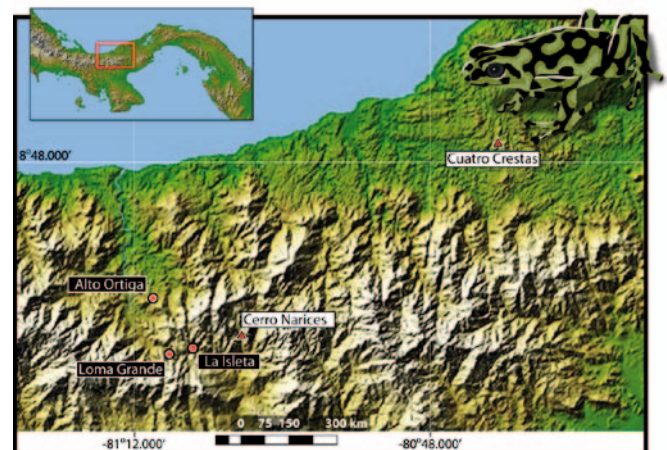


Figure 1. Distribution map of populations of the three focal *O. vicentei* study sites: Loma Grande (on the Continental Divide), La Isleta (on the Pacific slope), Alto Ortega (on the Caribbean slope), and the two additional sites included in the bioacoustics analysis: Cerro Narices (Pacific slope) and Cuatro Cuestas (Caribbean slope)

Biometric measurements

We located focal males by searching for their calls, and then climbing trees with the aid of a wooden ladder. We used nitrile gloves (Safe-touch: Dynarex New York, USA) for safe capture and handling. Snout-to-vent length was calculated

from photographed individuals (with Canon PowerShot D30: 12.1 megapixels: Canon Inc., Tokyo, Japan) using Image J (Rasband, 1997). Body mass was measured to the nearest 0.1 g using a portable balance (AWS-100: American Weigh Scales Inc., Georgia, USA).

Calling activity and environmental data

As with other dendrobatids, *O. vicentei* is active during the day, therefore the call-bouts of males were noted during daylight hours as a proxy for calling activity. Daytime was divided in two main periods; morning (07:00 h -10:00 h) and afternoon (14:00 h – 17:00 h) and the number of call-bouts heard was quantified in each three-hour period. Based on our field experience, upon arrival at the site, we waited 30 s before initiating acoustic recordings which did not cause any appreciable disturbance (Dorcas et al., 2010). We recorded the temperature and relative humidity every 8 minutes, using a portable thermo-hygrometer (model Atmos Skywatch: JDC Electronics, Switzerland). Site position and elevation were recorded with a GPS (Garmin eTrex 20x: Garmin International Inc., Olathe, KS, USA). Like other dendrobatids, *O. vicentei* is territorial (Lötters et al., 2007). We identified the territory of a single vocalising male at Loma Grande, and recorded its individual calling behaviour over the course of 24 h. We placed a long-term recorder (Wildlife acoustics Song Meter SM4) in front of the focal male (0.5 to 1.5 m away from it and 2 m above ground), and set it to record from 12:38 h to 12:15 h the next day (9 – 10 July 2017). Audio files were saved every 15 minutes and opened in Raven 1.5.0, as page sound (3 min duration, page increment 90 %); every call from the first day was analysed using the Band Limited Energy Detector tool, with the following preset: Minimum Frequency 4700 Hz, Maximum Frequency 6600 Hz, Minimum Duration: 0.19156 s, Maximum duration 0.3541 s, Minimum separation: 0.09288 s, Noise: SNR Threshold (dB) 28.0 above. The remaining parameters were set as default. It was not possible to analyse a single call from the next day, as the male moved farther from the microphone, and when trying to analyse calls under the interactive detectors, proper identifications were not possible due to an increase in background noise. As such, entire calls were only analysed for the morning recordings.

Bioacoustics

Male advertisement calls were recorded from the ground using a Marantz PMD660 handheld digital recorder (Marantz Professional, Cumberland, USA), using a Sennheiser ME66 microphone with a K6 powering module and a MZW66 foam windshield (Sennheiser Electronic GmbH, Germany) directing the microphone upwards. Recordings were made at a sampling rate of 44 kHz and 16 bits resolution in uncompressed PCM format and saved as wav-files. Ten calls from the middle of a call-bout were analysed per individual. For each call we analysed call duration, call interval, calls per bout, and maximum, minimum and dominant frequencies. The spectral and temporal parameters were analysed, and the power spectra were calculated in Raven Pro 1.5 (Window: Blackman, DFT: 2048 samples, 3 dB filter bandwidth: 158 Hz; Grid spacing 21.5 Hz; overlap 70.1 %; Charif et al., 2004).

Lowest and highest frequencies were measured 10 dB below peak frequency, avoiding overlap with background noise (Simões & Lima, 2011). Terminology used in the advertisement call description follows Zimmermann (1990) and Erdtmann & Amézquita (2009). The call repetition rate was calculated as number of calls per call-bout duration. In case of heavy rainfall and/or wind, no recordings were made. Data from only the individual male at Loma Grande was used here to describe the entire calling behaviour of the species during the day.

Diet

The stomach content of captured adult males was extracted using the flushing method which has been previously employed in amphibians with no evidence of harm to the animals (Born et al., 2010). A small-modified pipette tip was gently introduced inside the mouth to reach the pharyngeal area. To induce regurgitation an influx of tap water was introduced using a syringe. The content was emptied on a Petri dish, then collected using a plastic pipette, and deposited in 2 ml Eppendorf tubes with 10 % formalin.

Insects were identified under a stereomicroscope (NIKON, model SMZ445: Nikon Instruments Inc.) to order and when possible to genus using the keys of Palacio & Fernández (2003) and Krantz & Walter (2009). The vast majority of samples were heavily digested, making a precise identification difficult.

To determine the niche breadth we calculated the Levins index (Levins, 1968) which accounts for the uniformity in resources consumed (in our case, insect orders) for each individual in each population. We also followed the calculation of the standardised Levins index as indicated in Krebs (1999).

Data analysis

Data were transformed in order to meet parametric assumptions; otherwise non-parametric tests were used. The main effects of independent variables on calling activity was assessed using ANOVA and their interaction assessed using ANCOVA. Post-hoc testing was used to evaluate differences in air temperature among sites (Benjamini-Hochberg tests) and differences in call properties between populations of *O. vicentei* (Bonferroni tests). The relationships between climatic variables and the call properties were evaluated by linear regression analyses. A value of $P < 0.05$ was considered significant. All values reported are mean (\pm SEM) unless otherwise indicated. The analyses were undertaken in R v.3.4.3 (R Development Core Team, 2017).

RESULTS

Biometric differences

For biometric measurements, 21 adult males were captured at Loma Grande (SVL = 17.33 ± 0.01 mm, mass = 0.35 ± 0.01 g), 11 at Isleta (SVL = 18.23 ± 0.28 mm, mass = 0.43 ± 0.02 g) and 9 at Alto Ortiga (SVL = 17.82 ± 0.24 mm, mass = 0.48 ± 0.02 g). There were no differences in body size among the three frog populations controlling for body mass (ANOVA; Site: $F_{2,36} = 2.04$, $P = 0.14$, Mass: $F_{1,35} = 5.37$, $P = 0.03$, Site

x Mass: $F_{2,33} = 1.20$, $P = 0.31$). However, body mass was different among sites (ANOVA; Mass: $F_{2,36} = 16.84$, $P < 0.001$) the values of Loma Grande being smaller than those of Isleta (post-hoc Bonferroni test, $P = 0.004$) and Alto Ortiga (post-hoc Bonferroni test, $P < 0.001$) with no difference between Isleta and Alto Ortiga (post-hoc Bonferroni test, $P = 0.13$).

Calling activity

From all study sites combined we registered a total of 3042 call-bouts, averaging 31.09 ± 0.70 call-bouts per hour from 07:00 h to 10:00 h and 10.86 ± 0.30 call-bouts per hour from 14:00 h to 17:00 h. Overall the number of call-bouts was affected by time of the day, with more call-bouts in the morning than the afternoon (ANOVA, Time: $F_{5,93} = 3.92$, $P = 0.003$, Fig. 2). There was a calling peak between 08:00 h and 10:00 h, after which calling decreased with time across the day. In Isleta and Alto Ortiga in particular, peak calling activity occurred between 08:00 h and 09:00 h, while in Loma Grande peak was between 09:00 h and 10:00 h; in all three populations calling decreased in the afternoon (Supplementary material, Fig. 2). A single focal male from Loma Grande called 248 times from 06:17 h to 18:05 h, averaging 22.55 call-bouts per hour, with peaks in the morning between 07:00 h and 08:00 h ($n = 22$), around noon 12:00 h ($n = 30$) and around 17:00 h ($n = 38$). No calls for this focal male were recorded at 14:00 h (Supplementary materials, Fig. 3).

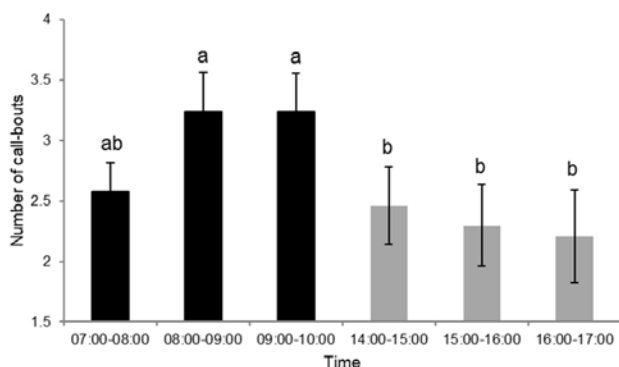


Figure 2. Calling activity differences between morning (black) and afternoon (grey) periods. To meet parametric assumptions number of call-bouts was transformed as $\log_{10}(\text{Calls h}^{-1} + 4)$. Values are means \pm SEM. Means without a letter in common are significantly different ($P \leq 0.05$).

Calling activity was affected also by season (ANOVA, Season: $F_{1,97} = 4.75$, $P = 0.032$), with higher calling activity during the dry season compared to the rainy season. However, there was no interaction between time of the day and season (ANCOVA, Time x Season: $F_{5,87} = 0.38$, $P = 0.86$).

In our dataset, air temperature and air relative humidity were inversely correlated (Spearman, $S = 272520$, $\rho = -0.73$, $P < 0.01$). Hence, to avoid autocorrelation, we chose one variable for inclusion in subsequent analyses. Air temperature differed among the three study sites (ANOVA, Site: $F_{2,95} = 19.63$, $P < 0.001$, Supplementary materials, Table 1). Air temperature was significantly higher in Alto Ortiga than at Isleta (post-hoc Benjamini-Hochberg test, $P < 0.001$)

and was significantly higher in Alto Ortiga than at Loma Grande (post-hoc Benjamini-Hochberg test, $P < 0.001$). Air temperature was not different between Isleta and Loma Grande (post-hoc Benjamini-Hochberg test, $P = 0.11$). The interaction between air temperature and site affected calling activity (ANCOVA, Temp x Site: $F_{2,92} = 5.41$, $P < 0.01$, Fig. 3).

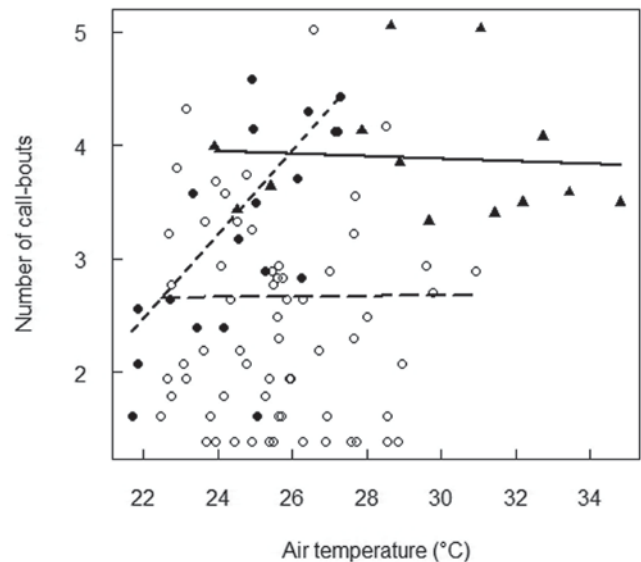


Figure 3. Interaction plot between site and air temperature on the number of call-bouts of *O. vicentei*. Filled circles and dash line represent Loma Grande; open circles and long dash line represent Isleta; the black triangles and solid line represent Alto Ortiga. To meet parametric assumptions number of call-bouts was transformed to $\log_{10}(\text{Calls h}^{-1} + 4)$.

Bioacoustics

A total of 7,322 calls from 41 male *O. vicentei* were analysed with a series of 29 to 290 pulsed calls in each call-bout (75.60 ± 51.31 pulsed calls; Supplementary materials, Table 2).

Bouts had a duration of 24.25 s (range 4.57–51.61 s, SEM 7.92, $n = 230$) with intervals between bouts averaging 108 s (range 5.04–717.34 s, SD 128.41; $n = 219$). The calls within a bout were repeated at a rate of 1.03 to 3.40 calls/second (2.35 ± 0.48 calls/second; Supplementary materials, Table 2). Call duration is variable (Coefficient of variation (CV): 28.52), ranging from 0.089–0.31 s. The dominant frequency is less variable (CV: 4.65), at $5736.9 \text{ Hz} \pm 266.76$ (Supplementary materials, Table 2). From the recorded calls of the single male at Loma Grande, we found that call-bouts are characterised by a large initial interval between individual calls; these intercall intervals become progressively shorter across the first approximately 10 calls (Supplementary materials, Fig. 4). After this the intercall interval becomes more homogenous, with the intercall intervals becoming roughly constant. There is also a progressive increase in call frequency, with the three first calls at lower frequency than the rest (pair-wise KW, $P < 0.05$, $n = 6329$, Supplementary materials, Fig. 5). It was not possible to measure the number of pulses per call, as we recorded in natural field conditions with

most recordings overlapped with background noise. Most recorded frogs called from high in the canopy (from 5 m to 15 m approximately); the distance between the microphone and the focal frog was too great to obtain a better signal to noise ratio.

Air temperature was positively correlated with call-bout repetition rates ($R^2 = 17.5$, $P < 0.05$), and negatively correlated with call duration, but only for the Loma Grande population ($R^2 = 0.59$, $P < 0.05$, $n = 6$; Supplementary materials, Fig. 6).

No correlation was found between these variables for Alto Ortega ($R^2 = 0.16$, $P > 0.05$, $n = 9$; Supplementary materials, Fig. 6) and Isleta ($R^2 = 7.13 \times 10^{-4}$, $P > 0.05$, $n = 21$; Supplementary materials, Fig. 6). The dominant frequency was different between the populations of Alto Ortega and Isleta (post-hoc Bonferroni test, $P < 0.05$; Fig. 4 & Fig. 5). Data from Cerro Narices and Donoso populations were not included in this analysis due to small sample size.

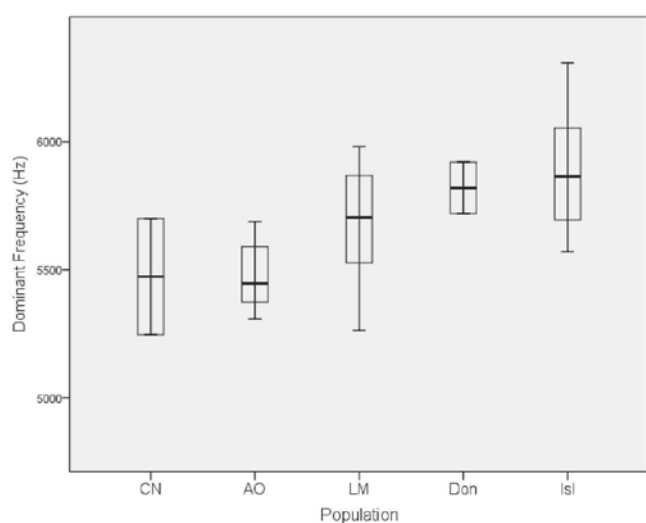


Figure 4. The dominant frequencies of the advertisement call of *O. vicentei* in our five study populations. We only found significant differences in dominant frequency between the Alto Ortega and Isleta populations (post-hoc Bonferroni test, $p < 0.05$). CN: Cerro Negro, AO: Alto Ortega, LM: Loma Grande, Don: Donoso, Isl: Isleta

Diet

A total of 27 males were sampled for their stomach content, of which 10 were from Loma Grande (dry season), 8 from Isleta (dry and rainy season) and 9 from Alto Ortega (rainy season). Although it was difficult to identify the items in the samples due to their advanced digested state, we found the arthropod orders Hymenoptera (ants), Diptera (flies) and Sarcophiles (oribatid mites) were common in all three populations, with Araneae (spiders) and Isopoda (isopods) present in smaller numbers (Fig. 6). In all three populations the ant genera identified were *Solenopsis*, *Crematogaster* and *Tapinoma* sp. near *ramulorum*, the last one a new inclusion in the diet of species of the genus *Oophaga* (Supplementary materials, Table 3).

There was no significant difference in niche breadth among the three populations according to the normal Levins index (ANOVA, Site: $F_{2,24} = 0.057$, $P = 0.94$, Supplementary materials, Table 4), nor the standardized Levins index (ANOVA, Site: $F_{2,24} = 1.58$, $P = 0.22$, Supplementary materials, Table 4).

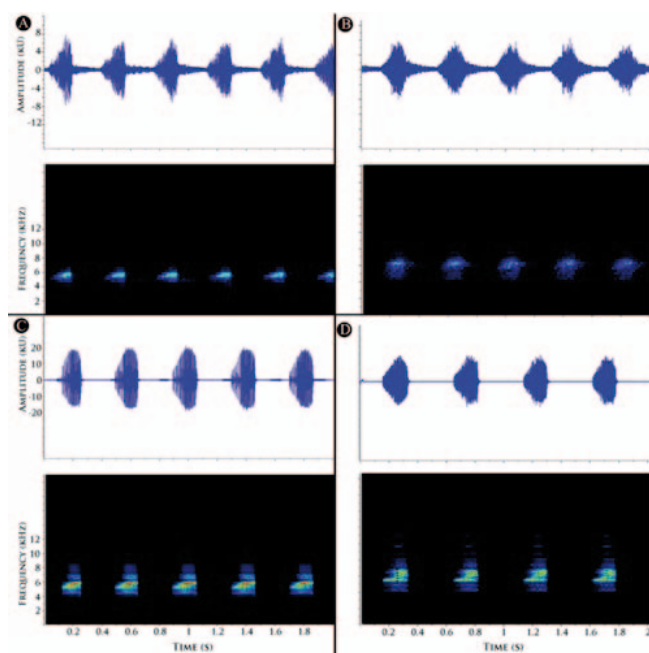


Figure 5. Oscillograms (above) and spectrograms (below, only dominant frequency is shown) of the advertisement calls for the populations of *O. vicentei* in this study; **A)** Isleta; **B)** Alto Ortega; **C)** Cerro Narices; **D)** Donoso, Colón

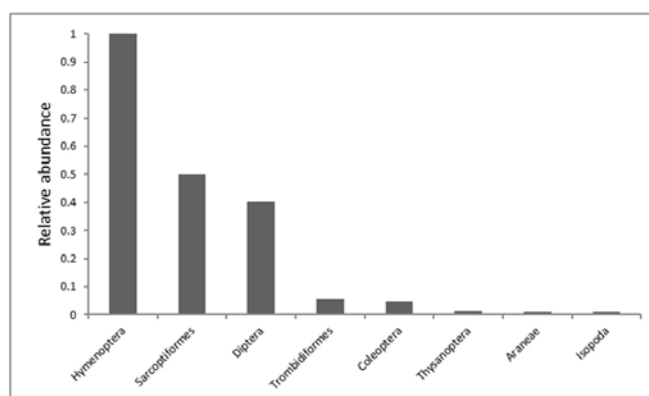


Figure 6. Relative abundance (proportion of frog stomach content) of arthropod orders found

DISCUSSION

Due to the logistical difficulties of studying this small, cryptic and arboreal species, we were unable to estimate with confidence the number of frogs calling at each site. With the obvious limitations we used number of call-bouts heard as an indicator of calling activity. To our knowledge this is the first time activity patterns have been described for an arboreal dendrobatid species. Overall our results indicate that *O. vicentei* males show peak calling activity between 08:00 h and 10:00 h, with decreased calling across the afternoon (Fig. 2). Our results are similar to findings for *Oophaga pumilio*, closely related to *O. vicentei*, which shows peak calling activity between 08:00 h and 10:00 h. In that species males call from spots on the ground or just above ground in the understorey vegetation (Bunnell, 1973) and this activity peaks in the morning between 07:45 h and 09:15 h (Graves,

1999). *Oophaga vicentei* spends most of its time high in trees (but see Peña et al., 2016), and as such may receive increased levels of early morning light triggering calling behaviour earlier than in ground dwelling frogs.

Overall calling activity during the dry season was higher than during the rainy season. Anuran calling activity is known to fluctuate with the onset of seasons especially in temperate environments (Saenz et al., 2006). The mountainous conditions in Santa Fe result in a dry season (December to April) marked by trade winds with increased hours of sunlight, and sporadic showers and fog during early morning at elevated sites (Macinnis-Ng et al., 2014). Although we did not measure rainfall during our study, mean values of relative humidity were above 80 % during dry season months, allowing dew formation to fill bromeliad axils with water, a suitable location for *O. vicentei* reproduction (Lötters et al., 2007).

In Loma Grande, air temperature was positively correlated with calling activity, unlike the other two sites (Fig. 3). Loma Grande was at the highest elevation (750 m a.s.l.), situated on the continental divide that separates the Pacific from the Caribbean slopes of Panama, and was subject to cooler air than the sites at lower elevations (Supplementary materials, Table 1). However, we cannot conclude that these results are due to population differences alone since, we have not estimated the number of calling frogs from each population. In amphibians, an increase in ambient temperature may elicit higher metabolism (Rome et al., 1992), hence for the frogs at Loma Grande a slight increase in air temperature could have boosted calling activity.

The calls of *O. vicentei* were similar among the populations, with differences primarily in dominant frequency. However, the calls of *O. vicentei* can be distinguished from the calls of other *Oophaga* spp. from western Panamá, by multiple acoustic parameters, including dominant frequency (DF), call duration (CD) and call rate (CR), as follows: *O. vicentei* (DF = 5736.9 Hz, CD = 0.17 s, CR = 5.32 Calls/2s; this study); *O. granulifera* (DF = 4010, CD = 0.38, CR = 4 calls/2s, in: Erdtmann & Amézquita, 2009; Myers & Daly, 1976), *O. pumilio* (DF = 4856, CD = 0.043 s, CR = 7.8 Calls/2s; in: Erdtmann & Amézquita, 2009; Myers & Daly, 1976) and *O. arborea* (DF = 4364, CD = 0.107, CR = 6.25 Calls/2s, n = 10, this study, see Supplementary materials, Figure 1).

Most published analyses of frog vocalisations usually include only single calls. *Oophaga* spp. from western Panama, however, calls in bouts. To our knowledge, ours is the first description of the entire series of call-bouts of an *Oophaga* spp. We found cline variation within the ten first calls within a call-bout in *O. vicentei* in dominant frequency, call duration and silent intervals. Particularly, the first call is spaced from the second call by almost double the silent interval between two calls in the middle of the call-bout. While the significance of this call structure is unknown, it is possible that the presence of this isolated call within a call-bout could convey information to receivers, but the importance of this in *O. vicentei* acoustic communication has yet to be determined.

We found differences in dominant frequency among the populations from Santa Fe National Park. Although we did not

measure the body size of specific calling males, there were no significant differences between the body sizes among specimens collected from different sites, only in terms of body mass. Body size can be negatively correlated with dominant frequency, with larger frogs calling at lower frequencies and vice versa (Gerhardt & Huber, 2002; Erdtmann & Amézquita, 2009). However, for at least some *Oophaga* spp., this is not the case as no correlation has been found between body size and dominant frequency (Prohl, 2003; Vargas-Salinas & Amézquita, 2013), even though other species can vary their dominant frequency by increasing their vocal cord mass or adjusting its tension (Ryan & Drewes, 1990; Vargas-Salinas & Amézquita, 2013). With the data we have, we could not determine whether the differences found in dominant frequency are an artefact of body size or mass alone, as we were unable to measure calling males. Differences in frequency could also be due to geographic distances. We found no frequency differences between the Loma Grande and Isleta populations, on the Continental Divide and the Pacific slope, respectively, but did find differences between these and the Alto Ortega population, on the Caribbean slope. Average dominant frequency values for the Cerro Narices population, which is on the Pacific slope, were however similar to those from Alto Ortega. Nevertheless, variation in dominant frequency has been found among other *Oophaga* spp., and could be explained by geographical distances, isolation or the selective pressure of habitat (Myers & Daly, 1976; Prohl, 2003; Vargas-Salinas & Amézquita, 2013). Despite this difference in dominant frequency we did not find differences in the coefficient of variation (CV), which was similar among all populations. Since the CV is a less variable trait (than dominant frequency itself), evaluating differences between individuals or populations could be important in taxonomic differentiation. We would argue that the differences we found in the raw values of dominant frequency among populations are not enough to differentiate these populations as distinct taxonomic units using this trait alone.

Temperature can affect temporal properties in ectotherms (Gerhardt & Huber, 2002). As in its congener, *O. pumilio*, temperature influenced the temporal call properties in *O. vicentei*: in both species, temperature was positively correlated to call repetition rates and negatively correlated to call duration (Myers & Daly, 1976; Prohl, 2003). Although call duration was significantly negatively correlated to temperature for the population at Loma Grande, only a slightly negative relationship was found for the other two populations. After removing the effect of temperature, we did not detect significant differences among populations in either call repetition rates or call duration.

The diet of our sampled males was dominated by arthropods, mainly ants and oribatid mites, which have been described in the diet of other species of dendrobatids linked to a bioaccumulation of defensive alkaloids (Saporito et al., 2009 & 2012). Ants were common in the diet of the three study populations (Supplementary materials, Table 3) including the genus *Solenopsis* which is known as a source of pumiliotoxin alkaloids for defensive purposes in poison frogs (Saporito et al., 2004). It was interesting to also discover ants

of the genus *Tapinoma* as part of the diet of *O. vicentei* as they are known to contain the repellent alkaloid actinidine (Tomalski et al., 1987) although this has not been identified as a defensive alkaloid in *O. vicentei*. Oribatid mites also represent an important part of the alkaloid provision in poison frogs (Saporito et al., 2007). There is evidence of a diverse array of alkaloids shared between mites and *O. pumilio* poison frogs, some of them also found in *O. vicentei*, e.g. indolizidines and pumiliotoxins (Saporito et al., 2004). Our samples represent just a snapshot of the potential variation in the diet in wet and dry seasons in each population.

The observation we have described on the ecology and behaviour of *O. vicentei* in the wild are an essential contribution to a better understanding of the conservation status of this species. This study has opened the door to future studies on this endemic, data deficient species.

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Are the habitat niches of female green pit vipers *Cryptelytrops macrops* and *Viridovipera vogeli* partitioned by vertical stratification?

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ABSTRACT - We used telemetry to track the movements of the females of two sympatric green pit viper species, *Cryptelytrops macrops* and *Viridovipera vogeli*, in north-eastern Thailand. This has generated information on their home ranges, lengths of displacements, and vertical niche partitioning. Five female vipers were tracked simultaneously within the same general habitat for 78 days in the cold, dry season for a mean of 113.6 ± 5.38 (range: 101- 129) fixes. The data show that *V. vogeli* had larger home ranges and greater displacements than *C. macrops* (0.49 ± 0.02 and 0.26 ± 0.07 ha, 38.01 ± 9.72 and 25.12 ± 2.40 m; respectively). Interestingly, *V. vogeli* selected arboreal sites significantly more frequently (mean 53.0 ± 14.4 % of observations, range 38.6- 67.5) than sympatric *C. macrops* (mean 23.4 ± 13.2 %, range 5.3- 49.2). We cautiously suggest that vertical stratification of these two sympatric vipers may be one factor facilitating the co-existence of otherwise morphologically similar species.

INTRODUCTION

If two or more species in an ecosystem occupy a similar niche then they will usually compete for resources (Gause, 1934) and if environmental factors remain constant, one species will eventually outcompete and exclude the other (Hardin, 1960). Interspecific competition for resources ultimately imposes higher energy costs on all competitors (Abramsky et al., 2001). Interspecific competition can be avoided by niche partitioning, when sympatric species develop different resource preferences (Wisheu, 1998), or when a population becomes regulated more by conspecifics than interspecifics (Chesson, 2000). In a landmark study on interspecific competition, Pacala & Roughgarden (1982) observed that *Anolis* species that partitioned feeding sites faced less conflict and competition than sympatric species that did not.

In snakes, food partitioning often takes greater priority over space between individuals, compared to other vertebrates (Toft, 1985), likely due to selection pressure for divergence in food types (Roughgarden, 1976). Intraspecific niche partitioning has been suggested for several snake species (Shine & Wall, 2007). Of the estimated 46 green pit viper species found in Asia (Vogel et al., 2014) many show sexual dimorphism of mass and snout-to-vent length. This includes the species of the current study, *Cryptelytrops macrops* Kramer 1977 and *Viridovipera vogeli* David et al. 2001 (Malhotra et al., 2004; Strine et al., 2015) and their dimorphism suggests some degree of intraspecific prey partitioning as males display different spatial ecology from females (Stuart et al., 2012; Barnes et al., 2017).

Viridovipera vogeli and *C. macrops* are both viviparous, typically arboreal and inhabit Thailand's Sakaerat Biosphere Reserve (Kramer, 1977; David et al., 2001; Strine et al., 2015). These two sympatric species normally forage near water for frogs and other potential prey (Orlov et al., 2002), and thus occupy similar niches (as defined by Gause, 1934). Here we present a telemetry study of females of the two viper species giving preliminary evidence of home range sizes, movement patterns and vertical niche partitioning. Males were not included as their small size makes them unsuitable subjects for telemetry (Strine et al., 2015; Barnes et al., 2017).

MATERIALS AND METHODS

The study was undertaken in the dry evergreen forest of the Sakaerat Biosphere Reserve (for details of the Reserve see Trisurat et al., 2009). The two green pit viper species of the study were captured during opportunistic searches at night. Morphometrics (mass, SVL, etc.) were determined the following day with the acrylic tube method and isoflurane anaesthesia as described by Wilkinson (2014). We surgically implanted Holohil BD-2 and BD-2T 1.8 g transmitters into the body cavities of vipers following Reinert & Cundall (1982) and Hardy & Greene (2000). Transmitter mass was less than 5 % of the viper body mass. Vipers were returned to their location of capture within 72 hours.

Vipers were sought daily, once during day light and once at night time, and during every fix we attempted to obtain visual confirmation. Daytime is typically the inactive period (when they were most likely to be sheltering or resting)

and night the active period (most frequently ambushing, moving, etc.). We minimised disturbance to individuals by approaching slowly, identifying the location of the snake, and then immediately retreating from the subject to approximately 5 m for data collection. We defined vertical strata as underground, on ground, groundstorey (<1 m above the ground), understorey (1–3 m), midstorey (3–10 m), and abovestorey (>10 m). The category “arboreal” referred to observations in the midstorey or abovestorey categories. Utilising categorical data allowed for simple data collection, thus minimising disturbance to the viper in the field. Snake locations were determined with handheld GPS units (Garmin GPSMap64s) to the highest accuracy possible (usually 5–12 m), confirmed later using ArcGIS 10.1 (ESRI, 2012). Displacements of >5 m were considered a “move” for consistency with the level of GPS accuracy. We calculated number of moves, mean distance per move, and mean daily displacement (MDD, number of days tracked divided by distance moved) and provide descriptive statistics.

We radiotracked three female *C. macrops* and two female *V. vogeli* between July 2014 and February 2015 for 92–215 days (mean 140.6 ± 26.12 , median = 110, Table 1) but analyses were confined to data collected from 11 November, 2014 to 27 January, 2015 (78 days) when all vipers were tracked simultaneously. This was the cold, dry season in north-east Thailand. We calculated minimum convex polygon (MCP, 100 %) and fixed kernel (50 and 99 %) home range size, using the adehabitatHR package (Calenge, 2006) in program R (version 3.1.2; R Development Core Team, 2010). Accuracy of fixed kernel and MCP methods have previously been questioned (Row & Blouin-Demers, 2006), but are used for comparative purposes in this work. To select the smoothing factor for core (50 %) and activity (99 %) area utilisation distributions, we employed the least-squares cross validation method (Tiebout & Cary, 1987).

Table 1. Summary of the radio-tracked *C. macrops* (CRMA) and *V. vogeli* (VIVO), including snout-vent length (SVL), total tracking duration of each viper, number of fixes during study period (11 November, 2014 to 27 January, 2015), and % fixes observed arboreal (> 3 m above ground level) during study period

Viper ID	SVL	Mass	Tracking		Number of fixes	% time arboreal
			Start 2014	End 2015		
CRMA174	534	43.7	7th Oct	27th Jan	122	5.3
CRMA178	594	44.5	11th Nov	13th Feb	129	15.7
CRMA186	592	44.8	8th Nov	12th Feb	113	49.2
VIVO002	729	200	7th Jul	12th Feb	101	67.5
VIVO003	720	202	29th Jul	13th Feb	103	38.6

We also used dynamic Brownian Bridge Movement Models (dBBMMs; Karelus et al., 2017; Kranstauber et al., 2012; Silva et al., 2018) for a more modern approach of quantifying utilisation distributions (UD) as home ranges and movement. Unlike MCP and kernel density estimates, which do not account for spatially autocorrelated animal movement data (Kranstauber et al., 2012), dBBMMs incorporate the temporal structure of animal locations to estimate UD based on their movement’s trajectory. We set the dBBMM moving window size to 9 (equivalent to 9 days) and margin size of 3 to detect possible changes in movement between 3-day periods for all vipers except for CRMA174 (window size = 21, margin size = 9), and telemetry location error to the average GPS accuracy obtained in the field (8 m). We used 90 % and 95 % dBBMM isopleth contour UD to represent areas of ‘core utilisation’ and a more generous ‘total utilisation,’ as these larger contours predict a more accurate area of use without over-smoothing (Walter et al., 2011). Movement and home range dBBMMs were calculated in R using adehabitatHR (Calenge, 2006), BBMM (Nielson et al., 2011), and ctm (Calabrese et

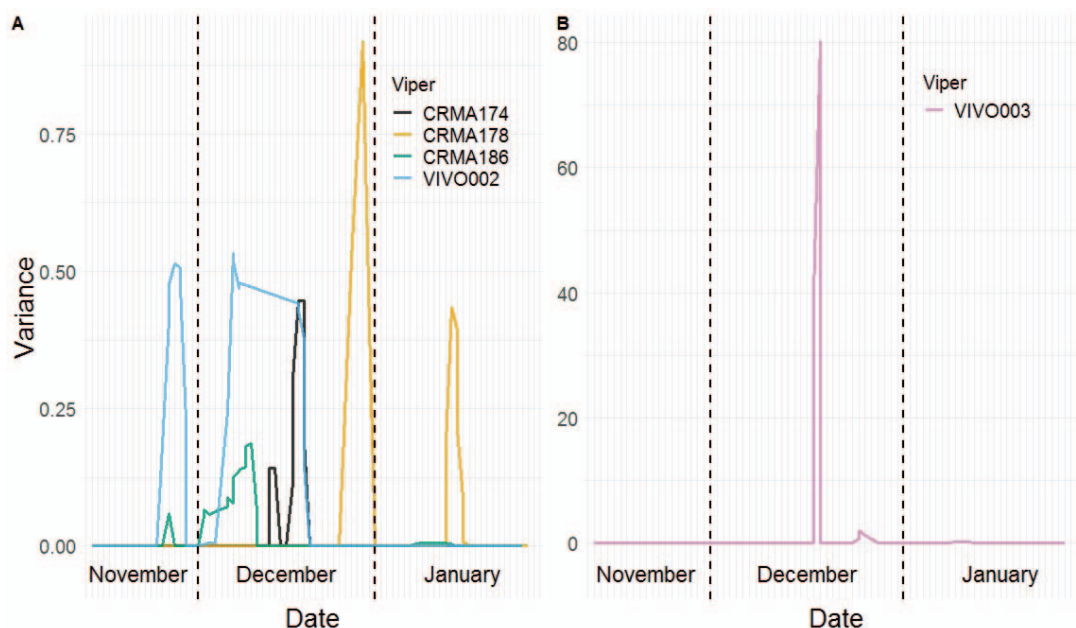


Figure 1. Movement variance estimation (σ^2m) over time for four green pit vipers with similar movement (A), and one viper with very high movement during the middle of December (B) during intensive study period between 11 November, 2014 to 27 January, 2015, with dashed lines indicating start and end of calendar months

Table 2. Movement and home range summary of radio-tracked *C. macrops* (CRMA) and *V. vogeli* (VIVO), including the number of displacements (moves), mean distance per displacement (m), mean daily displacement (MDD, m), and minimum convex polygon (MCP, 100%, ha), kernel (50 % FK and 99 % FK, ha), and dynamic Brownian Bridge Movement Model (90 % dBBMM and 95 % dBBMM, ha) home ranges

Viper ID	Moves	Mean distance	MDD	MCP	50 % FK	99 % FK	90 % dBBMM	95 % dBBMM
CRMA174	10	28.1	0.23	0.41	0.15	1.01	0.42	0.64
CRMA178	14	26.9	0.21	0.18	0.15	0.91	0.49	0.81
CRMA186	17	20.4	0.18	0.20	0.07	0.48	0.36	0.52
Mean	13.67	25.12	0.21	0.26	0.12	0.80	0.43	0.66
SE	2.03	2.40	0.01	0.07	0.02	0.16	0.04	0.08
Median	14	26.85	0.21	0.20	0.15	0.91	0.42	0.64
VIVO002	14	28.3	0.28	0.47	0.20	1.33	1.07	2.34
VIVO003	11	47.7	0.46	0.51	1.17	5.89	0.86	2.33
Mean	12.5	38.01	0.37	0.49	0.68	3.61	0.97	2.34
SE	1.5	9.72	0.09	0.02	0.49	2.28	0.04	0.01
Median	12.5	38.01	0.37	0.49	0.68	3.61	0.97	2.34

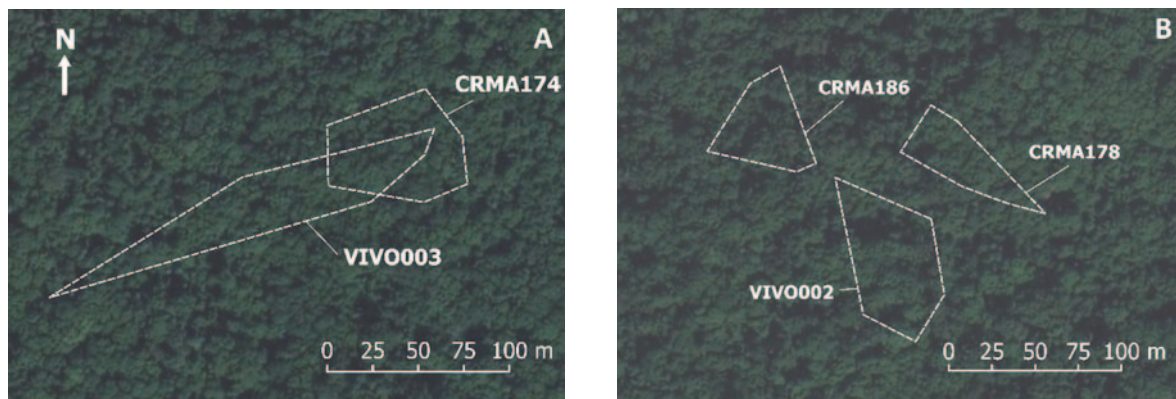


Figure 2. Maps of green pit viper minimum convex polygons (MCP, 100 %); **A.** CRMA174 and VIVO003, **B.** CRMA178, CRMA186, and VIVO002

al., 2016) packages.

Spatial overlap was calculated using UD overlap index (UDOI, Fieberg & Kochanny, 2005) with the R package KernSmooth for fixed kernels and the intersect function in QGIS (version 2.12.3; Quantum GIS Development Team, 2016) for MCP overlap analysis. Overlap is presented as area (ha and %) for MCP and as the UDOI for kernels. Values from the UDOI range from < 1 which suggests less overlap relative to uniform space use, 1 if both home ranges are uniformly distributed and have 100 % overlap, and values > 1 indicate higher than normal overlap relative to uniform space use. We also present results from 2 relatively infrequently used indices for comparative purposes; volume of intersection index (VI, Seidel, 1992; Kernohan et al., 2001) and Bhattacharyya's affinity (BA, Bhattacharyya, 1943), both of which range from 0 (no overlap) to 1 (identical home ranges); advantages and biases of all overlap methods are discussed in Fieberg & Kochanny (2005).

Due to small sample size, we compare overlap values and provide descriptive statistics for home ranges. Means are reported for data with standard error and medians. Due to our small sample size and categorical data, we used Chi-square tests with the chisq.test function in program R to assess differences in arboreality between the two study species.

RESULTS

In the 78 day period in which all vipers were tracked simultaneously, *C. macrops* were tracked more frequently (mean 121.3 ± 4.63 fixes, median = 122, range 113- 129 fixes) than the *V. vogeli* (mean 102 ± 1 fixes, median = 102, range 101-103 fixes). The two *V. vogeli* were longer and heavier than the three *C. macrops* (Table 1) and all were within the typical adult size range for their respective species.

The five vipers undertook an average of 13.2 ± 1.24 (median = 14, range 10-17) displacements, moved 30.28 ± 5.00 m (median = 28.14 m, range 20.38- 47.74 m) per displacement, and had mean daily displacements of 0.27 ± 0.05 m (median = 0.23, range 0.18- 0.46 m/day) (Table 2). Vipers moved most frequently during mid- December, however, peaks of movement activity were observed at the end of November and also middle of January (Fig. 1). Mean MCP home range size for all individuals was 0.35 ± 0.07 ha (median = 0.41, range 0.18-0.51 ha); 50 % and 99 % kernels were 0.35 ± 0.21 ha (median = 0.15, range 0.07-1.17 ha) and 1.92 ± 1.00 ha (median = 1.01, range 0.48-5.89 ha), respectively; and 90 % and 95 % dBBMM were 0.97 ± 0.04 ha (median = 0.97, range 0.86- 1.07 ha) and 2.34 ± 0.01 ha

Table 3. Home range overlap of *C. macrops* (CRMA) and *V. vogeli* (VIVO) with minimum convex polygon (MCP, 100 %) analysis of home range overlap (ha and %) and fixed kernel (FK, 50 and 99 %) overlap with Bhattacharyya's affinity (BA), volume of intersection index (VI), and utilisation distribution overlap index (UDOI). There was no overlap detected with any other combinations of individuals.

Viper IDs		MCP		50 % FK			99 % FK		
Overlapping pairs		Overlap (ha)	Overlap (%)	BA	VI	UDOI	BA	VI	UDOI
CRMA174	VIVO003	0.111	8.3	0.00479	0.0014	0.00002	0.16501	0.05628	0.07662
CRMA186	VIVO002	0	0	0	0	0	0.00762	0.00356	8.00E-05

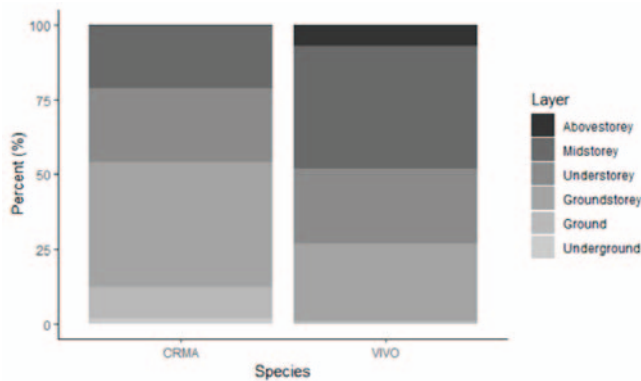


Figure 3. Summary of the percentage of fixes of *C. macrops* (CRMA) and *V. vogeli* (VIVO) that were below ground level (underground), on the ground (ground), <1 m above ground level (groundstorey), 1-3 m above ground level (understorey), 3-10 m above ground level (midstorey), and >10 m above ground level (abovestorey)

(median = 2.34, range 2.33-2.34 ha), respectively (Table 2, Fig. 2). *Viridovipera vogeli* had greater displacements (but did not move more frequently) and had larger home ranges than *C. macrops* (Table 2). Home range overlap between study vipers was minimal, and intraspecific overlap was not observed (Fig. 2 & Table 3).

During the 78 day study period, we observed that the two *V. vogeli* were in “arboreal” locations (> 3 m above ground level, mean 53.0 ± 14.4 %, median = 53.05, of observations) more often than *C. macrops* (mean 23.4 ± 13.2 %, median = 15.7, of observations, Table 1, Fig. 3). This difference was statistically significant ($\chi^2 = 25.0565$, $df = 1$, $p < 0.001$). The *C. macrops* were most frequently observed at the groundstorey level (< 1 m above ground, 101 observations, 41.6 % of total observations for this species), while the *V. vogeli* were most often observed at the abovestorey level (3- 10 m above ground, 46 observations, 41.1 % of total observations for this species).

DISCUSSION

Both green pit viper species in our study exhibited limited movement and small home ranges. This is consistent with their ambush foraging strategy (Macartney et al., 1988). However, *V. vogeli* moved further and exhibited larger home ranges than *C. macrops*. This was to be expected as larger individuals and species typically require larger home ranges for foraging (McNab, 1963).

Traditional home range estimators (MCP, kernels, etc.) are imperfect estimators for organisms with limited movement

and small home range size such as green pit vipers. The MCP method includes large areas of unused space and does not take movement into account (Nilsen et al., 2008), while kernels generally overestimate overall home range size (Row & Blouin- Demers, 2006). To date, the dBBMM method has only been used for one other snake species, the king cobra (*Ophiophagus hannah*), which displays significantly larger home ranges. However, this study suggests that dBBMMs are better at optimising the trade- off between Type I and Type II errors (under and over smoothing, respectively), displaying ecological patterns of habitat selection, addressing spatial autocorrelation of telemetry points, and providing insights into seasonal and habitat variation compared to MCP and kernel methodologies (Silva et al., 2018). All of our spatial methods, and coincidentally those currently used by snake ecologists, fail to incorporate vertical movement and space use which limits ecological inferences for arboreal species such as green pit vipers. Innovative home range and overlap techniques have been developed to incorporate vertical movement and stratification (i.e. Cooper et al., 2014); however, these methods require much more movement and continuous height measurements than traditional methods.

Snakes are not known to display territorial behaviour, and individuals of the same species and population usually exhibit widely overlapping home ranges (see review by Gregory et al., 1987; Weatherhead & Hoysak, 1989; Secor, 1994). It appears that both specimens of *V. vogeli* in our study had home ranges that overlapped those of *C. macrops*. No intraspecific overlap was observed, and one *C. macrops* (CRMA178) home range did not overlap any other study viper home range. Small home range size, particularly by *C. macrops*, may best explain the limited home range overlap observed but overlap with non- tracked individuals cannot be ruled out.

The two species in our study clearly used separate strata levels within the dry evergreen forest during the cold inactive season. We cautiously suggest that vertical stratification of these two sympatric vipers may be one factor facilitating the co-existence of otherwise morphologically similar species. However, there has been no previous comprehensive study of behaviour, prey selection, habitat selection, or thermal preference of either *C. macrops* or *V. vogeli*, which are factors that could influence this partitioning of space. It is clear that vertical niche partitioning of green pit vipers requires further investigation with larger sample sizes across all seasons. Intraspecific niche partitioning has been suggested previously for *C. macrops* (Strine et al., 2015), and similar studies with *V. vogeli* may further support both intraspecific niche partitioning as well as the vertical niche partitioning

that our results suggest. Further study may yield significant insight into green pit vipers (particularly *V. vogeli*), tropical species interactions, and snakes in general.

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Winter presence of adult male palmate newts (*Lissotriton helveticus*) in a pond in Scotland

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INTRODUCTION

The three species of newt that are native to Britain, palmate (*Lissotriton helveticus*), smooth (*Lissotriton vulgaris*) and great crested (*Triturus cristatus*), are also found throughout the temperate latitudes of western and central Europe (McInerney & Minting, 2016). Across this region, newt species are subject to wide seasonal air temperature variations which result in a phenological pattern characterised by adult migration from terrestrial over-wintering refugia to spring breeding ponds; and subsequent post-breeding migration back to over-wintering refugia (Griffiths, 1984; Verrell & Halliday, 1985). The specific cue that signals newts to commence the breeding migration is generally considered to be an increase in night-time air temperature above 4°C to 5°C following successive days of a stable temperature just below this level (Verrell & Halliday, 1985; Langton et al., 2001; English Nature, 2001; Dervo et al., 2016); additionally, once in breeding ponds newts are generally considered to be inactive at air temperatures below 5°C or, potentially, following cold nights.

It is well documented that newt larvae, those individuals that have not fully metamorphosed, may remain in ponds through the winter months, before completing metamorphosis the following year (van Gelder, 1973; Griffiths, 1997; McNeill & Downie, 2017). However, contrary to the established pattern, there are also reports of adult newts remaining in ponds during non-breeding months although these are much rarer. Dodd & Callan (1955) reported that the majority of a cohort of male palmate newts over-wintered in a pond in Fife, Scotland; while Edgar & Bird (2006) report that a small proportion of individuals may hibernate in the water. Notwithstanding, records of both adult over-wintering behaviour and of surveys from the post-breeding winter months generally appear in the literature much less frequently. Hence it remains to be seen whether the occurrence of adult newts overwintering in ponds is rare, undetected or under-reported.

MATERIALS AND METHODS

Surveys (n=14) were undertaken July 2016-April 2017 at a pond in Pumpherston, West Lothian, Scotland (NT 06647

70272). The pond is ovate in shape, with a 78 m perimeter and an area of 307 m², as defined by terrestrial vegetation; and 0.4 m at the deepest point. Newt populations at the pond have been documented previously (Jehle et al., 2013).

Environmental parameters were measured at the pond edge immediately prior to commencing a survey. Ambient air temperature (Aⁱ) at 1.5 m above ground level was measured using a Hyelec PeakMeter MS6508 digital thermometer ($\pm 0.5^\circ\text{C}$) exposed to the air for >60 s until the reading value had stabilised. Water temperature (Wⁱ) at a depth of c. 50 mm was measured 0.5 m from the pond edge of waterline using a TPI digital pocket thermometer ($\pm 1^\circ\text{C}$) and the stabilised temperature recorded. Equipment was calibrated at the University of Glasgow.

Newts were counted 30 minutes after sunset following a standard method described by Griffiths et al., (1996). Torching was preferred to bottle trapping as the latter should only be used when the night-time air temperature is >5°C (English Nature, 2001) and surveys were targeting colder, winter months. A 1 million candlepower Clulite Clubman CB3 LED spotlight was used to illuminate all areas of water which could be accessed (>90 %) along the pond perimeter (78 m). The surveyor walked the same route and direction around the pond each time. Newt species, life-stage (adult/juvenile/larvae) and sex (male/female) were classed where defining characteristics were observed (ARG UK, 2014), for example dark, webbed hind feet and tail filament in breeding adult male palmate, or recorded as 'unidentified'. Non-adult, juvenile newts are defined as individuals who are post-metamorphosed. Individuals that were not great crested newts but which could not be identified to gender level, were recorded as 'unsexed small newt' being palmate or smooth females, or males lacking secondary sexual characteristics. Newt classification was led by the same competent surveyor (author EP), holder of a Scottish Natural Heritage issued great crested newt survey licence, to control for recorder variability.

RESULTS

Palmate, smooth and great crested newts were recorded during pond surveys (Table 1). An adult newt of at least one species was present during every survey; juveniles were

Table 1. Environmental parameters (A^t = air temperature, W^t = water temperature) and newt counts (GCN = great crested newt, P = palmate, S = smooth, ♂ = adult male, ♀ = adult female, J = juvenile, L = larvae, Unsexed small newt = palmate or smooth females, or males without secondary sexual characteristics) for n=14 surveys

Date	A^t	W^t	GCN				P	S	Unsexed small newt	
			♂	♀	J	L	♂	♂	♂/♀	L
23/07/2016	16.6	18.1	1	5	4	44	9	0	3	0
14/08/2016	12.7	16.4	0	0	5	35	1	0	0	9
02/09/2016	12.8	16.3	0	0	2	38	0	0	1	6
08/10/2016	13.9	12.4	1	0	0	21	7	0	2	5
12/11/2016	7.2	6.7	0	0	0	0	8	0	3	4
15/12/2016	8.6	6.9	0	0	0	2	56	0	6	0
30/01/2017	4.5	1.9	0	0	0	0	1	0	1	0
11/02/2017	3.5	3.7	0	0	0	0	2	0	3	0
05/03/2017	2.0	6.5	6	0	0	0	5	3	4	0
11/03/2017	9.2	10	15	1	0	0	10	2	14	0
19/03/2017	7.9	9.7	1	0	0	0	4	0	2	0
02/04/2017	8.8	12.9	32	2	0	0	15	0	10	0
16/04/2017	7.7	9.6	41	7	0	1	22	4	30	0
30/04/2017	9.9	12.8	19	0	0	1	2	1	9	0

observed July-September; and larvae were recorded July-December and April.

The highest count of an adult of any confirmed species was palmate male (n=56) on 15th December (A^t = 8.6°C, W^t = 6.9°C) and the lowest count was a single palmate male recorded on 30th January (A^t = 4.5°C, W^t = 1.9°C). Adult newts were observed in negligible numbers (≤ 2) during counts in August, September and January. On 5th March (A^t = 2°C, W^t = 6.5°C) six great crested newts were recorded.

The following incidental records were noted: 15th December, one male palmate observed exhibiting tail fanning behaviour in an open area of the water column, interpreted as a courtship display; 30th January, one male palmate and one unsexed small newt observed swimming in the water column under surface ice (approx. 10 mm thick); 5th March, one male great crested newt observed in the torchlight before rapidly burying into the silt substrate; and, 19th March, breeding common toad (*Bufo bufo*) present in relatively high number (n=57) some in amplexus.

DISCUSSION

Adult male palmate newts were present in the pond during 13 of 14 surveys (Table 1), including winter months. The secondary sexual characteristics, which identify these adult males, regress fairly rapidly post-breeding (<2 months), disappearing in the terrestrial environment, and their formation takes 1-2 months upon returning to the aquatic environment (Griffiths & Mylotte, 1988). Therefore, we can infer that these male newts had likely not recently re-entered the pond after a long period away from the aquatic habitat.

We present strong evidence indicating likely winter residence in an aquatic habitat by a relatively large number

of adult male palmate newts at this location. This does not exclude the possibility that newts may have exited the pond for a short period between surveys or entered from an adjacent un-surveyed pond.

Retention of secondary sexual characteristics by newts during winter months does not necessarily imply breeding, as animals might strategically maintain their investment in sexual characteristics for opportunistic mating (Iglesias-Carrasco et al., 2016). However, the observation of a male palmate exhibiting tail fanning behaviour on 15th December, although no females were recorded on this date, suggests breeding may occur at this location during winter months. Palmate and smooth newts have been observed elsewhere undertaking early migration (i.e. mid-winter) following milder conditions (Jablonski, 2013); however, based on the developed secondary sexual characteristics, we do not believe the cohort of post-breeding newts exited aquatic habitat for terrestrial hibernacula in winter 2016.

During October-December surveys, male palmate newts were present in considerable numbers, i.e. ≥ 3 individuals, which on 15th December included the highest adult count (n=56) of any species. This result was quite unexpected as was a sevenfold increase on the previous palmate count from 12th November. Langton et al. (2001) suggest that air temperatures during the days preceding counts may influence the number of active newts in the pond, and our results appear to be in agreement. Specifically, the high count of palmate newts on 15th December was immediately following days with minimum air temperature 13°C and 6°C, recorded at a weather station 9.5 km east of the pond ('Edinburgh/Gogarbank'; Met Office; www.metoffice.gov.uk/). While this count also appears to indicate that there were more newts present than in summer months, it is

difficult to compare abundance between seasons as the presence of aquatic vegetation during summer may obscure any newts present, and newts may also be less visible to the surveyor due to behavioural changes when breeding activity ends (Langton et al., 2001). Further, if newts experience temperatures outside of thermal tolerance limits, individuals might bury themselves in the silt; remain in deeper areas of the pond to thermoregulate (Balogová & Gvoždík, 2015); or exit the pond and therefore be out of view of the surveyor. These behaviours might explain the low count during January–February when air temperatures <4.5°C.

Notwithstanding, it seems unlikely that these factors explain the relatively low numbers of adult palmate male recorded during the breeding season surveys in April 2017 (Table 1). It is possible that males may have exited the pond after December 2016 and only recently (<1 month) returned to the pond after hibernating in the terrestrial environment and thus have underdeveloped sexual characteristics. In this scenario, it would be more appropriate to aggregate male and unsexed small newt when comparing the counts from these dates: the total count of $n=52$ palmate newts is closer when compared to the high count of adult male palmate newts on 15th December ($n=56$).

On 10 of 14 surveys the water temperature was warmer than the air temperature and on 2nd April we recorded a water temperature which was 4.1°C greater than the air temperature (Table 1). Given that newts are known to adjust their position in the water column based on water temperature (Balogová & Gvoždík, 2015), which would affect their visibility, it is clear that water temperature is an important parameter in survey design, as suggested by Paterson (2018). Future research should examine the relationship between newt counts and local environmental parameters with increased temporal resolution, e.g. daily, and duration >10 months, to account for inter-annual variations.

There have been few previous reports of large numbers of adult newts in aquatic habitat during winter months. This behaviour is of interest because it is during winter months that conservation activities, for example removal of pond vegetation and substrate material, are undertaken with the aim of improving breeding habitat for newt populations. Our finding indicates that newts may be seriously disturbed or killed during such activities. Presence in ponds during winter alongside any changes in phenology resulting from climate change should be considered in the context of management (Dervo et al., 2016). Given that conservation measures for great crested newt are embedded in statutory legislation, such as the European Union's Conservation (Natural Habitats, &c.) Regulations 1994, there is clear merit in exploring the occurrence of winter presence in ponds over greater temporal and spatial scales.

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Play behaviour by captive tree monitors, *Varanus macraei* and *Varanus prasinus*

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Play describes suites of behaviour that are perceived as 'non-serious'. Play has proven to be difficult to define although there have been many attempts to do so (see Burghardt, 2014). Initially, play was thought to be limited to mammals and birds, but efforts to define it more objectively have led to the identification of play in a variety of other taxa as diverse as octopodes (Kuba et al., 2006), fish (Burghardt et al., 2015), and reptiles including monitor lizards (e.g. Hill, 1946), turtles (Burghardt et al., 1996; Kramer & Burghardt, 1998) and crocodilians (Lazell & Spitzer, 1977; Dinets, 2015). The widely accepted definition of play is 'repeated, seemingly non-functional behaviour differing from more adaptive versions structurally, contextually, or developmentally, and initiated when the animal is in a relaxed, unstimulating, or low stress setting' (Burghardt, 2014). Here we report the play behaviour of two species of monitor in captivity, *Varanus macraei* at ZSL London Zoo (Zoological Society of London) and *Varanus prasinus* at Bristol Zoo (Bristol Zoological Society).

At ZSL London Zoo, observations were made on a pair of adult captive-bred blue tree monitors (*V. macraei*) aged 6 (female) and 7.5 (male) years. The animals were housed in a 3x2x1.5 m enclosure with a heterogeneous structure including branches and artificial rockwork, a waterfall and a naturalistic substrate comprising layers of humus and leaf litter. Heating (mercury vapour lamps; Arcadia/Osram) and lighting (T5 UVB emitting fluorescent lamps; Arcadia Reptile) arrays provided an ambient temperature gradient from 25-28 °C (day), 23-26 °C (night) and a diurnal basking zone with temperatures between 39-43 °C and a maximum UVI of 5, in accordance with best husbandry practices for the species (Ziegler et al., 2009). The animals were fed a variety of invertebrate and vertebrate prey; these were generally broadcast in the enclosure to allow the animals to engage in natural hunting and feeding behaviour. The animals were also target-trained using operant conditioning with food items as a reward. The enclosure had been repeatedly planted with a variety of live plants, especially *Scindapsus* and *Philodendron*, in order to increase structural and environmental complexity, as well as for aesthetic appeal to the public.

At Bristol Zoo, juvenile (0.5-1.5 years), captive-bred *V. prasinus* were reared in custom glass-fronted fibreglass enclosures measuring 60x60x70 cm. Enclosures are furnished with live plants (e.g. *Ficus* sp., *Schefflera arboricola*, *Scindapsus* sp.), branches, cork bark tubes and damp refugia. The substrate consisted of humus and leaf litter and water

was provided with a small 10 cm water bowl. Lighting (T5 UVB emitting fluorescent lamps, Arcadia Reptile; T5 6500K lamps, Philips) and heating (75 W halogen bulbs, Sylvania/Osram) provided ambient temperatures of 27-31 °C (day) and 24-26 °C (night), a basking site of 38-40 °C with a maximum UVI of 6.0, and a photoperiod of 12:12. They were fed four times a week on a variety of insect prey that were usually broadcast in the enclosure.

Observations on *Varanus macraei*

Both specimens of *V. macraei* were repeatedly observed engaging in focussed destructive behaviour involving the leaves of plants for weeks or months after the plants were added. The behaviour included initial investigation and then removal of all or parts of individual leaves using mouth and front limbs to tear. The leaf sections removed were often transported some distance to a perch or hide where, using mouth and forelimbs, they were systematically shredded and then wiped in an exaggerated manner across rock work (Fig. 1 a). The fragments were typically not consumed (occasional, apparently incidental, ingestion of small fragments was observed), and once the leaf section is shredded, the lizards typically returned to the plant to obtain another piece. The behaviour was displayed at different times of day and was not associated with the presence of food.

Observations on *Varanus prasinus*

Several juvenile *V. prasinus* were observed removing leaves of *Schefflera arboricola* and *Ficus benjamina* and engaging in destructive behaviour (Fig. 2 a-c). It is not known whether these animals continued the behaviour past 1.5 years since by that age they were transferred from the collection. The behaviour was not observed in adult *V. prasinus* (n = 6) or *V. macraei* (n = 3) maintained under similar conditions at Bristol Zoo. Individuals investigated a leaf with tongue flicking before removing it with their mouth, often shaking the head side to side while pulling, and using forelimbs to assist if necessary. On one occasion a freshly-fallen leaf was picked up from the floor of the enclosure. The leaves were then transported to a favoured perching area where the mouth and forelimbs were used to bite and tear them apart, usually into two or three pieces. The front limbs were used to hold leaves in position to allow the animal to release the leaf from its jaws and reposition its bite location and to hold the leaf in place while biting/mouthing and pulling to assist in tearing. This posture

also allows the lizard to engage in forelimb raking, similar to prey handling behaviours described in several species of the *Haptesaurus* subgenus by Greene (1986), Irwin (1996) and Hartdegen (2000). Exaggerated wiping of the leaves from side to side against the perch was also witnessed. Occasionally the leaf would be accidentally dropped to the floor

of the enclosure and the individual would retrieve it, usually chasing after it as quickly as it could, and then returning to its original position to continue the aforementioned behaviours. One individual was observed collecting a second leaf with mouth and forelimbs while keeping hold of another, where the destructive behaviour described was exhibited on two



Figure 1. Stills image from mobile phone footage of a male *V. macraei*, surrounded by discarded leaf fragments collected from a *Scindapsus* plant c. 60 cm away from its retreat, systematically shredding a leaf fragment by wiping it across a rock face

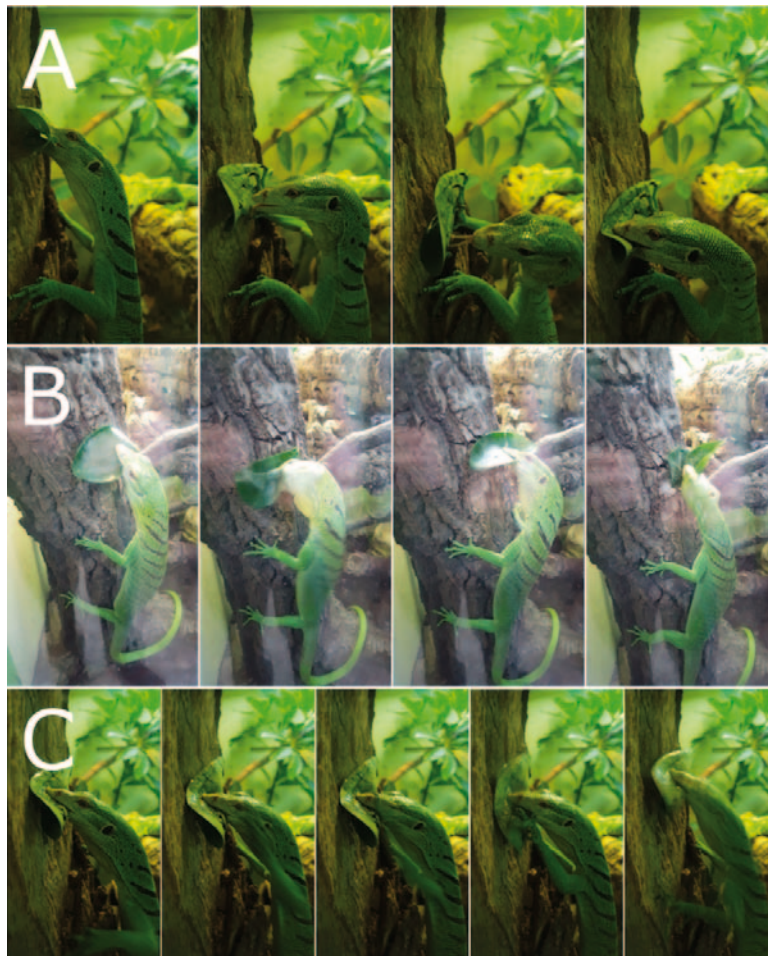


Figure 2. Stills images from mobile phone footage of a female *V. prasinus* using forelimbs in actions resulting in tearing a *Schefflera* leaf into several smaller fragments, **A.** repositioning bite location on leaf; **B.** wiping leaf across branching; **C.** forelimb raking leaf

leaves at the same time. No attempts to consume any leaf fragments were observed. The behaviour was not associated with the presence of food and has been observed at different times of day, both on feed and non-feed days alike.

Behavioural analysis

We consider the observed behaviour to be the same across both monitor species and tested it against Burghardt's (2014) five criteria of play (in bold) as follows:

1) Incompletely functional in the context in which it appears: The lizards in this study derived no food by engaging in the behaviour, as in the vast majority of cases no plant matter was ingested; when this does occur, it is in extremely small quantities relative to the quantity of leaf that is shredded.

2) Spontaneous, pleasurable, rewarding, or voluntary: The behaviour was voluntary and, given that it is engaged in frequently, this suggests that there is a degree of 'reward' or positive reinforcement for the lizards, i.e. they may 'enjoy' it.

3) Different from other more serious behaviours in form (for example, is exaggerated) or timing (for example, occur early in life, before the more serious version is needed): The behaviour differs in form from 'serious' behaviours in that it is a) directed against foliage, which is not a part of the lizards' diet, b) the fragments are not consumed, and c) the tearing and wiping behaviour is exaggerated compared with the treatment of prey items.

4) Repeated, but not in abnormal and unvarying stereotypic form (for example, rocking or pacing): The behaviour was repeated, but is not stereotyped in that it occurs with different plants, in different parts of the enclosure, with different bout lengths and at different times of day.

5) Initiated in the absence of severe stress: The behaviour was exhibited without consistent stimulus and under normal conditions, under which the lizards display no abnormal behaviours or lack of behaviours that might be associated with stress.

Given that this leaf shredding behaviour fulfils Burghardt's (2014) five criteria for play, we label it as a candidate object-based play behaviour (Held & Špinka, 2011). The selection of leaves as the object of play is likely incidental and may reflect the fact that of all the materials in the enclosure, live plant leaves are the most suitable for easy tearing. Artificial silk plant leaves, also present in the enclosure, are extremely difficult to tear, while dried leaf litter crumbles rather than tears. *Varanus komodoensis* has also been reported to exhibit object play behaviour not dissimilar from the behaviour we describe in *V. macraei* and *V. prasinus*, whereby objects were picked up and subjected to shaking, mouthing and destructive behaviour resembling exaggerated predatory behaviour but not leading to ingestion (Hill, 1946; Burghardt et al., 2002; Burghardt, 2005).

Burghardt (2010) described three play processes. Primary-process play is the simplest level and grows out of excess energy and low behavioural thresholds giving rise to behaviour with no long-term consequences, good or bad. Secondary-process play begins to develop immediate

physiological benefits for the animal, such as maintaining muscular function, or cognitive capacity. Tertiary-process play is necessary for the completion of developmental milestones. The transition from primary through to tertiary play processes underpins Surplus Resource Theory (SRT; Burghardt 1984; 2005; 2010; 2014). SRT provides a unifying approach to the different historic attempts to explain play behaviour and allows for the initiation of a play behaviour as a non-adaptive result of excess energy to become a fundamentally necessary part of an animal's development.

The behaviour reported here in *V. prasinus* and *V. macraei*, as well as similar behaviours reported in *V. komodoensis* (Burghardt et al., 2002), may fall into the primary-process category. Not all individual monitor lizards of the species described here engage in this sort of behaviour (even within the same collections as the animals observed exhibiting them), and leaf-shredding resembles prey-tearing and wiping behaviour, as well as removal of prey items to a perch for processing (Hartdegen et al., 1999; 2000) that these lizards already engage in. The high propensity for monitor lizards to engage in predatory behaviour may mean that this sort of general pattern of movement has the lowest behavioural threshold in this group, and therefore is the most likely to spill over into primary-process play.

Within animal groups, propensity for play appears to be correlated with brain size (Iwaniuk, 2001). Primates display the highest concentration of play behaviours of all mammals (see Burghardt, 2014), and this may be linked to their relatively high energy efficiency in the cost of maintenance and growth (Burghardt, 2014; Pontzer et al., 2014), which provides the excess energy required to facilitate both increased cognitive capacity and primary-process play. Monitor lizards, with their (for squamates) high cognitive capacity and problem-solving abilities (Cooper et al., 2000; Manrod et al., 2008; Gaalema, 2011; Mendyk & Horn, 2011), dextrous appendages and acute vision, may be regarded in some ways as being to squamates as primates are to mammals. Monitor lizards are relatively energy efficient (e.g. Dryden et al., 1990) and this may contribute to the appearance of primary-process play in this group of reptiles. Moreover, in captivity food is not a scarce resource and so conditions may be even more suitable for the development of primary-process play through SRT than in nature. It is important to note that the captive environment may be less stimulating than the wild one, and this may contribute to the development of behaviours such as those documented here, as animals may seek to create their own diversions in the absence of extrinsic ones.

The mapping of play behaviour in non-mammalian taxa is still only in its infancy. With only scattered reports of play behaviour, it is currently not possible to fully understand its phylogenetic distribution or diversity, and subsequently its evolutionary origins. We recommend that people working with these groups in the field or in captivity report similar observations to add to a growing catalogue of candidate play behaviour in non-mammals.

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Discovery of the first feeding area for adult and juvenile green turtles and loggerhead turtles in Greece

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Two species of sea turtle breed in the Mediterranean Sea, green turtles (*Chelonia mydas*) and loggerhead turtles (*Caretta caretta*). The green turtle is mostly restricted to the eastern Mediterranean, breeding in Cyprus, Turkey and Syria (Casale et al., 2018) but they can also be found at low densities in the Adriatic Sea (Lazar et al., 2004), and the coastal waters of Greece (Panagopoulos et al., 2001), Italy (Bentivegna et al., 2011) and north Africa (Stokes et al., 2015). The loggerhead turtle is found across the entire Mediterranean and breeds mainly in Greece, Turkey, Cyprus and Libya (Casale et al., 2018). Green turtles are Endangered in the Mediterranean due to their relatively low numbers and the genetic isolation from the Atlantic population (Encalada et al., 1996; Kasperek et al., 2001; Seminoff, 2004). The only published regular foraging location for green turtles in Greece is for juveniles in Lakonikos Bay, south Peloponnese (Margaritoulis & Teneketzis, 2001). All other observations are from strandings or captures that are not concentrated in one specific area (Panagopoulos et al., 2001). However, Corsini-Foka et al. (2013) found proportionally more green turtles in waters around Rhodes (in relation to all other Greek sites) which included three adults, but there were only 42 green turtle records from 27 years of data collection. Rhodes is the nearest island to my study site on the Greek island of Kastellorizo.

During a zoological survey of Kastellorizo island in August 2017, a number of green turtles (Fig. 1) and loggerhead turtles were recorded in the nearshore waters. Kastellorizo is a small Greek island situated 128 km east of Rhodes island and only 2 km from the south coast of Turkey. The turtles were present at the two small bays, green turtles in the shallow small-boat harbour (Fig. 2, A, 36° 8'55.17" N 29° 35'48.01" E) and loggerheads mostly in the deeper main harbour (Fig. 2, B, 36° 9'1.24" N 29° 35'27.38" E). The green turtles present in the area were mostly adult animals, but there were also some small juveniles. To count turtles, I stood on the best vantage point of the small boat harbour (Fig. 2, A) each afternoon from 9 August 2017 to 15 August 2017 and surveyed the water using binoculars. I estimated that there were 8-10 mostly adult green turtles swimming in the bay. It was difficult to count the animals because most of the time they were submerged and feeding, and only came to the surface to breathe for a few seconds. Surveys at the deeper harbour (Fig. 2, B) on three of the five same afternoons revealed that up to 5 individuals were present there. Just

outside the small shallow bay there is a small archipelago of 8 islands with shallow sea between them (Fig. 2, C), each with the same grassy bottomed habitat. On a third visit in July 2019, I snorkelled in this area and the shallow harbour, and estimated that in total, over 20 green turtles were present (mostly adult females and juveniles but I also observed two adult males). The turtles that I observed while snorkelling were feeding on the sea grasses *Cymodocea nodosa* and *Posidonia oceanica*. Some turtles would come to feed in very shallow water, no more than 60 cm deep (Fig. 1).

The only loggerhead feeding that I was able to witness was of two individuals that aggressively interacted in the main, deep harbour, while competing for food items given from the tavernas.



Figure 1. Green turtle in very shallow water feeding on *Cymodocea nodosa* sea grass

On another visit from 26 to 29 April 2019, to check if the turtles were present all year round, no turtles of any species were recorded. Local fishermen say that the turtles gather in the bays from May onwards.

The nearest nesting beach for green turtles is Patara beach in western Turkey, some 30 km west of Kastellorizo, which at the same time is believed to be the westernmost site of green turtle nesting in the Mediterranean (Kasperek et al., 2001). The beach is mainly a nesting site for loggerhead turtles but most years 1 to 2 nests of green turtles can be found (Olgun et al., 2016). The number of green turtles present at Kastellorizo is much higher than those nesting in the wider area, which may indicate that post-breeding and



Figure 2. The harbour area of the island of Kastellorizo, **A.** Small boat harbour, **B.** Main harbour, and **C.** Shallow grassy bay (image courtesy of Google Earth)

non-breeding adults spend time in these waters feeding. This is the first reported feeding ground for adult and juvenile green turtles and loggerheads in Greece, which is very important given the endangered status of the species globally. Inside the two harbours, boat traffic is dense, with possible deadly consequences for turtles; a speed limit for boats would be a welcome conservation measure.

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First observations on cave breeding in *Salamandra algira splendens* from north-eastern Morocco

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ABSTRACT - Two Moroccan subspecies of *Salamandra algira* were recently observed in caves- *S. algira splendens* and *S. algira spelaea*. Here, I report the first observations of cave breeding in *S. algira splendens*, in the Chaâra and Friouato caves of the north-eastern Middle Atlas (Taza region, Morocco). Twenty-two salamander and newt species are known to be facultative or obligate cave-dwellers in the Mediterranean ecoregion. They can inhabit or breed in caves so avoiding hot and very dry summer conditions. Caves with groundwater and stable humidity act as thermal refugia, winter or summer shelters, hiding places, feeding areas or, in some cases, as regular breeding sites.

INTRODUCTION

In Morocco, the genus *Salamandra* (Amphibia: Urodela) is represented by one species, *Salamandra algira* Bedriaga, 1883 which is widely distributed from northern Morocco to north-eastern Algeria (Beukema et al., 2013; Escoriza & Ben Hassine, 2015, 2019). A sub-species *S. algira splendens* is confined to humid, sub-humid and semi-arid mountainous habitats forested with *Abies pinsapo*, *Cedrus atlantica*, *Pinus* sp. and *Quercus* sp. (Beukema et al., 2013; Escoriza & Ben Hassine, 2015, 2019). This sub-species occurs in two well separated major groups: one in the Rif mountains the other in the Middle Atlas (Beukema et al., 2013; Raffaëlli, 2013; Hernandez, 2018a & b). Recently discovered locations for this endemic subspecies include the easternmost record from the Bokkoyas Massif, Al-Hocéima region, central Rif mountains (Hernandez & Escoriza, 2017) and Jbel Sidi Ali, central Middle Atlas, northern Morocco (Hernandez, 2018a). Recent records have also confirmed its presence in caves of the north-eastern Middle Atlas where adult salamanders were observed during speleological expeditions (Hernandez, 2018b). Here I report the first observations on cave breeding by *S. algira splendens* and discuss the caves inhabited by this subspecies in the Middle Atlas.

METHODS

During two periods, 12-15 February 2017 and 16-22 February 2019, we surveyed the natural caves of the north-eastern Middle Atlas (Taza region, Morocco) using the Visual Encounter Survey (VES) method (Heyer et al., 1994). Field work was conducted from approximately 10:00 h to 15:30 h. Survey sites with permanent or non-permanent water sources and streams were selected based on cave data from previous speleological expeditions, including the Association de Spéléologie Marocaine, Randoxygène, and data on cave topography (Camus & Lamouroux,

1981). On site, we surveyed almost all types of habitat including permanent and temporary streams and pools, and surrounding terrestrial habitats, stumps and stones to record *Salamandra algira splendens* larvae, juveniles and adults. Each taxon observed was photographed to aid identification (Sony Nex-5; Sony Ltd., Japan). Coordinates, geographic and elevational data were collected in situ using a Global Positioning System (Garmin Montana 680; Garmin Ltd., Olathe, KS, USA) and located on maps. Water chemical parameters were measured in situ using an Expresstech @ LCD pH Medidor Digital (Expresstech; Kingpow Company Limited; Hong-Kong; China) for pH and temperatures.

RESULTS

Five adults, one juvenile and 22 larvae of *S. algira splendens* were discovered at Grotte de Chaâra (33° 57'24.33" N, 4° 14'44.95" W) located 1,213 m a.s.l.. The adults and juvenile were walking on rocks (Fig. 1 A, B). They were located from a few hundred meters up to 2 km into the cave and near a large permanent stream in a large chamber (Fig. 2 A, B). Larvae were generally found inside the cave at 160-400 m from the entrance in five pools, about 68-185 cm in length and 32-55 cm deep (Fig. 3A, B). In all the pools containing larvae, water quality was acidic to neutral with a pH between 6.13 - 7.11, air temperature at midday was 13.1-16.2 °C and water temperature 11.1-12.6 °C. The habitat was characterised by various rocks and small to large pools in complete darkness. The external habitat comprised pine and oak forests with *Pinus* sp. and *Quercus suber*.

Twelve larvae, two juveniles and four adults were observed in the Friouato caves, Taza region, north-eastern Middle Atlas (34° 6'19.217" N, 4° 4'19.667" W) located 1,426 m a.s.l.. The active adults were found at depths of 115 to 450 m from the cave entrance. Both juveniles were active on rock ledges at several meters from the cave floor. All terrestrial individuals were found in large chambers of the caves with

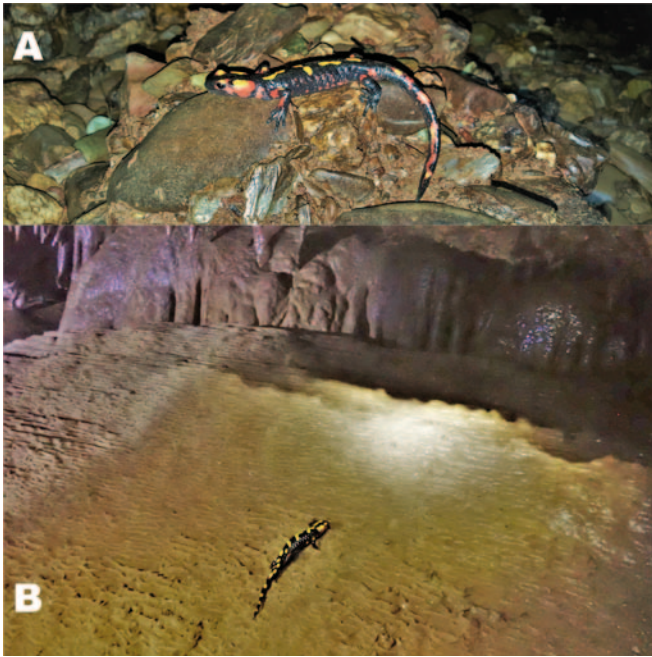


Figure 1. A. Adult female of *S. algira splendens* found at Grotte de Chaâra, Taza region, north-eastern Middle Atlas mountains, Morocco B. Adult male observed at the same site

relatively high air humidity (85-94 %) in the total darkness. Air temperature varied from 15.2 to 18.7 °C. The larvae were found in two small pools located in the cave at 120-230 m from the entrance. The pools substrates comprised sand, rocks and small gravel. The water quality was acidic to neutral with a pH between 6.62 - 7.16, air temperature at midday was 14.2-16.1 °C and water temperature 8.0-11.2 °C. The surrounding habitat comprised pine and oak forests with *Pinus* sp. and *Quercus suber*.

DISCUSSION

Cave fauna have long been a source of fascination due to their diversity and adaptations. Newts and salamanders are the only tetrapod vertebrates capable of having an exclusively subterranean life cycle (Gorički et al., 2019). In North Africa, two species were reported as facultative cave-dwellers: *Pleurodeles waltl* (Schleich et al., 1996; Hernandez, 2018b) and *Salamandra algira* (Pasteur & Bons, 1959). Two Moroccan subspecies of *Salamandra algira* were recently observed in caves *S. algira splendens* (Hernandez, 2018a, b) and *S. algira spelaea* (Escoriza & Comas, 2007). The current surveys found many adult individuals, juveniles and larvae in the Chaâra and Friouato caves of the north-eastern Middle Atlas (Figs. 1, 2 & 3). Observations of the species in other caves of the Taza region (e.g. Chiker and Zaerout) have been reported by local speleologists but need confirmation.

In both caves systems, larvae were commonly found at 120 – 350 m from the entrance, while adult individuals were observed all the year round in all parts of the caves from 0.2 – 2 km deep. At Grotte de Chaâra the main cave contains a large permanent underground stream which might have washed some larvae into the cave during periods of high rainfall. However, in the Friouato caves 12 larvae were in isolated

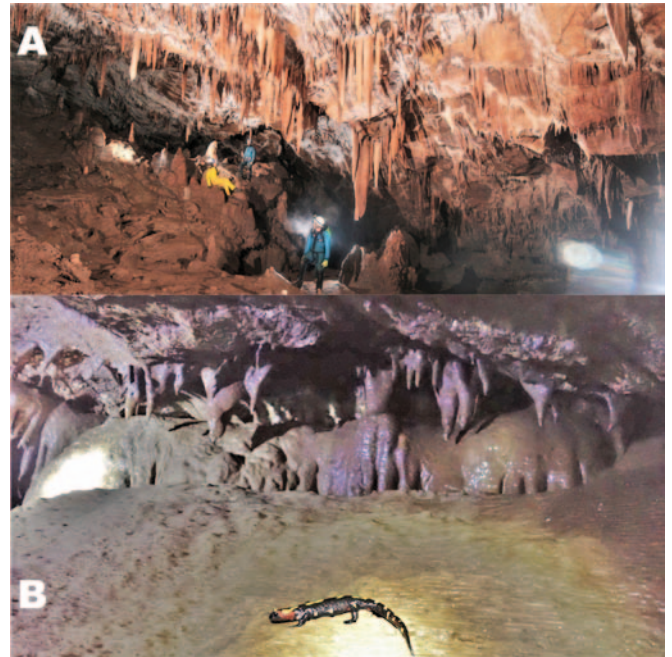


Figure 2. A. Habitat view of the largest chamber at Grotte de Chaâra where most of the adult specimens were recorded B. Another adult female specimen found in the same cave

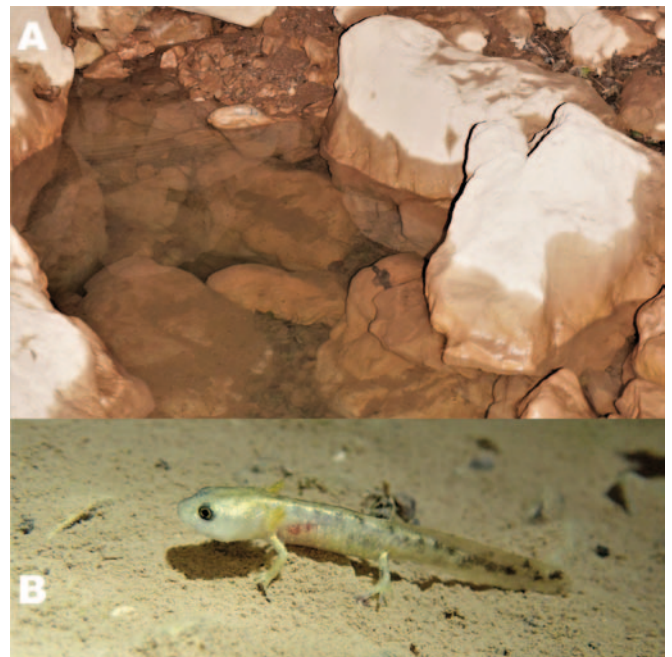


Figure 3. A. A small pool in the cave inhabited by many larvae of *S. algira splendens* B. Larva measuring 38 mm in the same pool

pools indicating that female *S. algira splendens* almost certainly deposited their larvae there. It is suggested that the species is breeding in both caves.

Within the Mediterranean ecoregion, twenty-two salamander and newt species are known to inhabit or breed in caves, especially during the hot, dry summers (Manenti et al., 2009, 2017; Hernandez, 2018b), others are more specialised cave dwellers (Table 1). Caves with groundwater and stable humidity play an essential role as thermal refugia for newts and salamanders (Hernandez, 2018b) and are also used

as winter shelters, hiding places during the active season, feeding habitats and as regular breeding sites (Herrero & Hinckley, 2014; Manenti et al., 2009, 2017; Balogová et al., 2017).

Table 2. Salamander and newt species of the Mediterranean ecoregion showing differing degrees of adaptation to cave-dwelling

Facultative cave-dwellers occasionally breeding in caves
<i>Calotriton asper</i> (Clergue-Gazeau & Martinez-Rica, 1978)
<i>Chioglossa lusitanica</i> (Gilbert & Malkmus, 1989)
<i>Euproctus platycephalus</i> (Lanza, 1983)
<i>Ichthyosaura alpestris</i> (Melega & Fusini, 2000)
<i>Lissotriton boscai</i> (Herrero & Hinckley, 2014)
<i>L. vulgaris</i> (Uhrin & Lesinsky, 1997)
<i>Pleurodeles waltl</i> (Herrero & Hinckley, 2014; Hernandez, 2018b)
<i>Salamandra salamandra</i> (Manenti et al., 2009, 2017)
<i>Salamandrina perspicillata</i> (Razzetti et al., 2001)
<i>S. terdigitata</i> (Lanza, 1983)
<i>Triturus cristatus</i> (Lanza, 1983; Uhrin & Lesinsky, 1997)
<i>T. marmoratus</i> (Giménez-Lopéz & Guarner Deu, 1982)
Highly adapted cave-dwellers usually breeding in caves
<i>Speleomantes flavus</i> (Raffaëlli, 2013)
<i>S. supramontis</i> (Raffaëlli, 2013)
<i>S. imperialis</i> (Raffaëlli, 2013)
<i>S. sarraabusensis</i> (Raffaëlli, 2013)
<i>S. genei</i> (Raffaëlli, 2013)
<i>S. italicus</i> (Raffaëlli, 2013)
<i>S. ambrosii</i> (Raffaëlli, 2013)
<i>S. strinati</i> (Raffaëlli, 2013)
Obligate cave-dwelling species
<i>Proteus anguinus</i> (Gorički et al., 2019)

These observations hint at the importance of subterranean habitats for salamanders living across the Mediterranean ecoregion but both they, and their associated invertebrate communities, require further study. This should consider the body condition of the animals to indicate how successful they are as cave-dwellers.

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Opportunistic feeding by house-dwelling geckos: does this make them more successful invaders?

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ABSTRACT - Various species of 'house' gecko are found in and around buildings, where they can be observed feeding opportunistically on the insects attracted to artificial lights. Most of the species are considered strict insectivores. Nevertheless, there have been several recently published observations of 'house' geckos feeding on non-insect food. In order to assess how common this behaviour is among geckos worldwide, we offered an online questionnaire to ecologists and herpetologists. Of the 74 observations received, most reported *Hemidactylus frenatus*, *H. platyurus* and *Gehyra mutilata* feeding on rice, bread, fruits, vegetables, dog food or chocolate cream, taken from tables, plates, and garbage bins. This opportunistic feeding behaviour is much more common than previously thought and is perpetrated by species considered to be highly invasive, possibly contributing to their success as invaders.

INTRODUCTION

Several gecko species (e.g. *Hemidactylus frenatus* and *Gehyra mutilata*) are often found in and around houses. These, so-called 'house' geckos, are very well adapted to urban life and are often observed feeding opportunistically on insects attracted to artificial lights at night (Tkaczenko et al., 2014). This provides them an easily accessible food source in locations generally lacking predators. In these conditions they reach higher densities in urban settings in comparison to their natural habitats (Weterings et al., 2018; Perry & Fisher, 2006).

Besides this interesting adaptation, common insectivorous gecko species have been reported recently to feed on food other than insects (Weterings, 2017; Weterings & Weterings, 2018; Tanalgo & Hughes, 2017). For example, *Hemidactylus platyurus* and *H. frenatus* were observed feeding on rice in a bin (Weterings, 2017) and *Gekko monarchus* feeding on bread from a plastic bag (Weterings & Weterings, 2018). To get an impression of how common this opportunistic feeding behaviour is among various insectivorous house-dwelling gecko species, we undertook an internet-based survey with ecologists, herpetologists and reptile enthusiasts that is reported here.

METHODS

We developed a short online questionnaire that participants could complete when they observed a gecko feeding on non-insect foods. It consisted of a short introduction to ensure that only non-insect foods were recorded followed by four questions:

1. Where did the gecko feed?
 - a. In a garbage bin
 - b. On a table
 - c. On dirty dishes
 - d. Other...

2. What did the gecko consume?

- a. Insects or other invertebrates
- b. Fruit or vegetables
- c. Rice
- d. Bread
- e. Eggs
- f. Unsure
- g. Other...

3. What species did you observe feeding on non-insect food items?

- a. Asian house gecko (*Hemidactylus frenatus*)
- b. Mediterranean house gecko (*H. turcicus*)
- c. Tropical house gecko (*H. mabouia*)
- d. Flat-tailed house gecko (*H. platyurus*)
- e. Brooke's house gecko (*H. brookii*)
- f. Indo-Pacific gecko (*H. garnotii*)
- g. Common four-clawed gecko (*G. mutilata*)
- h. Other unknown *Hemidactylus* species
- i. Not sure what species
- j. Others...

4. Where did you make the observations?

- 4.1 Locality (village, town, park, research station...)
- 4.2 District/County
- 4.3 Province/State
- 4.4 Country

The first three questions were multiple-choice, however, participants were given the freedom to submit alternative responses. The third question was accompanied by photographs of the various common house gecko species.

The questionnaire was shared on the ECOLOG listserver, on www.fieldherpforum.com, and on several Facebook pages and groups including the Facebook groups of the Societas Europaea Herpetologica and HerpResearch among others. The results of the questionnaire were plotted using the GGPlot2 (Wickham, 2009) library in RStudio v1.1.383 (RStudio, 2017) built on R v3.5.3 (R Development Team, 2019).



Figure 1. Map showing the location (black dots) where geckos were observed feeding on non-insect food. Circles show areas where the black dots overlap, numbers indicate the number of observations within a circle.

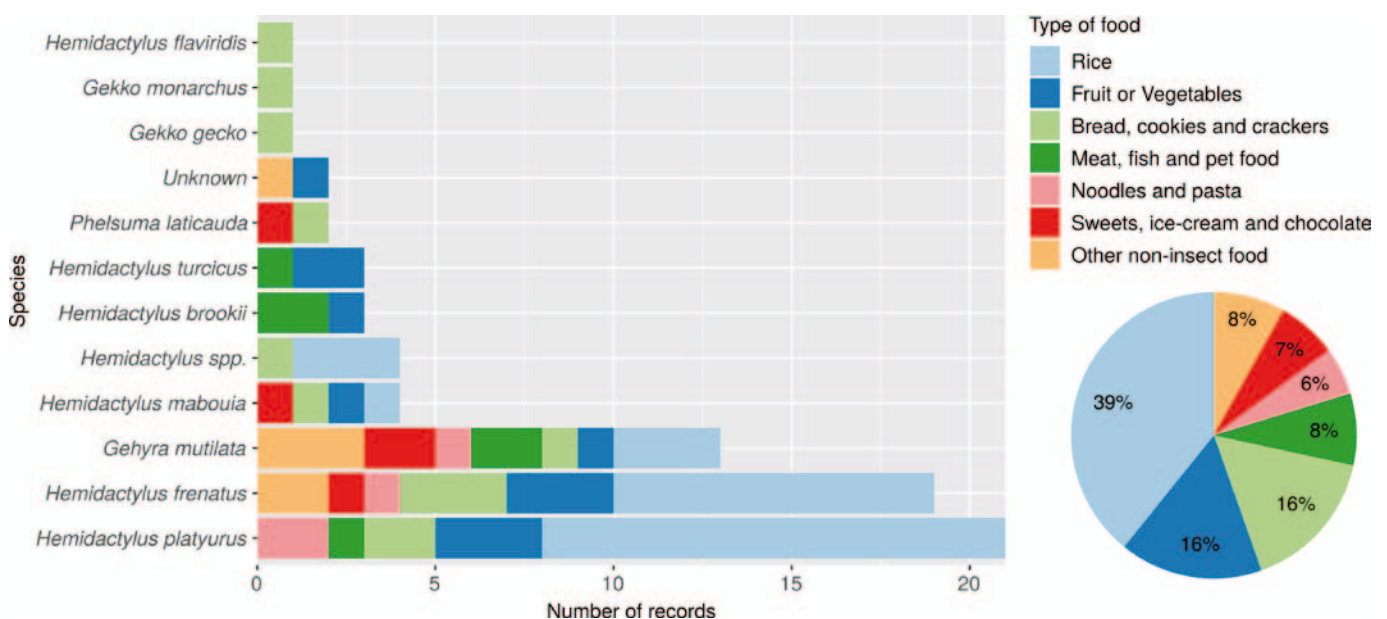


Figure 2. A bar chart showing the number of observations of non-insect food for different gecko species. The pie chart shows each food type as a percentage of all observations.

RESULTS

We received a total of 80 responses of which 74 included gecko feeding on non-insect foods. Most responses came from south-east Asia and the United States of America (Fig. 1). In total ten species, which are considered insectivorous, were observed feeding on non-insect foods. The species *H. platyurus*, *H. frenatus* and *G. mutilata* accounted for 72 % of the observations (Fig 2). The most commonly consumed non-insect food was cooked rice followed by fruit and vegetables and baked goods such as bread, cookies and crackers.

Some geckos were observed feeding on very particular food items. *Phelsuma laticauda* was, for example, observed feeding on an alcoholic tropical drink in Hawaii. *Gehyra mutilata* was observed eating garlic sauce from a bowl on a table top in Indonesia. On Vanuatu, a tropical paradise in the Pacific Ocean, *H. frenatus* was seen eating chocolate cream from a cake. Geckos were mostly observed feeding on the alternative food items on table tops (41 %), followed by dirty

dishes (11 %), floors (9 %), garbage bins (7 %), walls (3 %) and a variety of other locations (30 %).

DISCUSSION

Our survey shows that opportunistic feeding in geckos is more common than the incidental reports suggest (Weterings, 2017; Tanalgo & Hughes, 2017; Weterings & Weterings, 2018). Such feeding has been recorded for all common invasive house geckos, except for *Hemidactylus garnotii*, and also from other house-dwelling gecko species that are not considered invasive. These observations have largely come from south-east Asia, a direct consequence of the professional network of the authors, consequently Africa and South America are considered to be under-represented.

There have been several dietary studies of house geckos including *H. frenatus*, *H. mabouia*, *H. platyurus*, *G. mutilata* and *H. turcicus* (Tyler, 1961; Saenz, 1996; Ramires & Fraguas, 2004; Rocha & Anjos, 2007; Diaz Perez et al., 2012; Tkaczenko

et al., 2014; Barragán-Ramírez et al., 2015). In most of these studies, only insects were found in the diet although a few mention the presence of vegetal items within the stomach content (Iturriaga & Marrero 2013; Barragán-Ramírez et al., 2015). In these cases, vegetal content was considered to be ingested accidentally, i.e. not part of the gecko's diet. However, existing research into house gecko diet may be biased towards recording insects for two reasons. First, when gecko foraging is observed this is often on walls where artificial lighting attracts large numbers of insects (Tkaczenko et al., 2014; Aowpol et al., 2006). Second, when examining stomach contents, it is relatively easy to identify the hard exoskeleton of arthropods but identifying fruit, vegetables, ice-cream or dog-food from the stomach-gunk is often impossible.

Opportunistic behaviours and adaptability to new and changing environmental conditions facilitate the rapid colonisation of species in new habitats (Whitney & Gabler 2008, Chapple et al., 2012). Many of the gecko species reported in this study can be found in regions outside their native range and so are considered highly invasive (Weterings & Vetter, 2017). The opportunistic feeding behaviour that is observed in these gecko species may be an important factor in successful invasion. During periods of low insect abundance, for example during accidental transport (stow-aways), these species are able to eat other foods so allowing them to survive.

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Effects of different heat sources on the behaviour of blue tree monitors (*Varanus macraei*) in captivity

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INTRODUCTION

Captive reptiles are typically maintained using artificial heat sources. If reptiles are to achieve their optimal thermal working temperatures, and exhibit natural behaviours and physiologies then artificial heat sources should replicate sunlight as closely as possible and provide basking zones that are large enough to suit their needs (Autumn & De Nardo, 1995; Beaupre & Zaidan, 2012; Michaels et al., 2014). A wide variety of heaters are commercially available that provide combined electromagnetic radiation that approximate to the biologically relevant components of sunlight (Baines et al., 2016).

Self-ballasted mercury vapour lamps are typically used for heating in captivity and emit a mixture of ultraviolet-A (UV-A) and -B (UV-B) as well as visible light and infrared-A (IR-A).

Ultraviolet light is used by reptiles in a variety of ways such as recognition of conspecifics and food and the production of vitamin D₃ (Baines et al., 2016). A new development, an infrared lamp marketed as an 'Arcadia Deep Heat Projector' (Monkfield Nutrition Limited, Mepal, Cambridgeshire, UK), emits high levels of the medium wavelength infrared-B radiation (IR-B), a small amount of IR-A, very little visible light and no UV (Arcadia pers. Comms; information on packaging). This heat source is potentially much more energy efficient than mercury vapour lamps and so may represent a more economical approach to heating captive reptiles. Consequently, we compared the Arcadia Deep Heat Projector with mercury vapour lamps by recording the behaviour of captive blue tree monitors (*Varanus macraei*) when provided with these heat sources in succession.

MATERIALS AND METHODS

The study was undertaken at the Zoological Society of London using a male and a female blue tree monitor (*Varanus macraei*), both of which were 7.5 years (male) and 6 years (female) old at the time of study. An ethical review of the project was deemed unnecessary since it fell within the normal bounds of varanid husbandry and the project was approved on this basis (internally registered as ZDZ105). The lizards were housed in a concrete, mesh and glass enclosure (measuring 220 x 160 x 170 cm, L x W x H) furnished with a variety of décor, including natural branches, hides, a painted concrete tree trunk, rock

platforms, and a small water feature (Fig. 1). The substrate was a mixture of organic matter including peat-free compost and leaf litter. The ambient temperature in the enclosure was 26-30 °C in the day and 22-24 °C at night, which aligns with best practice husbandry for the species (Ziegler et al., 2009). The lizards' diet comprised of commercial feeder invertebrates and occasional defrosted vertebrates (mice, chicks etc.).

To record lizard behaviour, a time-lapse video camera (Plotwatcher Pro, Day6 Outdoors, USA) was installed in the front corner (from the public side) of the enclosure and set to take a photograph every 30 seconds. The camera was light activated and, as natural light penetrated the enclosure, it turned itself on roughly one hour before the enclosure lighting/heating came on, and off roughly one hour after it was switched off. The camera was left recording for eleven days, starting with a one day period for camera habituation for each treatment, hence yielding ten days of data. The first series of data were collected with two mercury vapour lamps (300W UltraVitalux, Osram, Germany) in place. The basking zone temperatures were 40-45 °C with a UV index of 3-6. This process was repeated when the heating system was changed to three IR lamps (Arcadia 50W 'Deep Heat Projectors', Monkfield Nutrition, UK). Under these lamps the basking zone temperatures were 39-43 °C with a UV index of 1.5-2. Lighting above the basking zone was provided by four fluorescent tubes (2x Arcadia 14 % T5 tubes and 2x 'ProLite' T5 tubes, both 550 mm) that gave ultraviolet B (UV-B) and small amounts of ultraviolet A (UV-A) throughout the study, set to a 12:12 photoperiod.

Surface temperatures were measured with a non-contact infrared thermometer (ZANMAX GM320, USA). We ensured that the expanse of the basking zones were identical (measuring 20 x 75 cm) between the two treatments. Filming of the lizards was then interrupted after the IR treatment and the IR lamps were replaced once again with mercury vapour lamps. After 14 days, filming was resumed to establish whether the lizards returned to a similar baseline pattern of behaviours.

Time-lapse videos were viewed using GameFinder Module software (V. 1.7.2, Day6 Outdoors, USA) which allows frame by frame playback. Data were entered into Microsoft Excel. An ethogram of relevant behaviours was created before the study through 3 h of lizard observation. The behaviours recorded were 'basking', 'resting', 'locomoting', 'out of sight'

and 'other'. From the time lapse video, a 30 minute period of lizard behaviour was sampled in the morning (09:30-10:00 h), midday (13:00-13:30 h) and afternoon (17:30-18:00 h) of each of the ten sample days, resulting in 30 observation windows per treatment. The two individuals could be distinguished easily based on colour markings (Ziegler et al., 2009), size and other anatomical details. The lizards' behaviour and location within the enclosure were recorded for each of the 30 second intervals in the 30-minute sample period, giving 61 individual records (including time zero) per sample window, per lizard. If a keeper entered the enclosure during one of these sample windows, the sample window analysed on that occasion was shifted forwards or backwards by 30 minutes to control for human disturbance to the lizards' behaviour.

The data were analysed for normality and homoscedascity in R (v.3.5.2; R Core team 2018) but the assumptions required for the use of ANOVA were not met. Consequently, data were analysed by Friedman tests for repeated measures using an online calculator (Social Science Statistics 2019).

RESULTS AND DISCUSSION

The lizards exhibited basking, resting and locomoting behaviours under both heating array treatments. In the male lizard, when the heating was changed from mercury vapour to IR lamps there was a large increase in the average count of basking behaviour (Fig. 2) which was statistically significant ($\chi^2 = 12.200$, $P = 0.002$). This was associated with a statistically significant reduction in resting behaviour ($\chi^2 = 18.617$, $P < 0.001$) but there was no change in the locomotory behaviours ($\chi^2 = 1.718$, $P = 0.42$). Similarly for the female, there was a large increase in basking behaviour (Fig. 2), although this was not actually statistically significant ($\chi^2 = 2.450$, $P = 0.294$), an associated significant reduction in resting behaviour ($\chi^2 = 7.267$, $P = 0.026$), and again no change observed in locomotory behaviours ($\chi^2 = 0.350$, $P = 0.839$).



Figure 1. Photograph of the enclosure in which the study took place. Basking zone area is indicated by the red circle.

Heating arrays that allow the animals to heat up faster, to reach a given surface temperature (within appropriate limits), should be beneficial by mimicking more natural conditions. The most likely explanation for the observed difference in

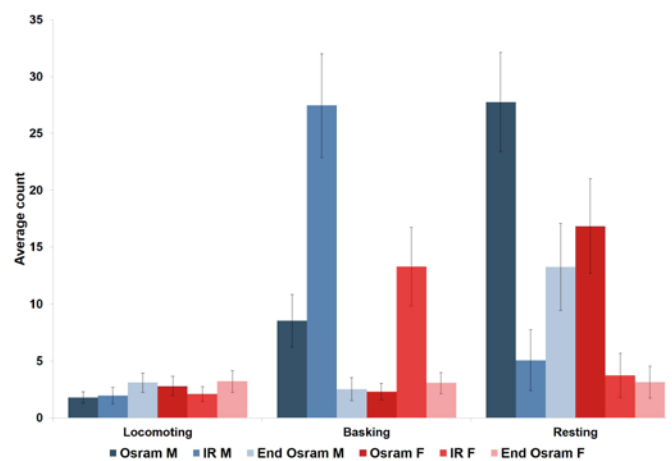


Figure 2. Average counts for each of the behaviours. Error bars show standard errors. Osram is the mercury vapour lamp and IR is the infrared deep heater. 'End Osram' is the return to mercury vapour lamp heating after the trial period with the infrared deep heater. M and F correspond to male and female.

basking time between the heat sources is the difference in proportions of IR-A and IR-B emitted by them. Different wavelengths of radiation have different effects on the warming of an animal (Barolet et al., 2016). Skin is the primary surface that is exposed to radiation and as such absorbs and reflects high proportions of it (Porter, 1967).

Between 60-70 % of incident sunlight is absorbed by the epidermis, compared to 10-20 % for the underlying muscle (Porter, 1967). The epidermis is heated most intensely by IR-B radiation, provided by the IR lamps. Here, the inner heating of the deeper tissues occurs through conduction of heat from the skin into the deeper tissues. In contrast, the mercury vapour lamps produce IR-A and visible light that have higher penetrative abilities than IR-B (Barolet et al., 2016). Some of the IR-A radiation is transmitted through the skin and directly into the deeper subcutaneous tissues, giving more rapid bodily warming than IR-B.

Although there was an increase in the amount of time spent basking by the female lizard, we did not find this to be statistically significant. As the female was smaller than the male there might have been a risk of competitive exclusion from the basking zone (Žagar et al., 2015) but given that the basking zone was large enough to accommodate both monitors this seems unlikely. More likely was that being smaller the female heated up more quickly than the male.

Overall, our data show a clear difference in the effects of the two heating arrays used in the study. From the perspective of optimising animal husbandry, the relatively new IR-B emitter, although more energy efficient, is likely to be less effective than mercury-vapour lamps for providing basking sites for captive *V. macraei* and likely other similarly sized lizards. We did not compare the IR-B emitter with a ceramic heater, which produces IR-C wavelengths that penetrate even less effectively into epidermis. It is likely that the IR-B emitter might perform better than IR-C emitters for reptile heating, but this remains untested. Practical decisions need to be made when designing enclosures for captive reptiles. These decisions must take into account species-specific

requirements and equipment costs. While the benefits of UV lighting in reptile husbandry are reasonably well known there is a surprising lack of information on IR heating. Future study should focus on IR heating arrays for the best benefit of captive stock.

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Chronicle of an injured mugger crocodile (*Crocodylus palustris*) surviving in the Vishwamitri River, India

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The mugger crocodile (*Crocodylus palustris*) is an apex predator in fresh water eco-systems with legal protection under the Indian Wildlife (Protection) Act, 1972 as a 'Schedule I' species. It is also considered 'Vulnerable' by IUCN criteria.

By the early 1960s in India, the species was depleted on account of illegal hunting, fishing and habitat loss (Whitaker & Andrews, 2003). However, mugger populations are now flourishing due to legal protection and successful conservation programmes (Singh, 1999). Consequently, at times there are conflicts between crocodiles and humans (Vijayakumar et al., 1999; Jayson, 2002; Whitaker, 2007; Vyas & Stevenson, 2017). The River Vishwamitri contains a substantial population of muggers, the last count shows the presence of 250 various sized specimens inhabiting a 25 km long stretch of this river in the largely human-dominated urban landscape of Vadodara city (Vyas, 2018). Every year a small number of muggers venture into human habitats and create panic amongst local people. This has inspired wildlife enthusiasts, to run 'save mugger' campaigns, an endeavour also supported by non-governmental organisations and the state Forest Department. Collectively, these agencies rescue and release troublesome muggers elsewhere in the same river system (Vyas, 2012). Between 2001 and 2010, many such rescued muggers were marked before release by caudal scute-clipping. This is a universally accepted method of individual identification, performed by clipping vertical caudal scutes in various combinations, with each combination designating a unique code number (Bustard & Choudhury, 1981).

On 25th July 2005, a badly injured mugger, measuring 1.8 m total length, was rescued from a small pool at Fokal, Zumkal, Vadodara (22° 25'55.47"N; 73° 12'19.14"E). The animal had lost the anterior part of the upper jaw (Fig.1), probably as a result of mutilation by local people using an axe-like weapon. This injured animal was tail clipped '247' by the Gujarat Society for Prevention of Cruelty to Animals, before release upstream of the Vishwamitri River in the waters of Lake Ajwa (22° 22'47.11"N; 73° 23'2.07"E); a man-made water body of 15 km². Considering the severity of its wound it would not have been expected to survive due to infection, starvation, or lack of defence against other crocodiles. The act of releasing a crocodile with such potentially fatal injuries, without any expert advice or veterinary consultation, was widely criticised by local conservationists and wildlife enthusiasts.

Eleven years later, in April 2016, a half-jawed mugger



Figure 1. Rescued *C. palustris* No. 247 marked by clipping tail scutes (vertical single row 2nd scute, 4th left and 7th right of double vertical row) before release, at Ajwa Lake, Vadodara. Inset at top, a lateral view of head showing missing one third of upper jaw.

was sighted (Fig. 2), along with 12 other muggers near the Vishwamitri river banks at Kirti Mandir, Vadodara (22° 18'33.16"N; 73° 11'23.31"E), 22 km (as the crow flies) west of Lake Ajwa. It was identified as mugger No. 247 with a completely healed upper jaw but without the nostril valve; the teeth in its lower jaw had grown large, and the animal seemed to be faring well. This sighting raised questions about how this mugger managed to travel so far after release and how it had survived the severe injury. It seems possible that the mugger had been washed out from the upstream area during the floods of 2014, when the water level of the river Vishwamitri reached 10.2 m leaving the entire area flooded. In the same year, about 48 various sized muggers were rescued from urban areas of Vadodara city (Vyas, 2018).

Another similarly injured mugger, with a missing upper half jaw, has been recorded (Vyas, 2014). That injury was caused by a territorial fight with a dominant, larger, residential male mugger. Subsequently, the victim survived a decade in captivity at the Sayajibaug Zoo, Vadodara. All crocodiles appear to have remarkably robust natural immune systems so that animals with extensive wounds apparently rarely succumbed to infection (Finger & Isberg, 2012). This fact indicates the



Figure 2. Mugger (*C. palustris*) No. 247 roaming along with a dozen of other large muggers in the waters of Vishwamitri near Kirti Mandir, Vadodara

importance of studies on the crocodilian immune system and wound healing. Happily, since its first reappearance in April 2016, No. 247 has continued to resurface to the present day.

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Albinism in *Elaphe sauromates*

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The Sarmanian Rat Snake, *Elaphe sauromates* (Pallas, 1814) is a rarely observed species from the Ponto-Caspian region. It is distributed from western-central Anatolia, through the eastern Balkan Peninsula of the northern Black Sea coast and Crimea to the western margins of Central Asia (Jablonski et al., 2019). This snake has a yellowish ground colour, often pronounced, sometimes orange-brown or very light. Characteristic is a dorsal dark pattern with transverse diamond to oval blotches. In older individuals this pattern is more diffuse. The ventral surface is often uniformly light (Schulz, 1996). Only one case of colour aberration has been recorded previously in this species and this was albinism in a 35 cm long immature specimen near Jambol town, ca 80 km west of Burgas (Petzold, 1975).

During a field survey on 12 October 2018 at 1:50 p.m. (local time), an adult *E. sauromates* was found that completely lacked intense pigmentation, the body appeared pale-yellow to light brown and the eyes scarlet (Fig. 1). The snake could not be captured for precise measurement but we noticed that it was an adult with an estimated total length around 1200 mm. This albino individual was found on a gravel road leading from route E87 to a local wind turbine platform, between the villages Kavarna and Hadzhi Dimitar in the Varna region, north-eastern Bulgaria (43° 45'37" N, 28° 41'34" E). The road forms an ecotone between a plant protection belt of *Fraxinus excelsior* and a corn field.

Interestingly, a second albino specimen of similar coloration was recorded on 30 May 2019 on the road between Kavarna and Balgarevo, again in NE Bulgaria (43° 43'36" N, 28° 35'95" E) at 5:27 p.m. This location was only 5 km from the first and on both sides of the road were growths of *Fraxinus angustifolia* and fields. Temperature during the observation was about 21 °C with weak wind. The individual was an adult with a total length of 985 mm, tail length 205 mm, and body weight 221 g.

These two cases of albinism are the first reported for adult *E. sauromates*. It is considered that albino snakes are rarely encountered in nature due to predation pressure on such individuals when juvenile (see Gezova et al., 2018). Nevertheless, these two new records show that survival of albino *E. sauromates* to the adult stage in the wild is possible. This may be due to the hidden lifestyle of this species.



Figure 1. An albino *E. sauromates* found near the village of Kavarna, NE Bulgaria

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Second observation of *Dermatemys mawii* in the upper Yucatán peninsula: a new population or an old individual?

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The Central American river turtle (*Dermatemys mawii*), or hickatee as it is known in Belize (Briggs-Gonzalez et al., 2018), is a large, freshwater turtle that represents the last remaining lineage of the family Dermatemydidae (Iverson & Mittermeier, 1980). Historically, this species was widely distributed throughout southern Mexico, Belize, and northern Guatemala (Alvarez del Toro et al., 1979; Iverson, 1992; Ernst & Barbour, 1989; Iverson & Mittermeier, 1980; Lee, 1996; Legler & Vogt, 2013; Vogt et al., 2011; Briggs-Gonzalez et al., 2018). However, due to harvesting for meat consumption (Moll, 1986; Polisar, 1994, 1995), numbers have declined drastically throughout much of its range, leading to its designation as a critically endangered species (Vogt et al., 2006; IUCN, 2019). The current distribution and many aspects of the general biology and life history of this turtle are not well known (Briggs-Gonzalez et al., 2018).

Dermatemys mawii was first reported from the northern coast of the Yucatan peninsula (21.281111° N, 89.643056° W) on 30 April 2010 (Chablé-Santos et al., 2011). This represented a 239 km northerly range extension for the species, although the specimen was presumed to be introduced (Vogt et al., 2011). Herein we document a second observation of *D. mawii* along the northern coast of the Yucatán Peninsula. On 6 March 2019, while hiking through La Reserva Ecológica El Corchito in the municipality of Progreso, Yucatán, Mexico (21.274754° N, 89.645159° W), we encountered and photographed an adult *Dermatemys* floating and swimming in the center of a cenote surrounded by mangroves and creeks (Fig. 1). This observation was made at a distance of roughly 740 m away from the 2010 record. It suggests either that more than one individual is present in the area or that we encountered the same individual as Chablé-Santos et al. (2011) nearly nine years later.

We attempted to determine whether the individual we encountered was the same as that in 2010 by referencing photographs of the two specimens. Both individuals appeared to be adult females, as indicated by the lack of yellow on the top of the head (Rainwater et al., 2012). The individual from 2010 was captured and marked with blue wire on the left supracaudal scute for identification at a distance (Fig. 2). While the individual in 2019 did not have this marking, it did have a conspicuous notch in the carapace where the wire would have been. As such, it may be impossible to determine whether the photographed individuals are different or if the individual from 2010 had simply lost the marking over the years.

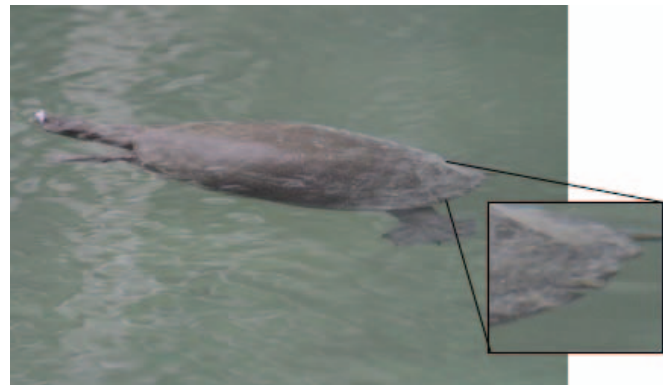


Figure 1. Adult female *D. mawii* observed in a freshwater cenote in 2019. Note the deep notch in supracaudal scute (inset).



Figure 2. Adult female *D. mawii* found in 2010 and marked with blue wire. Photos courtesy of Juan B. Chablé-Santos.

Regardless of whether the 2019 and 2010 observations represent one or two individuals, the current sighting has important implications for the conservation of *Dermatemys*. If the same individual was indeed observed nine years apart, then, given that the individual observed in 2010 was already an adult (Chablé-Santos et al., 2011), this would suggest that the longevity of adult *Dermatemys* in the wild is at least nine years. It may also imply that La Reserva Ecológica El Corchito is a suitable habitat for this species to thrive. If, in contrast, the two observations represent two different turtles, then this may indicate the establishment of a population in the El Corchito area. Identifying localities in which *Dermatemys* may persist, or are already persisting, could prove critical for conservation plans and potential head-starting or reintroduction programmes. We stress the need for further investigation into La Reserva Ecológica El Corchito and the surrounding areas (the home ranges of most aquatic turtles

are within a few square kilometers at most (Slavenko et al., 2015)) in order to determine which of the two scenarios proposed for our observation is more likely, as well as to lend insight into what factors might contribute to the apparent suitability of this habitat that is so distant from the primary range of this species.

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Contents: Issue 149, Autumn 2019

RESEARCH ARTICLES:

The Vanishing Viper: themes from a meeting to consider better conservation of <i>Vipera berus</i> ANGELA JULIAN & RICK J. HODGES.....	1
Vicente's poison frog (<i>Oophaga vicentei</i>) in the wild: calling activity, bioacoustics and diet ERIC E. FLORES, ABEL BATISTA, VITERBO RODRIGUEZ & RACHEL A. PAGE.....	11
Are the habitat niches of female green pit vipers <i>Cryptelytropis macrops</i> and <i>Viridovipera vogeli</i> partitioned by vertical stratification? CURT H. BARNES, WILLIAM FARREN, COLIN T. STRINE, JACQUES HILL III, SURACHIT WAENGSOOTHORN, & PONGTHEP SUWANWAREE.....	18

SHORT NOTE:

Winter presence of adult male palmate newts (<i>Lissotriton helveticus</i>) in a pond in Scotland GRANT WALKER, BRADLEY FAIRCLOUGH & ERIK PATERSON	24
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SHORT COMMUNICATIONS:

Play behaviour by captive tree monitors, <i>Varanus macraei</i> and <i>Varanus prasinus</i> DANIEL KANE, ADAM C. DAVIS & CHRISTOPHER J. MICHAELS.....	28
Discovery of the first feeding area for adult and juvenile green turtles and loggerhead turtles in Greece PHILIPPOS KATSIYIANNIS.....	32
First observations on cave breeding in <i>Salamandra algira splendens</i> from north-eastern Morocco AXEL HERNANDEZ	34
Opportunistic feeding by house-dwelling geckos: does this make them more successful invaders? ROBBIE WETERINGS & PREEYAPORN WETERINGS	38
Effects of different heat sources on the behaviour of blue tree monitors (<i>Varanus macraei</i>) in captivity OLIVER THOMAS, DANIEL KANE & CHRISTOPHER J. MICHAELS.....	41

NATURAL HISTORY NOTES:

Chronicle of an injured mugger crocodile (<i>Crocodylus palustris</i>) surviving in the Vishwamitri River, India RAJU VYAS	44
Albinism in <i>Elaphe sauromates</i> DANIEL JABLONSKI, JANKO JANKOV, KIRIL BEDEV & NIKOLAY NATCHEV.....	46
Second observation of <i>Dermatemys mawii</i> in the upper Yucatán peninsula: a new population or an old individual? JOHN DAVID CURLIS, MOLLY A. HIRST & MARCELLA D. BAIZ.....	47