The Herpetological Bulletin

Issue 131, Spring 2015

Published by the British Herpetological Society

THE HERPETOLOGICAL BULLETIN

The Herpetological Bulletin is produced quarterly and publishes, in English, a range of articles concerned with herpetology. These include society news, full-length papers, new methodologies, natural history notes, book reviews, letters from readers and other items of general herpetological interest. Emphasis is placed on natural history, conservation, captive breeding and husbandry, veterinary and behavioural aspects. Articles reporting the results of experimental research, descriptions of new taxa, or taxonomic revisions should be submitted to The Herpetological Journal (see inside back cover for Editor's address).

Guidelines for Contributing Authors:

1. See the BHS website for a free download of the Bulletin showing Bulletin style. A template is available from the BHS website **www.thebhs.org** or on request from the Editor.
2. Contributions should be submitted by email or as text files on CD or DVD in Windows® format using standard word-processing software.
3. Articles should be arranged in the following general order:

   Title
   Name(s) of authors(s)
   Address(es) of author(s) (please indicate corresponding author)
   Abstract (required for all full research articles - should not exceed 10% of total word length)
   Text acknowledgements
   References
   Appendices
   Footnotes should not be included.

4. Text contributions should be plain formatted with no additional spaces or tabs. It is requested that the *References* section is formatted following the Bulletin house style (refer to this issue as a guide to style and format). Particular attention should be given to the format of citations within the text and to references.
5. High resolution scanned images (TIFF or JPEG files) are the preferred format for illustrations, although good quality slides, colour and monochrome prints are also acceptable. All illustrations should be entirely relevant to the text and numbered sequentially with Arabic numerals. Images should be separate from the text file and in full resolution. Figure captions should be included within the text file, not embedded within images.
6. Authors will be informed promptly of receipt of their manuscript. Acknowledgement of receipt does not indicate acceptance for publication. All contributions are liable to assessment for suitability and ethical issues and all articles are subject to peer-review. The Editor reserves the right to shorten or amend a manuscript, although substantial alterations will not be made without permission of the primary author.
7. Authors will be supplied with a portable document file (pdf) of their published article and a complimentary copy of the full printed issue. Slides, artwork, and other original material will be returned following publication.
8. The Editor is keen to ensure that the Bulletin is open to as wide a range of contributors as possible. Therefore, if an author has concerns about compliance with submission guidelines or the suitability of a manuscript, or would like help in preparing it, please contact the Editor to discuss.
9. The significance and importance of some articles may be such that the Editor will offer a year's free subscription to the Society.

The views expressed by contributors to the Bulletin are not necessarily those of the Editor or the British Herpetological Society. All manuscript submissions and correspondence arising from the Bulletin should be sent to the Receiving Editors, **herpbulletin@thebhs.org.**

Front Cover: Male glass frog (*Hyalinobatrachium orientale tobagoense*) guarding his egg clutch on a leaf. See article on page 19. Photograph credit: Christopher Pollock.

Printed by: Bruce Clark (Printers), Units 7-8, Marybank Lane, Dundee, DD2 3DY. ISSN 1473-0928.

The Froglife Trust: Working for amphibians and reptiles in the UK and beyond

KATHY WORMALD*, J. ROGER DOWNIE, SILVIU PETROVAN & REBECCA NEAL

Froglife, 1 Loxley, Werrington, Peterborough, PE4 5BW
*Corresponding author email: kathy.wormald@froglife.org

INTRODUCTION

The Froglife Trust is a national wildlife conservation charity with a specific focus on reptiles and amphibians. Froglife began as Herpetofauna Consultants International in 1988, which evolved into Froglife in 2002. Today Froglife employs around 20 people and has an annual turnover in the region of £900,000. Froglife Trust's vision is strong, yet simple. We want to see a world in which reptiles and amphibians flourish as part of healthy ecosystems. Our strength is that we encourage people from all walks of life to get involved in wildlife conservation. Our aim is to make a real difference; which we do through our education and conservation teams working on-the-ground to inspire people and create and restore vital reptile and amphibian habitats.

The Key Strategies in our business plan are to:

1. Work internationally with relevant partners on reptile and amphibian conservation.
2. Continue to expand our learning and conservation project work both geographically and through programme development.
3. Extend links with universities, research institutions, and key technological centres to support external research, and expand Froglife's internal research capacity.
4. Build on communicating our messages to diverse audiences, and continue to demonstrate the positive impact our work has on wildlife and people.
5. Raise sufficient funds to continue and expand our work. Froglife collaborates actively with a range of partners within the wildlife conservation sector including a range of organisations in the UK and Europe, along with British government departments and statutory conservation agencies.

We collaborate extensively with organisations operating in other areas of the charitable sector including health, education, environment and social deprivation. Froglife is actively involved in forums including Scottish Wildlife LINK, a collective of organisations that lobbies the Scottish Government on conservation and environment issues; and The Cambridge Conservation Forum, which works on strengthening links between practitioners and researchers based in the Cambridgeshire area. Additionally Froglife is founding member of the European Network for the Protection of Amphibians and Reptiles from Transport Systems (ENPARTS), working with many partners on promoting best mitigation and prevention practice and collaborative research across Europe on road crossings. Froglife is also a founding member of Peterborough PLUS, a group of organisations drawn from across the charitable sector, which aims to ensure that local government tendering procedures recognise the ability of the charitable sector to deliver high quality service contracts. Froglife works closely with a number of UK Universities, primarily University of Glasgow, University of Hull, University of Leeds but also Salford University, Exeter and Anglia Ruskin.

Froglife operates a policy of placing all the data we collect in the public domain. We have data sharing agreements with most of the biodiversity records centres in the UK where we regularly submit our data. In 2001 Froglife produced the Great Crested Newt Conservation Handbook (Langton et al. 2001), which is still the main reference for both conservationists and ecological consultants working with this species in the UK. It has almost 10,000 downloads as a pdf as well as thousands of printed copies. We are preparing an update of this publication.

Hands-on conservation

Froglife's work centres on delivering on-the-ground actions for the benefit of reptiles and amphibians and as such we work extensively across the UK on creating and restoring standing water and terrestrial habitats. Since 2010 we have worked on 410 sites improving their biodiversity value. A total of 391 species surveys have been completed on these sites involving an amazing 2,308 volunteers (Fig. 1). We do not have the in-house capacity to maintain all of these sites so in order to ensure that the work that we do is sustainable all of our projects include an extensive volunteer training package. This includes training in species identification, surveying methodology and practical habitat maintenance.

Figure 1. Volunteers working on a habitat restoration project at Cardowan Moss Woodland, Glasgow, UK.

Traditionally, Froglife's work has focused on the more widespread UK species - great crested newt (*Triturus cristatus*), Smooth newt (*Lissotriton vulgaris*), palmate newt (*Lissotriton helveticus*), Common frog (*Rana temporaria*), Common toad (*Bufo bufo*), Viviparous lizard (*Zootoca vivipara*), Slow worm (*Anguis fragilis*), Adder (*Vipera berus*) and Grass snake (*Natrix natrix helvetica*). Although these are still relatively common some have undergone significant declines and have been listed as priorities within national biodiversity strategies. Our work to conserve these species is therefore crucial and all of our projects are delivering positive actions to achieve this. Froglife has produced a number of publications to aid others to deliver beneficial work such as the highly esteemed Great Crested Newt Handbook and our practical advice booklets Just Add Water and Urban Tails.

Froglife manages Hampton Nature Reserve (HNR) in Peterborough on behalf of landowners O&H Hampton Ltd. HNR is designated as being of European importance for wildlife (SSSI, SAC and Natura 2000 site) and has potentially the largest population of great crested newts in Europe. In addition to Froglife delivering substantive habitat work on the reserve to ensure that this important population is protected, the reserve also provides excellent research opportunities (more under Applied Research).

In 2015 Froglife will take on the management of the Boardwalks Local Nature Reserve in Peterborough. This used to have a healthy population of common toad; however, it has drastically declined in recent years. Froglife will deliver a comprehensive habitat improvement and management strategy in order to rescue the toad population and also other amphibian and reptile species on the site. We have secured substantial external funding from Heritage Lottery Fund and SITA Trust to carry out this work (see section on Froglife UK Wide for more information about our River Nene Dragon Finder project).

Although the sites that we work on are chosen for their amphibian and reptile interest, our work on these habitats delivers for a wide range of other important species. HNR is home to several rare invertebrate and plant species and many mammals including badgers and bats.

Toads on Roads

Froglife has for several years coordinated the Toads on Roads project. This involves supporting hundreds of volunteer groups across the UK aiming to stop toads being killed by vehicles during their migration season. In 2013 dedicated patrollers reported rescuing 85,997 toads.

Froglife keeps the official UK registry for crossing sites (http://www.froglife.org/what-we-do/toads-on-roads/) and we are using the database to try and understand more about the trends of toad populations (Carrier and Beebee, 2003) as well as spatial ecology projects.

Road mortality can have devastating effects on toad populations (Cooke, 2011), and it is important that in addition to assisting toads across roads, we also find alternative approaches to avoid toads having to cross busy highways. Thanks to grants from Patagonia, the Tides Foundation (grant making trust fund) and Biffa Awards (distributors of the Landfill Tax Credit Scheme) we have carried out habitat creation work and implemented strategies to encourage toads to use new ponds avoiding the necessity to cross a road as well as improvements to existing infrastructure to allow safe crossing of amphibians using road tunnels. We are currently monitoring this work to gauge its success. We provide an interactive map to allow very rapid contact with existing patrols and encourage more volunteers. Froglife also provides free insurance to all registered toad patrols, which can be vital for people working in the proximity of roads. We also help with providing specific equipment for night patrols, such as buckets, high visibility vests, torches, etc.

In order to streamline the coordination of the Toads on Roads patrols Froglife has, with the help of John Heaser, toad patroller and IT expert, developed an online facility on our website putting people interested in becoming patrollers directly in touch with the patrol coordinator. We will be developing an on-line database that we hope will be ready for the 2016 season. This will enable patrollers to carry out all administration for the patrols on-line.

In March 2012, Froglife hosted a conference to bring together 14 organisations from across Europe whose work focuses on protecting amphibians and reptiles from vehicles. As a result of the conference, ENPARTS was formed and currently has members from 17 European countries. The network is active through delivering research findings at conferences aiming to influence wider European policy.

Amphibian Disease

Froglife has for a number of years worked in partnership with the Zoological Society of London (ZSL) on amphibian diseases, primarily Ranavirus, through the Frog Mortality Project. Froglife has acted as the public portal for amphibian disease enquiries with ZSL carrying out vital research to establish the reasons, extent and impact on amphibian populations of the disease (Teacher et al. 2010). In 2012 Froglife's Amphibian Disease Project was incorporated into the new Garden Wildlife Disease Project. This is a partnership between Froglife, ZSL, The British Trust for Ornithology (BTO) and the Royal Society for the Protection of Birds (RSPB). This citizen science project asks people to report amphibians, reptiles, hedgehogs and birds that have died, been killed, or appear diseased. Those reporting mortality incidents are encouraged to enter their data onto a database through the BTO website. Reports are analysed by veterinary scientists at ZSL with some carcasses collected for further analysis.

Froglife UK wide

Froglife manages a number of projects operating in regions across the UK. These reflect the dynamic nature of Froglife, as an evolving organisation that is addressing the most pressing issues, whether it is on-the-ground conservation actions or through our learning programme to engage a wide range of individuals. Our Living Water Programme started in 2008 in London and as a result of its success it has expanded to Cambridgeshire, Northamptonshire, Sheffield, Glasgow, and North and South Lanarkshire. The programme focuses on standing water creation and restoration on wildlife sites, many of which are designated for their wildlife importance (Fig. 2).

We incorporate a strong volunteer training programme to assist with species surveying and habitat management.

The Living Water project in London lead to the Great Crested Newt Revisited project (2010-2012). Surveys carried out in 1984-1990 had identified sites in London with good crested newt populations. This project revisited those sites to establish the current status of the newts and where necessary to carry out habitat restoration to ensure that the sites continue to support these important populations. The findings were fairly bleak and although we found two new GCN sites six sites had also been lost.

Figure 2. Habitat creation at Foots Cray Meadow, Bexley, London, UK. Completed in 2009 part of the London Living Water project.

Froglife has a strong presence in London and has delivered a number of public education projects. Following a strategic meeting in 2010 we took the decision to consolidate all the different elements of our London work and to develop one substantial project to deliver activities across all boroughs of London. This culminated in the London Dragon Finder project, main funder being the Heritage Lottery Fund. We have also been supported by City Bridge Trust and several landfill tax credit scheme funders. The project runs from 2012 - 2016.

Following on from the London initiative we decided to take a similar approach to Scotland. Heritage Lottery Fund were very supportive and have provided us with a substantial grant, several other donors providing additional support. The programme (2014-2018) is operating across mainland Scotland and on some of the islands. These two projects have been followed by River Nene Dragon Finder (2015-2019) which is working all along the River Nene and its tributaries from its source in Northamptonshire through to it joining the sea at the Washes. These projects have attracted a total of £2.5m to date and will collectively be working on 93 sites and on most sites we will be carrying out multiple pond creation/restoration work meaning that this will result in a minimum of 500 new and restored ponds and substantial improvement in terrestrial habitats. This work runs alongside an engaging education programme including novel approaches to public engagement such as hosting Swimming with Dragon sessions, Dragons on the Move roadshow, Life Under the Surface exhibitions, Dragon Boat tours and races and the creation of a Mapestry (tapestry) depicting the natural heritage along twelve sections of the River Nene (Fig. 3). The programme is also delivering wildlife gardening workshops and a volunteer training programme.

Figure 3. Artists' impression, Lilian Taylor, of the Mapestry.

During 2011-2013 we investigated the variations in wildlife experiences between different generations. This involved young people interviewing older people about their wildlife memories and comparing these to the experiences of a younger generation. An output from the work is a booklet (unfortunately due to demand this is now out of stock) and a DVD that was shown at prestigious events organised by Froglife in London, Peterborough and Glasgow. It became evident that the older generation definitely had a more close up and complete experience with British wildlife than our younger generations, many of whom have never seen frogspawn! We found that this reminiscence work is a good way to engage with individuals who may not have the physical ability to get involved in wildlife actions but can share their past experiences. We have therefore incorporated a reminiscence element into the Dragon Finder programme through placing reminiscence seats at venues with high public footfall.

Froglife has been working with Peterborough City Council Youth Offending Services (YOS) since 2006. In 2012 we successfully secured a three year contract through a competitive tendering process. This work involves all young offenders in the Peterborough area attending Froglife conservation sessions to deliver their community service orders. The young people have contributed enormously to our conservation efforts from helping with habitat management tasks on HNR to building outdoor classrooms at schools. Last year these young people contributed a total of 762 hours to nature conservation. A recent Ofsted inspection of the YOS gave it an outstanding rating, even nominating it as the best service in the country with the report commending Froglife's contribution towards the success of the YOS. A few years back we noted a desire by many of the young people to continue working with Froglife after completing their community service orders but we did not have any suitable projects at that stage. This lead to the development of our Green Pathways Scheme, funded by BBC Children in Need and working with disadvantaged young people aged 10-15 within Peterborough. We also developed the Wildlife Ambassador project to work with any age group suffering from disadvantage. Our Green Pathways Scheme has proved so successful that BBC Children in Need have provided a further three year funding until 2018. The previous project worked with over 3,000 young people and transformed many green spaces around the city into wildlife havens (Fig. 4).

Figure 4. A group of young people involved in the Green Pathways Scheme doing some habitat management work at Hampton Nature Reserve, Peterborough, UK.

Thanks to grants from Young Start, Scottish Natural Heritage and The Robertson Trust in 2014 the scheme was expanded into Glasgow. It is working with young people in some of the highest socially deprived areas of Glasgow on improving local green spaces. We are often asked about vandalism of ponds and it is most certainly an issue that we do sometimes have to deal with. We find that the main issue is litter (trolleys, road signs, bottles left on site) but despite these issues our monitoring programme shows that generally the ponds are still functioning well for the species. Following the success of our Wildlife Ambassador project we have developed the Natural Achievers project and we are currently fundraising to get this work off the ground and working on-the-ground. Our Leapfrog Schools programme is creating and enhancing outdoor classrooms for a number of schools and once again we found Froglife being mentioned in an Ofsted report when the outdoor classroom at one school was reported as an excellent example of delivering educational lessons.

In keeping with Froglife's ethos all of our projects are working with a wide range of partners and particularly with partners who can refer individuals who are often excluded from taking part in wildlife conservation. For example, in Scotland we are working with TRELLIS, an organisation providing therapeutic gardens for individuals with a range of mental and/or physical health issues; in London we are partnering several borough Youth Offending Services and in the East of England we are working with a wide range of community groups.

Applied Research

A grant from the Esmee Fairbairn Foundation has enabled Froglife to benefit from having a Conservation Coordinator in post since autumn of 2011. This has greatly strengthened our research and monitoring capacity with Froglife managing several research projects. Wildlife road tunnels are increasingly being advocated in the UK and worldwide as a means of mitigation for development and are installed where new developments and roads will segment wildlife habitats in order to reduce roadkill and maintain connectivity. However, there has been little actual research into the effectiveness of these tunnels and which specifications are most effective and for which species. Froglife developed an innovative project using specially adapted cameras to monitor the wildlife using these tunnels. This has resulted in Froglife supporting a PhD student since 2013 and from 2015 also a Master by Research student. Three scientific publications are expected for 2015. As these tunnels are used by other wildlife the collected data will also inform wider conservation and mitigation practices (Fig. 5 & 6).

Figure 5. New tunnel at Hampton Nature Reserve, Peterborough, UK.

Figure 6. Landscape at Hampton Nature Reserve.

Since 2012, with funding from Natural England, Froglife has been working on using an improved and more robust method for estimating presence and abundance trends for GCN in a superabundant population using Hampton Nature Reserve, Peterborough, UK as a model. The site has the largest known population in the UK for this protected species but the reserve has over 350 ponds spread over 145 hectares and therefore quantifying what constitutes the most suitable pond habitat for newts and also trying to measure abundance trends can be extremely difficult. As a consequence we have been running a Masters by Research project using repeated newt surveys and occupancy modelling for understanding the distribution and abundance patterns of GCN on the reserve. We expect two publications describing the results.

In 2015 we are co-supervising one intern, an Erasmus Masters student from Belgium.

Our international reach is extending this year to Trinidad where we will have an association with the University of Glasgow's Trinidad Expedition, focusing on basic research and conservation work with the island's many amphibian species, several of which are listed as endangered by IUCN.

Our Learning Programme carried out research on public perception with reference to herpetofauna compared with other wildlife species. The Scale of the Problem research, funded through the Lottery Catalyst Fund, found a lack of appreciation of the importance of our species amongst the general public, highlighting a need for conservation organisations to give a higher priority to these species when promoting conservation needs. This is particularly the case for snakes, however not for all reptiles, as out of those that completed our survey, lizards proved the most popular of all the species.

Figure 7. Wildlife Gardening Workshop hosted in London as part of London Dragon Finder.

Species and habitat monitoring

In order for us to direct our conservation work it is important to have as much data as possible on our species. With the development of our London Dragon Finder project we were able to launch a free to download species recording and identification App. All of our projects are using the App alongside project participants to record species data. The wildlife workshops that form part of the London Dragon Finder project have proved very successful in not only training volunteers but also in assisting them to use the App (Fig. 7). The data will be analysed to enable us to map species distribution across the UK. To download the App visit http://www.froglife.org/dragonfinder/app/. We receive data from circa 160 toad patrol sites and this data is used for analysis into trend estimations as well as collaborations with other research projects, such as currently with Salford University, looking at infection rates and genetic background for common toads in different size populations and degree of isolation.

All of our on-the-ground conservation work incorporates a habitat monitoring programme. Surveys carried out at habitats after completion of the work often include surveys for aquatic invertebrates and plants providing us with a substantial body of evidence to establish the level of success of the work (Ramsay, 2014).

Spreading the word

Froglife manages a very active enquiry service with enquiries received through our website, on social media and by phone. The majority of our enquiries are from people wanting to help amphibians and reptiles and as such we put great store on answering every single enquiry and directing individuals to further information and our App. We are very popular on social media with over 12000 followers on Twitter and 2800 Facebook likes. Our website is packed with information and is very engaging and we receive many positive comments: visit www.froglife.org. Froglife produces a newsletter, Natterchat twice a year. Each edition is themed to cover a topical issue and we often feature guest writers.

We are passionate about getting out and about and over the past two years we have delivered 1,570 educational talks, training events and project sessions reaching a total of 18,000 people. We have also delivered 227 public events with 5,300 people attending.

As a result of the findings from the Scale of the Problem research Froglife has revamped the Just Add Water and Urban Tails publications to reflect comments received from those who took part in the survey (Fig. 8). Froglife staff frequently contribute to television and radio programmes and deal with a substantial number of press enquiries.

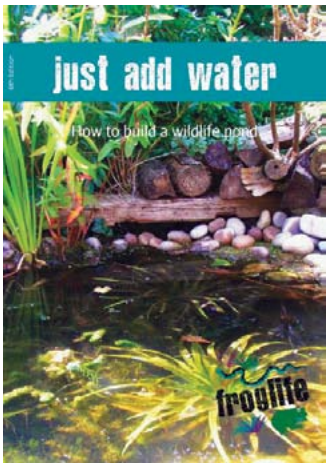


Figure 8. Just Add Water a booklet on how to build a wildlife pond and other wildlife gardening tips.

Evaluation

It is important that we know that our work is making a positive impact to the conservation agenda. It is easier to evaluate physical habitat work and the impact on species, and we do this through our monitoring programme. However, evaluating the impact of our work on people can be trickier but equally as important. We collect a lot of data on project participants but finding ways in which to evaluate these data to provide quantitative assessments has been the subject of much discussion at Froglife. This led us in 2014 to set up a partnership with the University of Hull, whose specialists are assessing the data from our Green Pathways Scheme. They will be drafting a report and plan to submit two articles to educational journals.

Friends and supporters

Froglife relies heavily on grants from grant making bodies and we are fortunate to have attracted these from a wide range of Trust funds, including those that fund social inclusion and health. We have also more recently received support from statutory bodies such as Natural England, Scottish Natural Heritage and Defra. This support is extremely valuable. However it is almost always restricted to a specific project. This means that we need to find additional resources to cover our core costs and also unfunded project work such as Toads on Roads, Amphibian Disease and our Enquiry Service. We cover these costs through our Friends scheme and through trading activities. Details on how to become a Friend are on our website and are open to anyone to join. Friends receive a welcome pack, copies of our newsletter, Natterchat, invites to events and other special offers as they occur. We have been very fortunate in that a number of celebrities have helped to promote our work (Fig. 9).

Figure 9. UK television presenter Charlie Dimmock with a group of Wildlife Ambassadors working at the Green Backyard, Peterborough, UK.

Figure 10. European Toads on Roads workshop hosted by Froglife in 2012 in Peterborough. The workshop lead to the formation of ENPARTS.

Future Aspirations

Froglife will continue to deliver the full range of work that it is currently involved in whilst also continuing to expand our successful Dragon Finder programme and Green Pathways Scheme. Our Living Water programme will be moving into phase 2 in Sheffield and in South Lanarkshire. We are keen to secure funding for our new Natural Achievers project that will provide opportunities for young people 17-25 years old and will complement our Green Pathways Scheme.

We will continue to expand our applied research capacity with our immediate target being to carry out research on 15 wildlife tunnels, and we will further develop partnerships with our European counterparts through ENPARTS (Fig. 10). Finding a long term solution to toad mortality is a top priority and we will further develop our initiatives on this front.

We are keen to be more active at a European level and we will be seeking opportunities to develop work with partners in other European countries.

REFERENCES

Carrier, J.A. & Beebee, T.J.C. (2003). Recent, substantial, and unexplained declines of the common toad *Bufo bufo* in lowland England. *Biological Conservation* 111: 395-399.

Cooke, A. (2011). The role of road traffic in the near extinction of Common Toads (*Bufo bufo*) in Ramsey and Bury. *Nature in Cambridgeshire* 53: 45-50.

Langton, T., Beckett, C. & Foster, J. (2001). *Great Crested Newt Conservation Handbook*. Froglife, Halesworth, UK.

Ramsay, A.J. (2014) *Gyrinus minutus* Fabricius (Gyrinidae): recent records from the Scottish Central belt. *The Coleopterist* 23:2

Teacher, A.G.F., Cunningham A.A. & Garner, T.W.J. (2010). Assessing the long-term impact of ranavirus infection in wild common frog populations. *Animal Conservation* 13: 514-522.

Accepted: 19 February 2015

A Comparison of the body sizes of Common toads *Bufo bufo L.* at two sites in Cambridgeshire, one with a Toad Patrol and one without

REBECCA ROSEMARY CATTELL

University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT
Current Address: Bourn, Cambridgeshire, CB23 2ST, UK
Email: rebecca_cattell@yahoo.co.uk

ABSTRACT - Common Toads *Bufo bufo* are declining in Britain and other areas of their range and road mortality is thought to be a contributing factor. In some areas, particularly in Europe and North America, 'toad patrols' have been set up to transport toads across roads during the breeding migration with the aim of reducing road mortality. This study measured body sizes of toads at two sites in Cambridgeshire, England, one with a long term toad patrol and one without, to determine whether the population at the patrolled site had a larger mean size because some individuals had been enabled to survive for longer. No significant difference in body size was found between the two sites, suggesting that mortality induced by factors other than traffic kill may be operating on the population and/or growth and that ultimate body size may be constrained by other environmental factors, which are not affected by toad patrols.

INTRODUCTION

Common Toads *Bufo bufo* are recognised as being of principal importance in Britain for the conservation of biodiversity under section 41 of the Natural Environment and Rural Communities (NERC) Act 2006 and were listed as a UK Biodiversity Action Plan species in 2007. However, they are still declining at present (Beebee, 2014). Amphibians can be particularly vulnerable to road mortality as they are often slow moving (Eigenbrod et al., 2009) and are believed to lack any behavioural ability to avoid roads (Eigenbrod et al. 2009; Beebee, 2013). In experimental tests many amphibian species stop moving when sensing engine noise and headlights of an approaching vehicle (Beebee, 2013).

In addition to the above factors, the vulnerability of *B. bufo* to road mortality is further increased by the species high terrestrial mobility, high migratory activity and breeding pond fidelity (Hels & Buchwald, 2001; Sutherland et al., 2010; Garriga et al., 2012; Beebee, 2013). Road mortality largely occurs during the breeding migration (Cooke, 1995; Meek, 2012) when as much as 100% mortality has been recorded (Semlitsch, 2003). The toads will not necessarily migrate through natural cover, such as rough grassland or scrub, to the breeding pond and often use open ground and roads, probably as a result of having few natural predators.

In many European countries *B. bufo* has the highest rate of road mortality of the amphibian species present (Santos et al., 2007) and the majority of amphibian road mortality research so far has been conducted on this species (Beebee, 2013). National authorities, non-governmental organisations and volunteer groups in Europe and North America manage mitigation measures against amphibian road mortality in the form of patrols and/or tunnels (Puky, 2005). Patrols consist of volunteers who collect and carry animals across the roads and release them at the breeding pond. A return procedure may also be in operation, returning animals back to the other side of the road after spawning has taken place. Patrols are most often used in species where a mass migration to a breeding site occurs, such as with some toad and salamander species, making hand collection effective. Tunnels are a permanent fixture under the road surface, intended for use by amphibians to and from the breeding migration and may be used by species which migrate in large numbers and also by those which do not.

The charity Froglife coordinates the toad patrol groups in Britain, providing advice and support to volunteers and managing a database of crossings and patrol data through the 'Toads on Roads' campaign, which was started by Flora and Fauna International in 1984. Many local Amphibian and Reptile Groups (ARGs) are involved in toad patrols. In Britain in the 1980s there were over 400 patrols, which between them moved more than 500,000 amphibians (mainly *B. bufo*) (Langton, 1989 in Beebee, 2013). By 2000 there were almost 900 patrols, which were moving a much reduced 100,000 toads (Froglife, 2012). The percentage killed averaged around 10% of those arriving at the road. For 2013 Froglife received patrol data from the largest number of patrols so far, with 141 returning data. In 2013, 80,923 were collected (and 7,327 reported dead) (Sivanesan, 2014). These numbers would seem to reflect what Beebee stated in 2014, that *B. bufo* has been and still is in decline in the UK.

After several anecdotal reports of toad populations where the adults were all noticeably small, a partnership between the Amphibian and Reptile Conservation Trust (ARC) and Amphibian and Reptile Groups UK (ARG UK) ran the first 'Toadsize' survey in 2013, a citizen–science project aimed at determining whether road mortality was preventing adults from reaching their full size. A total of 750 toads were assessed at 19 sites, with information about site locality and

the toad patrol recorded. The preliminary 2013 results have so far shown no difference in sizes either with or without a patrol or with or without traffic. However, patrol frequency and the distance of the road from the pond were found to be highly significant and associated with larger toads. A higher patrol frequency was associated with a greater size range of toads and the average size of toad is greater where the pond is further from the road (Inns, 2014).

The work reported here describes a similar study, carried out as part of the requirements for the M Sc degree at the University of Birmingham. The hypothesis being tested is that as road mortality may result in the death of a high percentage of the adult breeding population during the breeding migration, a regular patrol may prevent road mortality for a number of these individuals and so extend their lifespan, compared to a population similarly crossing a road and with no patrol. Because growth in toads continues throughout life, longer average lifespans should result in larger average body size.

METHODS

Study Sites

The body sizes of toads were sampled at two breeding locations in west Cambridgeshire, England. The first site in the village of Bourn (TL333592, 533380E, 259272N) was the pond at Great Common Farm on the Broadway road. This site had no current toad patrol and as far as could be ascertained has never had one. The second site had a voluntary toad patrol which has been in continuous operation since 1988. The patrol collect the vast majority of the breeding population through a combination of intensive searching and the use of temporary fencing, which keeps toads off the road until they can be collected. This pond was located at Madingley Hall (TL395604, 539499E, 260499N) on the High Street in the village of Madingley. Both ponds were located approximately 20 – 30 metres from the road. The surrounding areas of the ponds were composed of broadly similar habitat type, predominantly pasture with patches of scrub and hedgerow.

Sampling Method

The roads and surrounding areas of the ponds were surveyed for toads after dusk during the breeding migrations of 2013 and 2014. Survey dates were 11th April to 24th April 2013 and 18th February to 19th March 2014. All adult toads located were collected into buckets. Single females and amplexed pairs were kept in a separate bucket from single males to avoid fighting or smothering of the female. Toads which were incoming to the pond (pre-spawning) were kept in separate buckets from those which were outgoing (post-spawning).

At the end of the session at each site the collected toads were weighed (to the nearest gram) on a standard electronic scale (Duronic) and measured (to the nearest mm) from snout to urostyle. Four variables were recorded – female body weight (FBW), male body weight (MBW), female body length (FBL) and male body length (MBL). As there is some body weight loss after spawning in both sexes, whether the toad was pre or post-spawning was also recorded. This allowed only toads of the same state (and sex) to be compared.

The toads were then released either at the pond or on the other side of the road, depending on the direction of travel. To avoid the transmission of disease (such as Chytrid fungus) between sites the equipment, boots and hands were cleaned with a bleach based spray (Morissons Kitchen Spray with Bleach) and rinsed off thoroughly.

Analysis of Field Data

Data of body lengths and weights were first tested for normality using the Anderson-Darling test, which indicated 4 of the 8 data sets were not normally distributed. Subsequently, non-parametric Mann-Whitney U-tests (Minitab, v17) at n_1-1 + n_2-1 d.f. were used in the test for effect of site (patrol versus no patrol) on the median values of toad body lengths and body weights (pre-spawning toads only).

RESULTS

A total of 278 toads were recorded across the two sites, 178 in 2013 and 100 in 2014. No significant differences between years were found for any of the four variables, female mean body length (FMBL), female mean body weight (FMBW), male mean body length (MMBL) and male mean body weight (MMBW) and so the data for each of these variables for the two years have been combined. Table 1 shows the combined data, together with the results of Mann-Whitney U-tests for comparisons between medians. None of the differences were significant.

	Madingley (Patrol)	Bourn (No Patrol)	W	d.f. (n-2)	P
Females					
FMBL	72.6 (72)	73.1 (72)	3789.5	100	0.82
FMBW	43.3 (41)	45.3 (46)	3619.5	100	0.30
Males					
MMBL	59.7 (58)	60.1 (61)	11405.5	174	0.26
MMBW	22.7 (21)	22.6 (22)	11386.5	174	0.29

Table 1. Female mean body length (FMBL), female mean body weight (FMBW), male mean body length (MMBL) and male mean body weight (MMBW) for toads at Madingley and Bourn. Numbers in parenthesis show median values with W the Mann-Whitney statistics for tests of differences between medians. Data for 2013 and 2014 combined.

DISCUSSION

The results comparing body length and weights of toads between the patrolled and unpatrolled sites were not significant, meaning the null hypothesis could not be rejected. No significant difference in the lengths or weights of toads between the patrolled and unpatrolled sites was found. It is possible that there was a difference in the quality and quantity of food items between the sites. In theory better feeding conditions at Bourn could have enabled the toads there to have reached the same size as those that were enabled to grow larger at Madingley by virtue of a toad patrol effect but this seems unlikely. The terrestrial habitat within 1000 m of the

breeding ponds (the typical migration range) was varied, with a larger proportion of mature woodland at Madingley and more rough grassland at Bourn. Both are favoured terrestrial habitats for common toads.

A previous study (Inns, 2014) also found no significant difference in sizes of toads from populations with or without patrols, but it did find that larger toads were significantly correlated with patrol frequency. The Madingley site is patrolled frequently - almost constantly during the breeding migration and return journey - but this study did not find larger toads there. The Inns (2014) study also found a greater size range at patrolled sites: this again was not evident at Madingley. If patrolling did exert an effect on toad body size, it could be argued that it should be evident at the Madingley site where there has been a continuous patrolling effort since 1988. The combination of intensive collection and temporary fencing means that relatively few toads succumb to road mortality during the breeding migration at this site.

One possible explanation for the results recorded here is that that toads at Madingley are experiencing a high adult mortality due to factors other than road mortality during the breeding season. A low annual survival rate for *B. bufo* has been documented, with many surviving for just a single breeding season (Gibbons & McCarthy, 1984; Kuhn, 1994; Scribner et al., 1997, cited in Brede & Beebee, 2006). Gittins (1983), Gittins et al., (1984) cited in Beebee (1996) and Hemelaar (1984) cited in Beebee (1996) found that adult male survivorship between years to be 0.52 and for adult females 0.40 (the lower survival of the female was thought to be due to the increased burden of egg production). Scribner et al. (2001) found an even lower annual survival of adult toads, of under 15% and under 5% at two sites in England.

Changes in survival of adult and juvenile stages is thought likely to drive amphibian population dynamics as typically these stages have higher survival than earlier stages (Biek et al., 2002). Franz et al. (2013) found that road mortality of adult Natterjack Toads *E. calamita* in Poland was a key process affecting the dynamics of the meta-population. The majority of previous amphibian research has focussed on embryo and larval survival. Very little is known about the effects of road mortality on juvenile amphibians. It would be beneficial to have an increased focus on other life history stages to identify which are the most sensitive to the driver/s of a species decline. Targeted conservation efforts may then be enacted more widely.

In the face of declining amphibian populations, toad patrols are arguably worth pursuing even if most adults experience only one breeding season. Although Beebee & Griffiths (2005) suggest that the chances of reversing amphibian declines seem poor, as the threats are complicated and difficult to mitigate against, patrols are likely at the very least to increase the chances of more toads spawning at least once in those areas affected by road mortality, even if they do not increase individual toad survivorship longer term. Further research on the effectiveness of toad patrols on the lifespan of toads and the overall population size would be useful in determining future conservation measures for this declining species. This comment is speculative and the results of this study must be regarded as preliminary, and as with much work in applied ecology, they highlight the need for further research.

ACKNOWLEDGEMENTS

I would like to thank several people for their help with this work: William Seale of Madingley Toad Patrol for his help in collecting and measuring toads and providing advice on many aspects of toad ecology, Beckie Maclellan for assisting with toad collection, Dr John Wilkinson of Amphibian and Reptile Conservation for advice and for inspiring the idea for this study and Alex Lockton for supervising the project and providing guidance.

REFERENCES

Beebee, T. J. C. (1996). *Ecology and Conservation of Amphibians*. London: Chapman and Hall.

Beebee, T. J. C. (2013). Effects of road mortality and mitigation measures on amphibian populations. *Conservation Biology* 27: 657–668.

Beebee, T. J. C. (2014). Amphibian conservation in Britain: A 40 - year history. *Journal of Herpetology* 48: 2–12.

Beebee, T. J. C. & Griffiths, R. A. (2005.) The amphibian decline crisis: a watershed for conservation biology? *Biological Conservation* 125: 271–285.

Biek, R., Funk, W. C., Maxell, B. A. & Mills, L. S. (2002). What is missing in amphibian decline research: insights from ecological sensitivity analysis. *Conservation Biology* 16: 728–734.

Brede, E. G. & Beebee, T. J. C. (2006). Large variations in the ratio of effective breeding and census population sizes between two species of pond breeding anurans. *Biological Journal of the Linnean Society* 89: 365–372.

Cooke, A. S. (1995). Road mortality of common toads (*Bufo bufo*) near a breeding site, 1974-1994. *Amphibia - Reptilia* 16: 87–90.

Eigenbrod, F., Hecnar, S. J. & Fahrig, L. (2009). Quantifying the road - effect zone: threshold effects of a motorway on anuran populations in Ontario, Canada. *Ecology and Society* 14: 24.

Franz, K.W., Romanowski, J., Johst, K. A & Grimm, V. (2013). Ranking landscape development scenarios affecting natterjack toad (*Bufo calamita*) population dynamics in Central Poland. *Plos One* 8: 5.

Froglife [online] www.froglife.org [Accessed April 2014]

Garriga, N., Santos, X., Montori, A., Richter-Boix, A., Franch, M. & Llorente, G. A. (2012). Are protected areas truly protected? The impact of road traffic on vertebrate fauna. *Biodiversity Conservation* 21: 2761–2774.

Gittins, S. P. (1983). Population dynamics of the Common Toad (*Bufo bufo*) at a lake in mid-Wales. *Journal of Animal Ecology* 52: 981–988.

Hels, T. & Buchwald, E. (2001). The effect of road kills on amphibian populations. *Biological Conservation* 99: 331–340.

Inns, H. (2014). Wildlife Reports. *British Wildlife* 25: 354–356.

Meek, R. (2012). Patterns of amphibian road-kills in the Vendée region of Western France. *Herpetological Journal* 22: 51–58.

Natural England Environment and Rural Communities (NERC) Act 2006 [online] www.naturalengland.org.uk/ourwork/conservation/biodiversity/protectandmanage/habsandspeciesimportance [Accessed May 2014]

Puky, M. (2005). Amphibian road kills: a global perspective. Proceedings of the 2005 International Conference on Ecology and Transportation. North Carolina State University: Center for Transportation and the Environment, 325–338.

Santos, X., Llorente, G. A., Montori, A., Carretero, M. A., Franch, M., Garriga, N. & Richter–Boix, A. (2007). Evaluating factors affecting amphibian mortality on roads: the case of the Common Toad *Bufo bufo*, near a breeding place. *Animal Biodiversity and Conservation* 30: 1.

Scribner, K. T., Arntzen, J. W., Cruddace, N., Oldham, R. S. & Burke, T. (2001). Environmental correlates of toad abundance and genetic diversity. *Biological Conservation* 98: 201–210.

Semlitsch, R. D. (Ed.) (2003) Amphibian Conservation. Washington: Smithsonian Institution.

Sivanesan, S. (2014). Toads, Tunnels and Technology. *Natterchat* spring/summer 2014: 12–13.

Sutherland, R. W., Dunning, P. R. & Baker, W. M. (2010). Amphibian encounter rates on roads with different amounts of traffic and urbanization. *Conservation Biology* 24: 1626–1635.

Accepted: 11 December 2014

Cardiac thrombus in a Burmese python (*Python molurus bivitattus*)

ALINE LUIZA KONELL, BARBARA CRISTINA SANSON, AMÁLIA TURNER GIANNICO, JULIANA WERNER, FABIANO MONTIANI-FERREIRA,TILDE RODRIGUES FROES & ROGÉRIO RIBAS LANGE*

Universidade Federal do Paraná, Rua dos Funcionários, 1540 Juvevê, CEP 80035-050, Curitiba, Paraná – Brazil
*Corresponding author email: rrlange@ufpr.br

ABSTRACT - A ten-year-old male Burmese python (*Python molurus bivitattus*) was evaluated for coelomic distension in the second third of the body in the area around the heart. On radiographic examination an increase in soft tissue opacity was seen superimposed over the cardiac silhouette. Ultrasonographic examination revealed a cystic mass in the heart. The snake died after the examination. Necropsy revealed severe distension in cardiac region with coelomic membrane adhesion to the pericardium, which was thickened and filled with a massive blood clot. The heart was increased in size with a mass inside the right atrium. Histological presentation was consistent with septic cardiovascular thrombus with signs of recanalization, diffuse necrosis and acute diffuse inflammatory reaction. Gram staining technique revealed the presence of Gram negative bacteria. The exact cause of the thrombus and the entry site for bacteria could not be determined, but chronic stomatitis that was present for more than seven years in this patient was suggested as a possible origin. Nevertheless, there are only a few reports of cardiac diseases in snakes and generally with scant information concerning the aetiology. In this work, besides reporting details of the case itself possible causes for this cardiac disease are discussed.
Key words: Heart disease, thrombus, septic, snake, stomatitis

INTRODUCTION

Cardiovascular system disease in reptiles is infrequently diagnosed. However, both primary and secondary heart diseases have been described including congenital disease, myocarditis, heart disease associated with nutrition, neoplasms and aneurysms, arteritis, and endocarditis caused by bacteria, parasites or viruses (e.g. Jacobson & Kollias, 1986; Rishniw & Carmel, 1999; Jensen & Wang, 2009; Stumpel et al., 2012). To the author's knowledge, this is the first case report demonstrating diagnostic imaging features and histopathological results of a cardiac thrombus in a Burmese python (*Python molurus bivitattus*).

CASE REPORT

A ten-year-old male Burmese python (*P. m. bivitattus*) from Curitiba´s municipal zoo was presented to the Universidade Federal do Paraná´s Wild and Exotic Animal Service for clinical examination of a coelomic distension in the second third of the body, most precisely in the area around the heart, which was noticed two weeks previously (Fig. 1). The animal weighed 8.25 kg and was dehydrated, lethargic, thin and weak. The snake had been anorexic for three months and had been treated with subcutaneous gentamicin injections for pneumonia and topical riphamicin (Rifocina Spray, Laboratório Sanofi, São Paulo, SP) on oral lesions for chronic stomatitis, diagnosed seven years before. The animal was housed at the Curitiba´s Zoo facility for seven years but originally, the snake came from animal trafficking having being held for an unknown period in a triage center for wildlife (Centro de Triagem de Animais Selvagens - CETAS). Therefore, history prior the time that the animal was housed at CETAS was not available.

Figure 1. A male Burmese python (*P. m. bivitattus*) with coelomic distension in the second third of the body. The animal´s head is to the left.

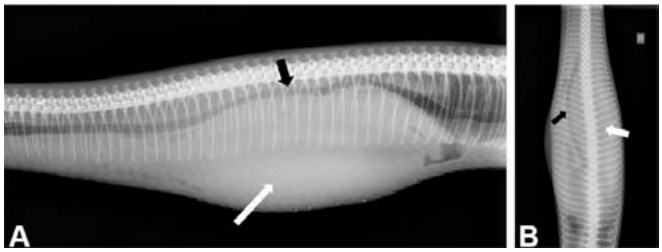


Figure 2. Radiographs of the cranial third of the male Burmese python (*P. m. bivitattus*) body. a) Lateral projection radiography showing an increase in soft tissue opacity superimposed over the cardiac silhouette (white arrow) and dorsal displacement of the trachea (black arrow). Cranial end is to the left. b) Ventrodorsal projection. Note again the soft tissue opacity over cardiac silhouette (white arrow) and lateral displacement of the trachea (black arrow).

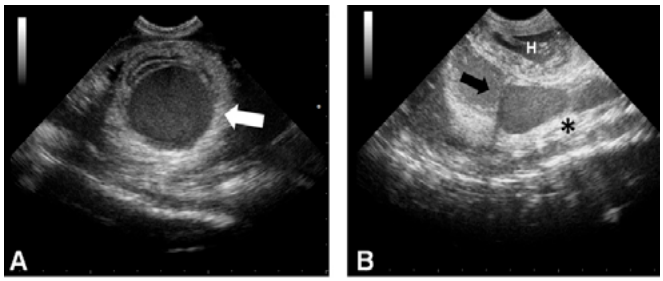


Figure 3. Ultrasonograms of a male Burmese python (*P. m. bivitattus*). a) Multiple layers mass filled with heterogeneous fluid (white arrow) inside the right atrium. b) The heart (H) was ventrally displaced. Note thickening of pericardium (asterisk) and the pericardial effusion (black arrow).

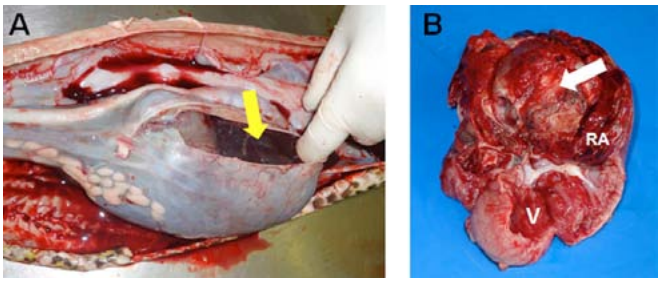


Figure 4. Postmortem examination pictures of a male Burmese python (*P. m. bivitattus*). a) Note the severe distension in cardiac region with coelomic membrane adhesion to the pericardium. The pericardium was thickened and with a blood clot inside (yellow arrow). b) The mass was observed inside the right atrium measuring 51 x 40 x 35 mm, with reddish coloration fibrous appearance and with a cystic cavity (white arrow). RA, wall of right atrium; V, ventricle.

For radiographic examination, lateral and ventrodorsal projections were performed using X-ray intensifying screens (Agfa Healthcare CR-30-X, DS5302, Mortsel, Belgium) with a tabletop technique of 55 kVp and 100 mAs. Radiographs of the cranial third of the body revealed an increase of soft tissue opacity superimposed over the cardiac silhouette, making it lose details and outline, with a dorsal-lateral displacement of the trachea (observed in lateral and ventrodorsal projections, respectively) (Fig. 2).

Ultrasonographic examination was carried out using an ultrasound system (MyLab 30 - Esaote, Genova, Italy) equipped with a 9-3 MHz micro-convex probe. This revealed a rounded structure measuring 44 vs 50 mm, with multiple layers (Fig. 3a) attached to the heart filled with heterogeneous free fluid (Fig. 3b). Pericardial thickening with large quantity of turbulent effusion was observed. At this point, differential diagnosis included a cardiac thrombus, left aortic arch aneurysm and a neoplastic mass.

The animal died immediately after the ultrasonographic examination. Necropsy revealed severe distension in the cardiac region with coelomic membrane adhesion to the pericardium, which presented as thickened with a very large blood clot present (Fig. 4a). The heart seemed to be increased in size (102 x 67 x 55 mm) with a significant dilation of the right atrium. Inside the right atrium, attached to its wall a mass measuring 51 x 40 x 35 mm was present, with reddish discoloration, firm consistency and gristly appearance. After cutting this mass a cystic cavity was seen (Fig. 4b). Mucous membranes in the oral cavity were erythematous with petechiae, plaques with caseous material appearing along the dental arcade, characterizing gingivitis, glossitis and palatitis. The liver was slightly yellow. No other major gross findings in other organs were noticed. The heart and liver tissues were collected and fixed in 10% neutral buffered formalin solution. After the heart tissue was fixed the wall of the cystic cavity mass was seen to be formed by a cluster of overlapping layers loosely bonded (Fig. 5). Tissue samples were routinely processed, paraffin embedded, sectioned at 5 mm, and stained with hematoxylin and eosin.

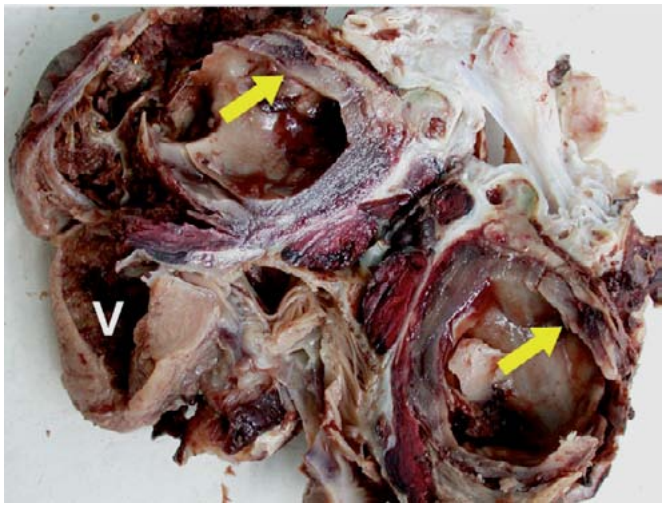


Figure 5. The heart of a male Burmese python (*P. m. bivitattus*) fixed in 10% neutral buffered formalin solution. Observe the wall of the cystic cavity formed by a cluster of overlapping layers loosely bonded (yellows arrows). (V) ventricle.

Histologically, the heart tissue was hemorrhagic and edematous, with relatively severe diffuse infiltration by lymphocytes, plasma cells, macrophages and heterophils. Muscle fibres were hyalinised but without signs of necrosis. An extensive mixed (of fibrin and erythrocytes) thrombus with the formation of sharp lines of Zahn and signs of recanalization (formation of new channels) occasionally seen, especially near the wall (Fig. 6a). Several regions exhibited deep tissue necrosis of coagulation with the presence of colonies of bacteria (bacilli) (Fig. 6b). There was no evidence or signs of neoplasia. In general, the histological presentation of this heart was consistent with a septic cardiovascular thrombus with signs of recanalization, disseminate necrosis and acute diffuse inflammatory reaction. In the liver tissue histologic changes included cytoplasmatic lipid vacuolation of hepatocytes, characterizing hepatic lipidosis. No other significant microscopic findings were seen in this animal. Gram staining technique was carried out in the thrombus tissue and showed Gram negative bacteria.

DISCUSSION

Although a great body of knowledge has been accumulated regarding the function of the reptile heart, its clinical implications in veterinary medicine have received limited attention. Literature related to the physiologic and evolutionary importance of the reptile heart is available, but there is little

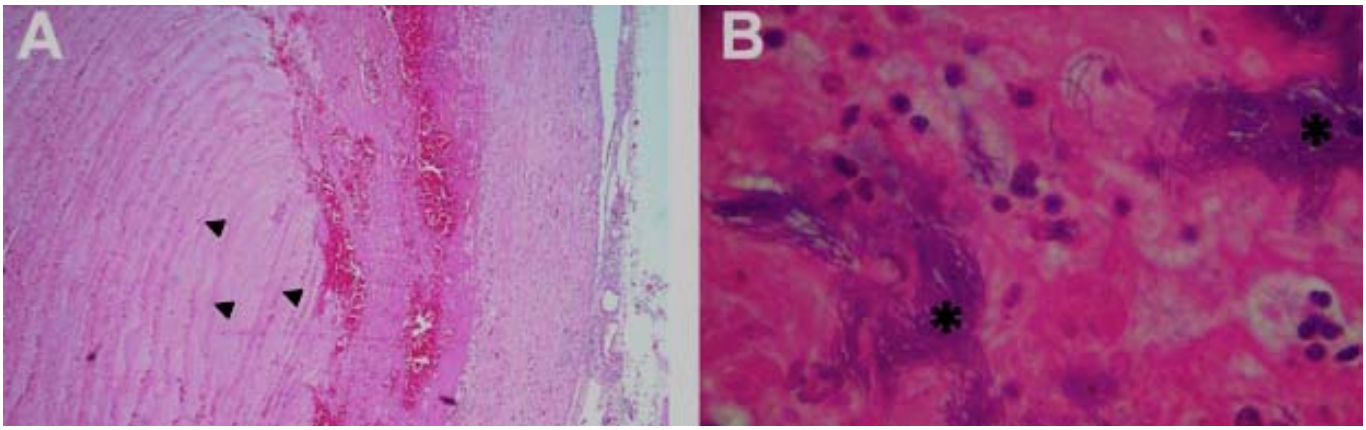


Figure 6. Photomicrographies of the intracardiac thrombus in a male Burmese python (*P. m. bivitattus*). a) Well organized, probably old blood clot evidenced by Zahn lines (arrow head) (HE 100x). b) Colonization by bacillary bacteria (asterisks) (HE 1000x).

clinical research about reptile cardiology (White, 1976). It is important to be aware of the normal anatomy and physiology of reptiles prior to diagnostic imaging or postmortem examinations. A detailed description of the snake cardiac anatomy and physiology has been described and reviewed by various authors and can be consulted for better understanding (Farrell et al., 1998; Girling & Hynes, 2004; Murray, 2006; Wyneken, 2009; Jensen et al., 2010).

A possible explanation for the presence of this contaminated thrombus would be a Gram negative septic embolus dislodged from the blood supply of the mouth as the snake had a long standing clinical history of chronic stomatitis. This septic embolus may have attached to the right atrium and initiated the process. A large number of different bacteria may be found in the oral cavity of affected reptiles, especially snakes (Fonseca, 2009). Draper et al. (1981), studying the microbiota from oral cavities of healthy and sick snakes, found Gram positive bacteria in healthy animals, whereas the sick ones presented, most frequently, Gram negative bacteria. Thus, the bacteria found in the intracardiac thrombus that have been classified as Gram negative, may have had oral origin. In cases where stomatitis is chronic and necrosis and ulceration are deep, a blood clot loaded with bacteria can be released into the bloodstream, resulting in septicemia (Jho, 2011). Despite the information about snake oral flora, investigations concerning the stomatitis influence in systemic diseases in several reptile species are scarce. One of our limitations was the lack of bacterial culture to identify which bacteria were responsible for the stomatitis and the microorganisms in the thrombus. Due to this limitation, it was not possible to confirm the origin of these bacteria.

Chronic stomatitis generates sense of pain and reduces the feeding capacity of the animal, leading to stress due starvation and weakness. Pythons have a low metabolism and capable of slowing down their metabolism for several months between each feed in order to conserve energy (O'Malley, 2005). In this case, the animal was anorexic for three months and was not cachectic, supporting O'Malley's proposition. In addition to cardiac abnormalities, we could also observe hepatic lipidosis. It may have occurred because the rapid weight loss resulted in secondary disease processes such hepatic lipidosis (Mitchell, 2009). This problem is seen most often in patients that have been starved, especially if they are obese (Donoghue, 2006).

An additional explanation for the formation of a cardiac thrombus could be blood collection by cardiocentesis. In this case report, we do not have a historical report of this technique during the seven years that the animal lived at the zoo or CETAS. Cardiocentesis is the most commonly used blood collection technique in snakes because it allows collections of large sample volumes, it carries a relatively low risk of lymphatic contamination and it can be used on snakes ranging from small neonates to large constrictors (Hernandez-Divers, 2006). Several researchers suggest that it is possible to collect blood safely by cardiocentesis in snakes, but care should be taken with asepsis and there should be few consecutive attempts (McFadden et al., 2011; Nardini et al., 2013). However, some authors suggest that risk of cardiac complications as a consequence of cardiocentesis exists, but it is poorly described. Some reports describe a dilated ventricle, lesions in the pericardium and myocardium, tissue adhesion and cardiac tamponade (Isaza et al., 2004; Selleri & Di Girolamo, 2012). The type of thrombus and observation of bacteria give us indications that blood collection by cardiocentesis may have been poorly performed.

The bacteria found in the thrombus, detected by histopatology were Gram negative. Usually, bacteria found in the body surface of most reptiles have been associated with unusual subtypes of *Salmonella* sp., a Gram negative bacterium (Mitchell, 2006). This finding could reinforce the fact that it is possible that cardiocentesis can introduce bacteria into a reptile's heart, if the technique is not carried out correctly, for instance with antiseptic care, restraint and right positioning. There are other safer techniques to collect blood samples, such as using the ventral coccygeal vein. The use of a poor technique requiring several attempts to collect blood samples has been suggested as a cause of trauma to the heart and associated structures.

Jacobson et al. (1991) reported a septic thrombus resulting in congestive heart failure, also in a Burmese python. This is the only report previously published in the literature about intracardiac thrombus in a snake. Similar to our report, the dilatation and the thrombus in the snake's heart were in the right atrium. In Jacobson's case, they found granulomas and urates deposited in the kidney, spleen and myocardium and small intestine congestion. In our case report, no other organs

had lesions, despite vacuolar degeneration in hepatocytes, a sign of lipidosis, found in both cases. *Salmonella arizona* and *Corynebacterium* sp. were isolated from the thrombus in Jacobson's case report, confirming hematogenous infection. In both cases it was not possible to affirm the source of infection, but the two reports provide important information on complementary tests for diagnosis.

This description reported an intracardiac thrombus in a snake and although the origin has not been clarified, possible causes were discussed. For several years the hypothesis that oral problems induce cardiac problems has been raised (Basilio, 2004), therefore, this would be one of many reasons to maintain appropriate oral health. Thus complete and frequent clinical examination of reptiles in order to rule out oral infections such as stomatitis is indispensable due to its systemic implications. Considering the other possible cause suggested, we concluded that antiseptic care as well as positioning and technical experience during blood collection are essential, especially in cardiocentesis.

ACKNOWLEDGMENTS

We would like to thank Prof. Pedro Ribas Werner for his assistance in evaluating the macroscopic lesions and interpretation of pathological findings of this case and the Curitiba Zoo. We also thank Christine Tilley and an anonymous reviewer for very useful comments on earlier versions of the manuscript.

REFERENCES

Basilio, R. C., Loducca, F. E. & Haddad, P. C. (2004). Medical dental prophylaxis of endocarditis. *The Brazilian Journal of Infectious Diseases* 8: 340-47.

Donoghue, S. (2006): In: *Reptile Medicine and Surgery*, pp. 284. Mader, D.R. (Ed). Saunders Elsevier, St. Louis, MO.

Draper C.S, Walker R.D & Lawler H.E. (1981): Patterns of oral bacterial infection in captive snakes. *Journal of the American Veterinary Medical Association* 179:1223-6.

Girling, S.J. & Hynes, B. (2004): Cardiovascular and haemopoietic systems. In: S. J. Girling and P. Raiti (Eds.). *BSAVA Manual of Reptiles*, 2nd ed. British Small Animal Veterinary Association, Gloucester, United Kingdom, pp. 243-260.

Hernandez-Divers, S.J. (2006): Diagnostic techniques. In: *Reptile Medicine and Surgery*, pp. 513-516. Mader, D.R. (Ed). Saunders Elsevier, St. Louis, MO.

Isaza, R., Andrews, G., Coke, R. & Hunter, R. (2004): Assessment of multiple cardiocentesis in Ball pythons (*Python regius*). *Contemporary Topics in Laboratory Animal Science* 43: 35-38.

Fonseca, M.G., Moreira, W.M.Q., Cunha, K.C., Ribeiro, A.C.M.G. & Almeida, M.T.G. (2009). Oral microbiota of Brazilian captive snakes. *Journal of Venomous Animals and Toxins Including Tropical Diseases* 15: 154-160.

Farrell, A.P., Gamperl, A.K. & Francis, E.T.B. (1998). Comparative aspects of heart morphology. In: *Biology of the Reptilia, Morphology G, Visceral Organs*. pp. 375-424. Gans, C., Gaunt, A.S. (Eds.). Society for the Study of Amphibians and Reptiles, Ithaca, New York. vol. 19

Jacobson, E.R. & Kollias, G.V. (1986). Adenovirus like infection in a Savannah Monitor. *Journal of Zoo Animal Medicine* 17: 149-151.

Jacobson, E.R., Homer, B. & Adams, W. (1991). Endocarditis and congestive heart failure in a Burmese python (*Python molurus bivittatus*) *Journal of Zoo and Wildlife Medicine* 22: 245-248.

Jensen, B. & Wang, T. (2009). Hemodynamic consequences of cardiac malformations in two juvenile ball pythons (*Python regius*). *Journal of Zoo and Wildlife Medicine* 401: 752-756.

Jensen, B., Nyengaard, J.R., Pedersen, M. & Wang, T. (2010). Anatomy of the python heart. *Anatomical Science International* 85: 194-203.

Jho, Y., Park, D., Lee, J. & Lyoo, Y.S. (2011), Aerobic bacteria from oral cavities and cloaca of snakes in a petting zoo. *Korean Journal of Veterinary Research* 51: 243-247

McFadden, M., Bennet, R.A., Reavill, D.R., Ragetly, G.R. & Clark-Price, S.C. (2011). Clinical and histologic effects of intracardiac administration of propofol for induction of anesthesia in ball pythons (*Python regius*). *Journal of the American Veterinary Medical Association* 239: 803-807.

Mitchell, M.A. (2006). Salmonella: diagnostic methods for reptiles. In: Mader, D. R. (Ed.). *Reptile Medicine and Surgery*, 2nd ed. Saunders Elsevier, St Louis, Missouri. pp. 900-905

Mitchell, M.A. (2009): Snakes. In: Mitchell, M.A. & Tully Jr, T.N (Eds). *Manual of Exotic Pet Practice Saunders*, St. Louis, MO. pp. 136-162

Murray, M.J. (2006). Cardiopulmonary Anatomy and Physiology. In: Mader, D. R. (Ed.). *Reptile Medicine and Surgery*, 2nd ed. Saunders Elsevier, St Louis, Missouri. pp. 124–134.

Nardini, G., Leopardi, S. & Bielli, M. (2013). Clinical hematology in reptilian species. Veterinary Clinics of North America: *Exotic Animal Practice* 16: 1-30.

O'Malley, B. (2005). Snakes. In: *Clinical Anatomy and Physiology of Exotic Species*, O'Malley, B. (Ed) Edinburgh: Elsevier Saunders. pp. 77-93.

Rishniw, M. & Carmel, B.P. (1999). Atrioventricular valvular insufficiency and congestive heart failure in a carpet python. *Australian Veterinary Journal* 77: 580-583.

Selleri, P. & Di Girolamo, N. (2012). Cardiac tamponade following cardiocentesis in a cardiopathic boa constrictor imperator (*Boa constrictor imperator*). *Journal of Small Animal Practice* 53: 487.

Stumpel, J.B., Del-Pozo, J., French, A. & Eatwell, K. (2012). Cardiac hemangioma in a corn snake (*Pantherophis guttatus*). *Journal of Zoo and Wildlife Medicine* 43: 360-366.

White, F.N. (1976). Circulation. In: *Biology of the Reptilia*, pp. 273-334. Gans C. (Ed). Academic Press Inc, London.

Wyneken, J. (2009). Normal reptile heart morphology and function. Veterinary Clinics of North America: *Exotic Animal Practice* 12: 51-63.

Accepted: 13 February 2015

A record of captive reproduction in the Red bellied toad *Melanophryniscus klappenbachi* with notes on the use of a short-term brumation period

ADAM W. BLAND

The Manchester Museum, Oxford Road, Manchester M13 9PL
Email: adam.bland@manchester.ac.uk

INTRODUCTION

The South American genus *Melanophryniscus* is represented throughout Argentina, Bolivia, Brazil, Paraguay and Uruguay by 26 species (Frost 2013) of small to medium sized Bufonids. These are collectively and commonly referred to as the red-bellied toads, as many species possess red or orange flash markings upon their ventral surface and the undersides of the hands and feet. Some species are also in possession of a brightly coloured dorsum; much like members of the *Dendrobatidae*, although most remain less conspicuously marked. These toads are mostly diurnal, spending the day foraging for small invertebrates. Their reproduction is seasonal and opportunistic; many species occur in usually dry habitats where explosive breeding takes place after heavy rains, where many individuals emerge to reproduce in temporary water bodies (Aquino et al. 2004, Garcia et al. 2004, Lavilla et al. 2004). The resulting larvae have been known to display rapid development to ensure metamorphosis before the disappearance of these breeding sites in the usually dry environment (Kurth et al. 2013).

Melanophyniscus klappenbachi was described in 2000, following taxonomic separation from *M. stelzneri* (Prigioni & Langone 2000) and occurs in shrubland habitats within the Chaco region of Argentina and Paraguay at elevations of 50 to 100 masl. It is a small diurnal species, reaching an average adult size of 2.5 - 3cm. The dorsal colouration consists of irregular yellow blotches on a black base colour (Fig. 1). This species is considered locally common within its range (Aquino et al. 2004). At the time of writing it is present within the international pet trade, and although this is not currently considered to be a major threat to the species, methods to produce regular captive reproductions should be established as to reduce the collection of wild specimens to be maintained in captivity. This species does not seem to be regularly reproduced within zoological collections (pers. obs.), and developing methodology to reproduce this more common species of *Melanophryniscus* in captivity may also benefit future conservation efforts for more threatened related species.

Kurth et al. (2013) report captive reproduction of this species, although only describe briefly the methods used to induce a successful spawning and subsequent rearing of offspring, focusing on the description of the species larvae and development. This included providing a three week brumation period for all specimens prior to breeding. This paper describes a method used to maintain and successfully reproduce *Melanophryniscus klappenbachi* in captivity using a much shorter brumation period for the female specimen only; the benefits of this method are discussed.

Figure 1: Adult *M. klappenbachi* showing black and yellow aposematic colouration.

METHODS

The adult group of *M. klappenbachi* used in this instance comprised two males and one female maintained within the Manchester Museum Vivarium. They were housed in a naturalistic vivarium measuring L 46cm X H 30cm X W 39cm, which was furnished with *Philodendron scandens*, flat rocks were provided which the toads used as shelters and also served as open feeding areas. A substrate mixture of soil and sphagnum moss was used above a drainage layer of loose clay balls. The top layer of substrate was furnished with leaf litter and sheet moss, which was also frequently utilised as shelter by the toads. A shallow water dish was available at all times and was replaced daily. Temperatures maintained fluctuated between 22–26˚C and UVB was provided through the use of Arcadia 6% T5 lighting with a photoperiod of 12/12. The humidity was maintained between 50-60% through a light daily misting provided by hand and the toads had access to fresh water via their water bowl if needed. Food was provided daily and consisted of hatchling crickets, *Drosophila* sp, and tropical springtails (*Siera* sp). All food offered was supplemented with Repashy Calcium Plus and

Figure 2: Newly metamorphosed *M. klappenbachi*.

Figure 3: Basic rearing containers used to house newly metamorphosed *M. klappenbachi.*

gut loaded with fresh fruit and vegetables when possible.

To attempt inducing reproductive behaviour in the specimens it was decided to follow Kurth et al. (2013) and provide a winter brumation period where the temperature was reduced to 4 - 9˚C. In the description of this period by Kurth et al (2013) the brumation of specimens lasted three weeks and was provided for both males and females. In this instance, it was decided to provide this temperature reduction but only for the female whilst the males remained in normal conditions, and this period was only provided for four days. The female *M. klappenbachi* was housed within a ventilated container containing 10-15cm of sphagnum moss and placed within a refrigerator where temperatures fluctuated between 5-8˚C. Each day the toad was inspected to ensure no negative effects of conditioning were to be observed and as expected activity was reduced almost completely. Upon being removed from the refrigerator after the four day conditioning period was over, the female *M. klappenbachi* was then placed directly back into the regular vivarium where within minutes she became active and even began to feed. All specimens remained within normal conditions for a further four days allowing the female to feed and reacclimatise until all being placed within a semi-aquatic rain chamber.

The rain chamber created for this species measured L 60cm X H 45cm X W 45cm and contained 10cm of water maintained at temperatures between 20-25˚C. Floating pieces of cork bark were used as land areas and the water was well planted with *Elodea densa*, densely filling the rain chamber with plants aided the toads when moving through the water as they were not adept swimmers for long periods, they also provided egg deposition sites. An aquarium pump was used to circulate the water as artificial rain over the toads during the daytime hours between 10.00am and 4.00pm.

RESULTS

All three specimens were introduced to the rain chamber simultaneously and reacted to the change in environment immediately. Within 20-30 minutes both males began to call and actively pursued the female. Amplexus was achieved within 2 hours of introduction to the rain chamber, the amplectant pair continued to pace around the enclosure whilst the single male persisted with loud calls and attempts to displace the successful male.

After 24 hours egg deposition began and multiple clumps of spawn each containing in excess of 20 individual eggs were deposited in various areas of the rain chamber, attached to the aquatic plants and submersed areas of cork bark. During spawning and fertilisation the male cradled the eggs with his feet and carefully attached them to the chosen surface. The aquarium pump was turned off at this time to reduce the risk of rainfall causing damage or displacement of the eggs. Once the female finished depositing eggs, approximately two hours after egg deposition began, the male remained firmly in amplexus and they were separated in an effort to avoid the risk of exhausting the female. At this stage all specimens were then placed back into their regular vivarium, where breeding activity almost immediately ceased; short intermittent calls were heard as they began to settle back into their regular environment. The spawn was left to develop within the rain chamber.

Egg and larval development followed the description by Kurth et al (2013) with larvae hatching after 48 hours and developing to metamorphosis in a relatively short amount of time. The larvae remained in the rain chamber for the duration of their development. No water filtration was used, but instead 50% water changes were undertaken every 24-48 hours and an air pump was used to oxygenate the water. The diet for the larvae consisted of finely powdered foods such as sera micron and powdered Spirulina algae dusted onto the surface of the water twice per day. The larvae spent much of their time clinging to the sides of the aquarium or objects in the water using their specialised mouthparts. The water temperature was maintained at 25˚C, which appeared to ensure healthy and consistent growth. The first specimen metamorphosed after 19 days of development, although others took double this length of time to metamorphose. When leaving the water, the toadlets climbed out onto the floating cork without any problem and care was taken to ensure newly emerged young were removed promptly as to avoid the risk of drowning if they were to fall back into the water; during the peak of metamorphosis the aquarium was inspected regularly.

The newly metamorphosed young measured between 5-7mm and lacked the colouration seen in the adults; they were mostly uniformly black in colour (Fig. 2). They were

housed in small partially ventilated plastic containers measuring L 15.5cm X H 6cm X W 7cm with a substrate of damp paper towel and moss for shelter (Fig. 3). Groups of up to 10-11 toadlets were housed in these containers. The containers were lightly misted with water once per day and the damp paper towel was replaced every second day. Feeding began 24 hours after metamorphosis and was provided by supplemented tropical springtails (*Siera* sp.) of varying sizes; young and adult springtails were readily consumed although young toads were unable to consume hatchling crickets. The use of the small rearing containers appeared essential in successful rearing of young, as this enabled toadlets to easily locate their prey items. It was found that feeding opportunities were required constantly for toadlets to maintain and support growth and body condition, large quantities of supplemented springtails were required daily and under this regime initial growth during the first 7-14 days was rapid. Early stages of the development of adult colouration also appeared during this time. Temperatures provided were between 22–26˚C, as with the adults. Size of rearing container was increased according to growth until specimens could be housed in naturalistic vivaria as used with the adults. This was achieved when young toads reached over 1cm in total length, at which time they also began to display their full adult colouration (Fig. 4) and feed upon hatchling crickets.

Figure 4: Adult colouration showing in toadlets measuring over 1cm in total length.

DISCUSSION

There was little sexual dimorphism between the specimens and males were not often observed calling outside of breeding conditions; only infrequent short calls were observed occasionally when in normal conditions. The most useful characteristic to identify sex was size; females are slightly larger than males, the female measured only 4-5mm larger in SVL than the males of this group. Compared directly with one another the female was easily recognisable. It was also found that this species is one that prefers to feed on extremely small food items, any food item offered larger that a 1st instar cricket or even the larger *Drosophila hydei* was a struggle for the adult *M. klappenbachi* to easily swallow and usually refused. It was also evident that to successfully raise toadlets it was essential to provide large quantities of small supplemented invertebrates to satisfy their voracious appetites and high metabolism during this age. Failure to do this will result in inconsistent weights during growth, metabolic problems and loss of specimens. Establishing cultures of tropical springtails in advance to maintain a constant supply was of high importance.

This species does not seem to fare well when maintained within a too humid or wet environment for long periods; as previously mentioned they are inhabitants of sub-tropical shrublands Once settled within the vivarium these mostly diurnal toads were observed to utilise hide areas within the enclosure underneath rocks, foliage and even small burrows in the substrate seemingly made by the toads themselves; although this behaviour was not observed. Activity at times was quite low and localised around their chosen hides where they would be observed feeding; although they would occasionally make use of the floor space of the vivarium during their periods of activity, deciding to rest and feed out in the open. During reproduction presumed multiple paternity was also observed as the competing male amplexed with the pair whilst the female began to spawn.

The decision to induce brumation and attempt breeding was only made when the toads maintained seemed in peak condition and of excellent health. Providing a short brumation period for only the female was a decision made after a previous attempt at reproduction using the same method, although without providing any brumation beforehand, was unsuccessful. This resulted in excellent reproductive behaviour in the male specimens although the female produced no spawn. It was clear that the males did not seem to require a temperature reduction to induce reproductive behaviour and it was shown that they were capable of successful egg fertilization without brumation; the change to a semi aquatic environment was a sufficient stimulus. However, a short brumation period seemed important to stimulate egg production in the female. It was noted that during this period the female began to swell and became noticeably rounder in appearance within 48 hours of being inside the refrigerator.

The length of time the female was maintained at low temperatures in this instance was also significantly shorter than provided by Kurth et.al (2013), showing that providing low temperatures for multiple weeks is not necessary for a successful reproduction and is also not required for both sexes but for females only. Species such as this that occur within a relatively dry environment depending on temporary breeding sites and which also show large seasonal and regular temperature fluctuations must become opportunistic in their reproductive habits. This species seems to remain opportunistic in captivity in its willingness to reproduce, and providing that temperature fluctuations are within the parameters that they would naturally experience in the wild, it may not be necessary for the cold brumation period to last quite as long as it does in situ. As the short term temperature reduction seems to be a sufficient stimulus, and the length of time it persists for in the wild is a constraint of nature, as the toads will explosively breed immediately once conditions are favourable. Therefore, in the captive environment it would seem that providing that the temperature reduction they

would naturally experience in the wild is given, it would not be required to last as long.

Using this short term brumation period method for females may be beneficial for the specimens in the long term, as it provides successful reproductions without the potential health risks of wintering all adult specimens for extended periods of time. This method has been used multiple times with the same success in the Manchester Museum during 2014. Further investigation would be required to study the use of this method on other species inhabiting similar environments that also breed opportunistically and explosively, as it may prove beneficial in the success of their captive breeding, particularly those of conservation concern where ex-situ conservation breeding may be necessary and have little margin for error. This method could produce healthy captive reproductions without unnecessarily risking the health of important specimens by inducing a long term brumation period. It may also be used to quickly induce reproduction as and when the specimens are healthy and in good condition to breed, as creating a prolonged season that would lengthen the process would not be necessary. This method has shown to yield healthy larvae as early as twelve days from the beginning of the brumation period, as apposed to the process extending over one month, and has also shown no negative effects or health issues in the breeding adults which remain much of their time within stable conditions.

M. klappenbachi is currently listed as Least Concern by the IUCN and considered to have a stable population within its natural range (Aquino et al. 2004). However, the explosive breeding habits of this species, and other members of the genus, lend themselves to rapid population declines should an infectious disease such as *Batrachochytrium dendrobatidis* (Bd) become prevalent within their natural environment. As large aggregations of breeding adults coming together in water bodies could consequently spread infection and potentially lead to die offs of adult individuals, causing a sudden population decline. It is reasons such as this, which make it important to establish proven methods of captive reproduction should ex-situ conservation programmes become required for these species in the future. It is also possible that methods established using a more common species of a genus may be of use for these purposes for the more threatened related species. Of the 23 assessed species of *Melanophryniscus*, three species are considered to be critically endangered; *M. admirabilis*, *M. langonei* & *M. peritus* (IUCN 2015) and it is possible that knowledge of the requirements of one species may prove transferrable to another with regards to conservation breeding.

ACKNOWLEDGEMENTS

The Author would like to thank Dave Perry of Peregrine Livefoods for providing specimens from his personal collection to the Manchester Museum and Andrew R. Gray of the Manchester Museum for his insightful comments on the manuscript.

REFERENCES

Aquino, L., Kwet, A., Baldo, D. & Céspedez, J. (2004). *Melanophryniscus klappenbachi*. The IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Downloaded on 31 January 2015.

Frost, D.R. (2013) Amphibian Species of The World, an online Reference.http://research.amnh.org/vz/herpetology/amphibia/index.php//Amphibia/Anura/Bufonidae/Melanophryniscus. Version 6.0 accessed 07/08/2014.

Garcia, P., Segalla, M.V., Lavilla, E. & Baldo, D. (2004). *Melanophryniscus tumifrons*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded on 31 January 2015.

IUCN (2015). The IUCN Red List of Threatened Species. Version 2014. 2 <www.iucnredlist.org>. Downloaded on 31 January 2015.

Kurth, M., Hörnes, D., Rödder, D. (2013). Race against desiccation: rapid larval development in *Melanophryniscus klappenbachi* (Anura: Bufonidae). *Salamandra* 50: 117-124.

Lavilla, E., Baldo, D. & Rodríguez, L. (2004). *Melanophryniscus stelzneri*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded on 29 January 2015.

Prigioni, C.M. & Langone, J.A. (2000). Una nueva especie de *Melanophryniscus Gallardo*, 1961, de Argentina y Paraguay (Amphibia, Anura, Bufonidae). *Comunicaciones Zoologicas del Museo de Historia Natural de Montevideo* 195: 1-12.

Accepted: 5 February 2015

The tadpole of the glass frog *Hyalinobatrachium orientale tobagoense* (Anura: Centrolenidae) from Tobago, West Indies

J. ROGER DOWNIE[1*], MOHSEN NOKHBATOLFOGHAHAI[2] & LYNDSAY CHRISTIE[1]

[1]School of Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK
[2]Biology Department, Faculty of Science, University of Shiraz, Shiraz, Iran 71345
[*]Corresponding author email: roger.downie@glasgow.ac.uk

ABSTRACT - We describe the tadpole of the Tobago glass frog *Hyalinobatrachium orientale tobagoense* for the first time. Like the few other *Hyalinobatrachium* species tadpoles described so far, it lives hidden in sand and gravel at the bottom of stream beds. The tadpoles have relatively long tails and slender lightly pigmented bodies with tiny eyes. They appear to grow very slowly and hind limb buds were not developed in the six week old Gosner stage 25 individuals we describe.

INTRODUCTION

The glass frog *Hyalinobatrachium orientale* has been identified from two localities, the oriental sector of northeastern Venezuela and the north of the West Indian island of Tobago. Jowers et al (2014) felt that Hardy's (1984) original designation of the Tobago population as a sub-species, *H. o. tobagoense* was justified based on Braby et al.'s (2012) suggestion that the use of sub-species be 'restricted to evolving populations representing partially isolated lineages of a species that are allopatric, phenotypically distinct and have at least one fixed diagnosable character state.' Braby et al. felt that well characterised sub-species can be particularly useful in defining biodiversity units that need conservation.

As Hoffmann (2010) has noted, glass frog larvae are very difficult to locate in the field because, after hatching, the larvae burrow into the sand and gravel at the bottom of flowing streams and generally do not emerge until metamorphosis. Hoffmann's (2010) descriptions of 13 species of Costa Rican glass frog tadpoles are therefore mainly based on captive reared specimens. The tadpoles of *H. orientale* have not previously been described either from Venezuela or Tobago. Here, we provide a description based on captive reared specimens, using the same main characters as Hoffmann (2010).

METHODS

Two egg clutches of *H. orientale* were collected during June 2014 from the undersides of leaves overhanging a stream close to Spring Trail in Tobago's Main Ridge Forest Reserve (*H. orientale* is the only Tobago frog species showing this mode of reproduction: Hardy, 1984). The eggs were incubated until hatching and then the larvae were grown in tanks for six weeks as described by Nokhbatolfoghahai et al. (submitted). Surviving larvae (9 from 15) were then recovered, lethally anaesthetised in benzocaine, then preserved in formol-saline. Specimens were examined and measured using a Wild M3Z dissecting microscope fitted with an eyepiece scale. Photographs were taken using a Nikon D5100 DSLR camera with a Nikkor 40 mm lens. Two specimens were embedded in wax, sectioned and stained using H and E in order to examine limb development. For the labial tooth row formula, we have followed the recommendation of Altig and McDiarmid (1999a). The remaining specimens have been deposited in the University of Glasgow's Hunterian Zoology Museum, accession number 1437.

RESULTS

During the six weeks we allowed the larvae to grow they had increased in length by 36.6% to a mean (+ SD) of 15.3+1.4 mm (from a hatching length of 11.2 +0.2, n=10) and in wet weight had tripled to a mean of 0.03+0.007 g (n=9). Their intestines were visibly full of particulate material. Even after six weeks growth, all specimens remained at Gosner (1960) stage 25, with no external sign of hindlimb development.

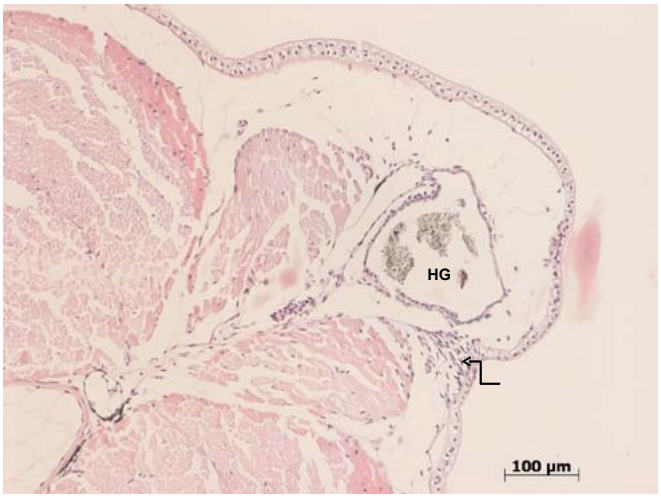


Figure 1. H & E stained transverse section of a stage 25 *H. orientale* tadpole in the hindgut region, showing a condensation of mesenchymal cells beneath the epidermis, but no protruding limb bud. HG = hindgut; arrow points to mesenchymal condensation.

Sections in the region where hind limb buds would be expected (junction of body and tail, close to vent tube) show condensations of mesenchymal cells near the hind gut, but no sign of any protruding buds, indicating that the limbs are in the pre-bud stage (Fig. 1). The detailed tadpole descriptions and measurements which follow are based on the five specimens with the most intact tails.

Figure 2 shows dorsal, left lateral and ventral views of one of the measured tadpoles. The body shape is flat in the dorso-ventral plane rather than rounded, with a mean width/height ratio of 1.19+0.03. The tail is long and slender: tail length as a proportion of total length is 64%; tail width as a proportion of total tadpole length is 14.3%. The dorsal tail fin originates at the base of the tail and is initially low, increasing in height about half-way along the tail, as the musculature of the tail begins to narrow. The ventral tail fin originates at the vent tube and is a little higher initially than the dorsal fin, again widening posteriorly. At maximum height, the dorsal fin is 20.7% of tail width and the ventral fin is 22.0%. Maximal tail width is 22.4% of tail length. The tail tip is rounded after gradual narrowing.

Figure 2. Fixed, unstained complete *H. orientale* stage 25 tadpole showing a)left lateral with spiracle visible, b) ventral and c) dorsal views. See text for details.

The position of the sinistral spiracle on the body is slightly ventral to the midline and 58% along the body from the snout. The dorsal side of the body is fairly uniformly pigmented except for clear areas ventro-lateral to the eyes and lateral to the anterior of the intestine. The ventral side of the body is unpigmented and transparent. The lateral side of the tail has a discontinuous pigment line along the middle of the muscle half to two-thirds the length of the tail; at the tail tip, there is diffuse pigment covering the muscle. The tail fins are unpigmented. In dorsal view, the tail has a broad band of pigment along the midline, narrowing at the tip; in ventral view, the tail has a sparse discontinuous pigment line from base to tip.

The snout is rounded. The eyes are dorsally positioned and very small, 0.45 mm in diameter (in comparison,

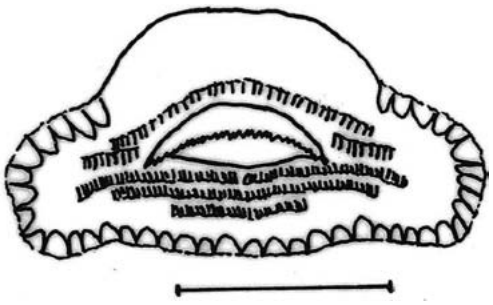


Figure 3. Drawing of the oral disc of *H. orientale*, made with the aid of a drawing tube. Mouth opened out to make all tooth rows visible. Anterior above. Disc has a single row of papillae all round its margin except at anterior end. Scale line = 0.5 mm.

Leptodactylus fuscus stage 25 tadpoles of similar size have eyes of 1.3 mm in diameter; JRD, unpublished data). The ratio of interorbital width to maximum body width is 0.35, and the position of the interorbital axis is 20.4% along the body from the snout tip. The nares can just be seen with a dissecting microscope using intense lateral illumination and are about half-way between the interorbital axis and the snout tip.

Figure 3 shows the morphology of the oral disc. The disc is ventral in position, with a short length of snout sometimes protruding anterior to it in ventral view. The margin of the disc bears a single row of papillae laterally and posteriorly, but the anterior margin is papilla-free. The tooth row formula is 2(2)/3(1); i.e. the second anterior row has a gap, which is wide; the first posterior row has a narrow gap; the remaining rows are continuous; the lengths of the posterior rows reduce progressively from P1 to P3. The jaws have serrated edges. The mean ratio of oral disc width to maximum body width is 0.46+0.008.

DISCUSSION

In several respects: particularly body and tail shape, spiracle position, interorbital width and eye diameter, oral disc morphology and relative size - *H. orientale* tadpoles closely resemble other *Hyalinobatrachium* species described by Hoffmann (2010). Altig and McDiarmid (1999b) gave descriptions of the tadpoles of three glass frog genera, *Centrolene*, *Cochranella* and *Hyalinobatrachium*, noting that generic and specific differences are poorly known. Our findings fit well with the features they noted for *Hyalinobatrachium*.

As Hoffmann (2010) has noted, glass frog tadpoles remain relatively undescribed because of the problems of locating them in the field and the difficulties of rearing them under laboratory conditions. We present here the first description of the tadpole of the Tobago glass frog *H. orientale*. Our specimens were collected as eggs in the field, allowed to hatch then transferred to aquaria with river rocks and gravel covering the bottoms. The water was continually aerated and a pinch of tropical fish food flakes was provided every second day. The tadpoles were never visible in the water column (about 20 cm deep) and were only retrieved after removal of all the rocks and gravel: after 6 weeks, they were still at Gosner stage 25

but had grown significantly in length and weight, and their intestines were packed with particulate material, indicating food consumption. The growth rate achieved seems very slow. In comparison, several Trinidad frog species reach metamorphosis in 2-3 weeks (Downie, 2013). However, the rate is probably comparable with Hoffmann's (2010) findings, with two Costa Rican *Hyalinobatrachium* species taking 265 and 394 days respectively to reach Gosner (1960) stage 41, just before metamorphosis. The lack of progressive development of the hind limb buds in our specimens was striking and not noted in Hoffmann's (2010) descriptions. Relatively late development of the hindlimbs does occur in other species (for example, in *Mannophryne trinitatis*; Downie, unpublished observations), but it is not clear why this should happen. It will be of interest to attempt to grow *H. orientale* tadpoles as far as metamorphosis in order to determine how long it takes and when progressive limb development occurs.

ACKNOWLEDGEMENTS

The University of Glasgow and the Blodwen Lloyd Binns Bequest helped fund JRD's visit to Trinidad and Tobago. Several students on the University of Glasgow Tobago expedition 2014 assisted with fieldwork. We thank Chris Pollock for taking the photographs shown in Fig. 2. The specimens used in this investigation were collected and exported under the Trinidad Government's Wildlife Section permit number 001741.

REFERENCES

Altig, R. & McDiarmid, R.W.(1999a). Body plan: development and morphology. In: *Tadpoles; the biology of anuran larvae*, pp. 24-51. McDiarmid, R.W. & Altig, R.(Eds.). Chicago: Chicago University Press.

Altig, R. & McDiarmid, R.W. (1999b). Diversity: familial and generic characteristics. In: *Tadpoles; the biology of anuran larvae*, pp.295-337. McDiarmid, R.W. & Altig, R.(Eds.). Chicago: Chicago University Press.

Braby, M.F., Eastwood, R. & Murray, N. (2012). The sub-species concept in butterflies: has its application in taxonomy and conservation outlived its usefulness? *Biological Journal of the Linnean Society* 106: 699-716.

Downie, J.R. (2013). How long do Trinidad's frogs take to reach metamorphosis? *Living World: Journal of Trinidad and Tobago Field Naturalists' Club* 2013: 54-56.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190.

Hardy, J.D. Jr. (1984). A new sub-species of *Centrolenella orientalis* (Anura: Centrolenidae) from Tobago, West Indies. *Bulletin of the Maryland Herpetological Society* 20: 165-173.

Hoffmann, H.(2010). The glass frog tadpoles of Costa Rica (Anura: Centrolenidae): a study of morphology. *Abhandlungen der Senckenberg Gesellschaft für Naturforschung* 567: 1-78.

Jowers, M.J., Lehtinen, R.M., Downie, J.R., Georgiadis, A.P. & Murphy, J.C. (2014). Molecular phylogenetics of the glass frog *Hyalinobatrachium orientale* (Anura: Centrolenidae): evidence for Pliocene connections between mainland Venezuela and the island of Tobago. *Mitochondrial DNA* (published online 3/2/14).

Accepted: 4 February 2015

Reproduction in the Schokari sand racer, *Psammophis schokari* (Squamata: Colubridae) from Israel

STEPHEN R. GOLDBERG

Department of Biology, Whittier College, Whittier, California 90608, USA
Email: sgoldberg@whittier.edu

ABSTRACT - In this study data on reproduction from a histological examination of gonadal material of *Psammophis schokari* from Israel is reported. Male and female reproductive activity is synchronous and occurs in spring. Five is a new smallest reported clutch size for *P. schokari* from Israel. Published clutch sizes for *P. schokari* from other parts of its range are presented.

INTRODUCTION

The Schokari Sand Racer, *Psammophis schokari* (Forskal, 1775) occurs through the entire state of Israel (Bar & Haimovitch, 2011) and is broadly distributed occurring in northern Africa from Mauritania to Egypt; East Africa, south to Somalia; the Sinai and Arabian Peninsula, Near East, to western India, southern Turkmenistan in the north and north-western India in the east (Ananjeva et al., 2006; Schleich et al., 1996). In Israel it is a ground-dwelling, diurnal snake (Bar & Haimovitch, 2011). There are currently 34 recognised species of *Psammophis* (Uetz & Hosek, 2014). Despite its wide distribution, little information is available on its reproductive cycle. Bar & Haimovitch (2011) reported *P. schokari* deposited 6-10 eggs per clutch in Israel. The purpose of this paper is to supply additional information on the reproductive cycle of *P. schokari* from a histological examination of museum specimens from Israel, as part of an ongoing series of studies on the timing of events in the reproductive cycle of Middle-Eastern Snakes. Due to the difficulty in obtaining collecting permits for large monthly samples of reptiles, utilization of previously collected specimens in museums has become increasingly important.

MATERIALS AND METHODS

A sample of 30 *P. schokari* consisting of 11 males (mean snout-vent length, SVL =533.6 mm ± 70.7 SD, range = 452-693 mm, and 19 females (mean SVL = 548.1 mm ± 58.9 SD, range = 472-681 mm) collected in Palestine/ Israel between 1946-2012 and deposited in the Zoological Museum of Tel Aviv University (TAUM) was examined (by region): A'rava Valley, TAUM: 1277, 14355, 15936, 16196; Central Negev, TAUM 109, 115, 1325, 1326, 12847, 13306, 15996, Dead Sea Area, TAUM: 1024, 1424; HaSharon, TAUM: 2492, 2799; Karmel Ridge, TAUM: 2026; Northern Coastal Plain, TAUM: 1513, 1793; Northern Negev, TAUM: 1816, 1818 , 2844; Southern Coastal Plain, TAUM: 117, 1336 , 1677, 1678, 1817, 3835, 8357, 12177, 14051; Southern Negev, TAUM: 1828.

A small slit was made in the left side of the abdomen and the left testis was removed from males and the left ovary was removed from females for histological examination. Enlarged ovarian follicles (> 7 mm) were counted in situ. No histology was performed on them. There was a high probability that follicles of > 7 mm size would have completed yolk deposition and ovulated. Removed gonads were embedded in paraffin, sections were cut at 5μm and stained by Harris' hematoxylin followed by eosin counterstain (Presnell & Schreibman, 1997). Slides of the testes were categorised as to the stage of the testicular cycle. Slides of ovaries were examined for yolk deposition; oviductal eggs or enlarged ovarian follicles were grossly noted. Mean body sizes (SVL) of male and female *P. schokari* were compared using an unpaired t-test (Instat vers 3.0b, Graphpad Software, San Diego, CA).

RESULTS AND DISCUSSION

There was no significant size difference in mean SVL length between male and female samples of *P. schokari* (unpaired t-test, t = 0.61, p = 0.55). Three stages were noted in the testicular cycle (Table 1): (1) spermiogenesis (seminiferous tubules lined by clusters of sperm and/or metamorphosing spermatids; (2) recrudescence = renewal (proliferation of germ cells for the next period of spermiogenesis). Primary spermatocytes predominated; (3) regressed, seminiferous

Month	N	Regressed	Recrudescence	Spermio-genesis
February	1	0	1	0
April	4	0	0	4
May	1	1	0	0
July	1	1	0	0
November	2	2	0	0
December	2	0	2	0

Table 1. Monthly stages in the testicular cycle of 11 *P. schokari* males from Israel.

Month	N	Quiescent	Early yolk deposition	Enlarged follicles > 7 mm	Oviductal eggs
January	2	2	0	0	0
February	1	1	0	0	0
March	1	1	0	0	0
April	5	3	1	1	0
May	3	2	0	0	1
September	1	1	0	0	0
November	3	3	0	0	0
December	3	3	0	0	0

Table 2. Monthly stages in the ovarian cycle of 19 *P. schokari* females from Israel

Clutch Size	Locality	Source
2-6	Iran	Latifi, 1991
5-6	North Africa	Schleich et al. 1996
oviparous, no clutch	Turkmenistan	Szczerbak, 2003
4-10	Pakistan	Khan 2006
5-6	Ethiopia, Eritrea	Largen & Spawls, 2010
5-6	Oman, United Arab Emirates	Gardner, 2013
5-6	Israel	This paper

Table 3. Clutch sizes of *P. schokari* from different parts of its range.

tubules contain spermatogonia and interspersed Sertoli cells. All males undergoing spermiogenesis (N = 4) were collected in April, indicating mating occurs during spring. The smallest reproductively active male (spermiogenesis) measured 452 mm SVL (TAUM 109) and was collected in April.

The smallest reproductively active female (five oviductal eggs) measured 509 mm SVL (TAUM 1513) and was collected during May. Five is a new minimum clutch size for *P. schokari* in Israel. Spring reproductive activity in *P. schokari* (Table 2) occurred in April and May, as was evidenced by one female from April exhibiting early yolk deposition (TAUM 1024) and one (TAUM 1817) containing six enlarged follicles (> 7 mm length) that would have constituted a clutch later in the spring. Clutch sizes of *P. schokari* from different parts of its range are in Table 3.

The presence of sperm-containing males and reproductively-active females in spring indicates synchrony in the reproductive cycles of both sexes. The reproductive cycle of *P. schokari* most closely fits the category "III. Spermatogenesis Vernal and Prenuptial A" of Saint Girons (1982) in which spermiogenesis runs from February to April, matings occur in April, followed by ovulation at the end of May. The female reproductive cycle of *P. schokari* would appear to fit the "monoestrous" type of Schleich et al. (1996) in which one egg clutch is produced in a fixed (spring) reproductive period.

ACKNOWLEDGMENTS

I thank Shai Meiri (TAUM) for permission to examine *P. schokari*; Erez Maza (TAUM) for facilitating the loan and the National Collections of Natural History at Tel Aviv University for providing samples of *P. schokari* for this study.

REFERENCES

Ananjeva, N.B., Orlov, N.L., Khalikov, R.G., Darevsky, I.S., Ryabov, S.A., & Barabanov, A.V. (2006). *The Reptiles of Northern Eurasia, Taxonomic Diversity, Distribution, Conservation Status*. Sofia: Pensoft.

Bar, A., & Haimovitch, G. (2011). *A Field Guide to Reptiles and Amphibians of Israel*. Herzilya: Pazbar Ltd.

Gardner, A.S. (2013). *The Amphibians and Reptiles of Oman and the UAE*. Frankfurt am Main: Edition Chimaira.

Khan, M.S. (2006). *Amphibians and Reptiles of Pakistan*. Malabar, Florida: Krieger Publishing Company.

Latifi, M. (1991). *The Snakes of Iran*. Ithaca, New York: Society for the Study of Amphibians and Reptiles, Contributions to Herpetology, Number 7.

Largan, M. & Spawls, S. (2010). *The Amphibians and Reptiles of Ethiopia and Eritrea*. Frankfurt am Main: Edition Chimaira.

Presnell, J.K. & Schreibman, M.P. (1997). H*umason's Animal Tissue Techniques*, 5th Edition. Baltimore: Johns Hopkins University Press.

Saint Girons, H.S. (1982). Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. *Herpetologica* 38: 5-16.

Schleich, H.H., Kästle, W. & Kabisch, K. (1996). *Amphibians and Reptiles of North Africa*. Koenigstein: Koeltz Scientific Publishers.

Szczerbak, N.N. (2003). *Guide to the Reptiles of the Eastern Palearctic*. Malabar, Florida: Krieger Publishing Company.

Uetz, R. & Hosek, J. (eds.). The Reptile Database, www.reptile-database.org. accessed October 17, 2014.

Accepted: 15 December 2014

Notes on egg laying sites of *Calodactylodes aureus* (Beddome, 1870) in Tirupattur Forest Division, Southern India.

AYUTHAVEL KALAIMANI

Aarohi, No.192,7th Block, Jayanagar, Bangalore 560011 Karnataka, India
Email: manikalai16@yahoo.com

INTRODUCTION

The Indian golden gecko *Calodactylodes aureus* (Beddome, 1870) is endemic to the Eastern Ghats mountain range of peninsular India (Bauer & Das, 2000; Kalaimani & Nath, 2012, 2013; Reddy et al., 2013; Srinivasalu et al.,2014). It prefers rocky areas with deep stream valleys and has been observed at elevations between 50 and 1000 m and reported to lay eggs in communal egg deposition sites (Bauer & Das, 2000; Javed et al., 2007) on rocky surfaces, mostly on vertical rocks in both natural and human-inhabited areas. This paper gives information on mass egg laying sites of *C. aureus* in Tirupattur Forest Division, Eastern Ghats, Tamil Nadu.

METHODS

The study was conducted in Tirupattur Forest Division 12°32'35.69" N 78°37'13.96" E (300-1300 m a.s.l.).Tamil Nadu. These forest divisions have four forest ranges; Alangayam, Ambur, Tirupattur and Singarapettai. The study area has 42 forest beats all of which were surveyed for the study. The area has six forest types namely, southern dry mixed deciduous forests, southern dry deciduous forests, southern dry savanna forests, dry bamboo brakes, dry tropical riverine forests, southern tropical thorn forests and southern scrub forests (Champion & Seth, 1968). The survey period was from September 2011 to January 2012 (over five months) for a total of 41 days (~5 hrs per day). In these forest divisions caves/rock boulders were carefully observed with powerful torches. The following details were recorded: geo-coordinates, elevation (meters), number of sightings with identification of male and female *C. aureus* using the key given by Bauer & Das (2000). The number of *C. aureus*, cave height, cave length, distance to the nearest water source, number of egg laying sites and potential threats and sympatric herpetofaunal species were recorded.

RESULTS AND DISCUSSION

During the study period a total of 855 sightings of *C. aureus* were made in 229 rock boulders/caves in Tirupattur Forest Division. Of these 133 were golden coloured males (Table 1 & Fig. 1). *C. aureus* were recorded in rock boulders/caves at a height 0.12-36 m, distance of 0.08-38m into the cave and distance from water source of about 0.01-180m. A total of

Figure 1. Example of an adult male *C. aureus* found at Tirupattur Forest Division, State of Tamil Nadu, India

Forest Range	Number of males	Total number of lizards	Number of egg laying sites
Ambur	61	266	51
Alangayam	34	268	75
Tirupattur	14	169	52
Singarapettai	24	152	51
Total	133	855	229

Table 1. Number of *C. aureus* recorded in Tirupattur Forest Division.

932 egg laying sites were recorded. The eggs were situated at a height of 0.2 - 15m from the ground on rock boulders/caves. In the caves of Yelagiri foot hills, Alangayam Range, Tirupattur Forest Division, an estimated several thousand empty egg shells and some fertile eggs in the same cave were found. (Fig. 2). The elevation range of *C. aureus* above sea level is recorded from <50m to >1250m with habitat association primarily of rocky areas with deep stream valleys (Sreekar et al., 2007). Communal egg deposition sites on rocky surfaces have been recorded previously (Bauer & Das, 2000; Javed et al., 2007) with fertile eggs recorded between late June to September (Sreekar et al., 2010). However, this study found eggs deposited between September 2011 and January 2012. During the study period ants were seen predating upon fertile eggs of *C. aureus* on two occasions

Figure 2. Photograph of the large number of empty egg shells of *C. aureus* observed in a cave in the Yelagiri Foothills, October 2011.

and *C. aureus* used wasp nests and swallow nests for laying eggs. This agrees with studies of geckos in Sri Lanka, where *C. illingthiworthorum* and *Hemidactylus depressus* use swallow and wasp nests respectively to prevent egg predation (Bauer et al., 2004). Sympatric herpetofauna observed during the study included *Psammophilus dorsalis*, *Hemidactylus* sp., *H. triedrus*, *H. gigantecus*, *H. graniticolus*, *Eutropis carinata* and *Lygosoma cf. punctatus*.

Human disturbance is a major threat to *C. aureus* in Andhra Pradesh (Dutta et al., 2004; Rajsekhar & Nandhakumar, 2007; Sreekar et al., 2010) and in our study area *C. aureus* also faces threats from man-made fire and woodcutting. This species is the only member representing the genus in India (Bauer & Das, 2000) with the taxon categorized as Least Concern but population monitoring necessary (Bauer et al., 2013).

ACKNOWLEDGEMENTS

Thanks to Pathmawadhe, District Forest Officer, Tirupattur Forest Division and M.S.Chaitra from Aarohi, Bangalore. To S. R. Ganesh for comments that improved the earlier version of the manuscript. My sincere thanks also to Dr Todd Lewis and an anonymous reviewer for their critical comments. To K.V. Sudhakar, Anukul Nath, Gandhla Chethan Kumar, Lakshmi Narayana and Shek Dhavooth Ali for their support

REFERENCES

Beddome, R.H. (1870). Description of some new lizards from the Madras Presidency. *Madras Monthly Journal of Medical Science* 1: 30-35.

Bauer, A., & I. Das. (2000). A review of the gekkonid genus *Calodactylodes* (Reptilia: Squamata) from India and Sri Lanka. *Journal of South Asian Natural History* 5: 25-35.

Bauer, A., de silva, A., & Austin, C. C. (2004). The use of abandoned wasp nests by *Hemidactylus depressus* in Sri Lanka. *Lyriocephalus* 5: 221-222.

Bauer, A., Srinivasulu, C., Agarwal, I., Roy, A.D., Srinivasulu, B. & Mohapatra, P. 2013.Calodactylodes aureus. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 27 March 2014.

Champion, H.G & A.K. Seth. (1968). *A Revised Survey of Forest Types of India*. Government of India, New Delhi.

Dutta,S.K., B.Mohanty & P.P.Mohapatra. (2005). Niyamgiri unraveled. *Sanctuary Asia* 25: 56-57.

Javed, S.M.M., Waran, A. & Tampal , F. (2007). On the occurrence of golden gecko *Calodactylodes aureus* in Papikonda Hills, Eastern Ghats, India. *Zoo's Print Journal* 22: 2727-2729.

Kalaimani, A. & Nath , A. (2012). A new locality and microhabitat usage by *Calodactylodes aureus* (Beddome,1870) from Tamil Nadu, Southern India. *Herpetotropicos* 8: 49-54.

Kalaimani. A. & Nath, A. (2013). The Indian Golden Gecko *Calodactylodes aureus* (Beddome,1870) in Tamil Nadu, India. *Taprobanica* 5: 81-84.

Rajshekhar, M. & Nanda kumar,N.V. (2007). A new finding on the occurrence of golden gecko (*Calodactylodes aureus*) in the rock boulders near Vellore town. *The Bioscan* 2: 61-62.

Reddy, Y. Amarnath, P. Indira, T. Pullaiah, B., Sadasivaiah, Swamy,K. R. K & Rani, S. S. (2013). Range extension of Indian Golden Gecko *Calodactylodes aureus* in Andhra Pradesh, India. *Reptile Rap* 15: 41-42.

Sreekar, R., C. Srinivasulu, M. Seetharamaraju & Srinivasulu, C.A. (2010). Selection of egg attachment sites by the Indian Golden Gecko *Calodactylodes aureus* (Beddome, 1870) (Reptilia: Gekkonidae) in Andhra Pradesh, India. *Journal of Threatened Taxa* 2: 1268-1272.

Srinivasalu, B., C. Srinivasalu,T.A.Shah, G. Devender & Kaur, H. (2014). First confirmed record of *Calodactylodes aureus* (Reptilia) from Karnataka, India. *Taprobanica* 6 :53-54.

Accepted: 28 November 2014

Leptophis ahaetulla marginatus (Southern green parrot snake): Behaviour.

JONATHAN R. CLEGG

Para La Tierra, Reserva Natural Laguna Blanca, Santa Rosa del Aguaray, San Pedro, Paraguay.
Email: clegg.jonathan@rocketmail.com

Leptophis ahaetulla marginatus, the southern green parrot snake, is a diurnal, arboreal snake that occurs from southeastern Bolivia to western Sao Paulo State in Brazil, through Paraguay and southern Brazil to northeastern Argentina and Uruguay (Peters and Miranda, 1970). It is a large snake, up to 1500mm, and is an active forager in trees and bushes (Cei, 1993; Lopez et al., 2003). The diet of *L. a. marginatus* is reported to consist primarily of semiarboreal and arboreal hylid frogs, (particularly species from the genus *Scinax*) supplemented by birds (Lopez et al., 2003)

Trachycephalus typhonius (previously *T. venulosus*), the marbled tree frog, is an arboreal hylid species that *L. a. marginatus* is known to prey upon (Prado, 2003; Albuquerque and Di Bernardo, 2005). This is despite *T. typhonius* exuding a sticky, toxic secretion that deters predation in some snakes (*Drymarchon corais*, Leary and Razafindratsita 1998; *Leptodeira annulata*, Manzanilla et al., 1998)

On December 18th, 2010, the distress call of *T. typhonius* drew our attention to an adult *L. a. marginatus* attempting to predate an adult *T. typhonius* in Atlantic Forest at Reserva Natural Laguna Blanca, Departamento San Pedro, Paraguay (S23°49'44.3", W056°17'32.3"). The snake and frog were on branches approximately 4m from the ground. The *L. a. marginatus* had the posterior of the dorsum and legs of the frog in its mouth when we first located it at 2.55pm.

The struggle between the snake and frog lasted over an hour, and during this time the frog continued to perform the distress call as it began to break free from the snake (Fig 1). The frog eventually broke free and jumped from its position on the branch directly to the forest floor in one motion. The snake immediately chased the frog and tracked it the 4m distance from the branch to the forest floor. The frog had barely moved from its position on the forest floor when the snake once again grabbed the frog head first and consumed it without a struggle. Recapture predatory behaviour of snakes in snake-frog predator-prey interactions is rarely reported and is particularly interesting in this observation given the noxious skin secretions of *T. typhonius*.

It is likely that the skin secretion of *T. typhonius* acts as a viscous glue that loosens the grip of the snake and does not have a lasting toxic effect, at least in *L. a. marginatus*. Considering the immediate recapture and consumption of the frog, the defensive secretion of *T. typhonius* may not change the outcome of predation events between these two species.

Figure 1. Adult *T. typhonius* breaking free from adult *L. a. marginatus*. Photograph; J. Clegg.

Prado (2003) described another predation of *T. typhonius* by *L. ahaetulla*, in the Pantanal, Mato Grosso do Sui, southwestern Brazil, without struggle. Ingestion was complete after 28 minutes, despite glue like skin secretions of the frog visible on the snout of the snake. This predation event took place 1m from the ground, with the snake having consumed the frog head first (Prado, 2003). The present observation therefore suggests that despite the toxic skin secretion of *T. typhonius* having anti-predation effects on other snake species, predation on *T. typhonius* by *L. a. marginatus* is a frequent occurence and the secretions likely have limited or no effect.

ACKNOWLEDGMENTS

I thank logistic support from Karina Atkinson and help from the interns and volunteers at Para La Tierra, Reserva Natural Laguna Blanca, Departmento San Pedro, Paraguay.

REFERENCES

Albuquerque, N.R., & Di-Bernardo, M. (2005) *Leptophis ahaetulla marginatus*. Natural History Note. *Herpetological Review* 36: 325.

Cei, J. M. (1993). Reptiles del noroeste, nordeste y este de la Argentina. Herpetofauna de las Selvas subtropicales, Puna y Pampas. Monografie di Museo Regionale di Scienze Naturali, *Torino* 14: 575.

Leary, C. J., & Razafindratsita, V. R. (1998). Attempted predation on a hylid frog, *Phrynohyas venulosa*, by an indigo snake, *Drymarchon corais*, and the response of conspecific frogs to distress calls. *Amphibia-Reptilia* 19: 442-446.

López, M. S., Giraudo, A. R., & Arzamendia, V. (2003). *Leptophis ahaetulla marginatus*. Diet. Natural History. *Herpetological Review* 34: 68-69.

Manzanilla, J., La Marca, E., Villareal, O. & Sanchez, D. (1998). *Phrynohyas venulosa* Antipredator Device. *Herpetological Review* 29: 39–40.

Peters, J. A., & R. D. Orejas-Miranda. (1970). Catalogue of the Neotropical Squamata: Part I. Snakes. *Bulletin of the United States National Museum* 297: 1-347.

Prado, C.P.A. (2003): Natural History Notes: *Leptodactylus chaquensis* (NCN), *Pseudis paradoxa* (paradox frog) and *Phrynohyas venulosa*. Predation. *Herpetological Review* 34: 231–232.

Accepted 18 February 2015

Hemidactylus agrius (Country leaf-toed gecko): Polydactyly and tail bifurcation

MARIA JAQUELINE MONTE DE ANDRADE[1,2*], JOSÉ ROBERTO IZIDIO LOPES[2], RAUL FERNANDES DANTAS DE SALES[2] & ELIZA MARIA XAVIER FREIRE[2].

[1]Programa de Pós-graduação em Ciências Biológicas, Universidade Federal do Rio Grande do Norte, Campus Universitário Lagoa Nova, Natal, RN, Brazil.
[2]Laboratório de Herpetologia, Departamento de Botânica, Ecologia e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte. Campus Universitário Lagoa Nova, Natal, RN, Brazil.
[*]Corresponding author email: jackeline_monte@hotmail.com

Polydactyly is a congenital malformation affecting the extremities of individuals, causing the appearance of a greater number of fingers, which occurs frequently in populations of tetrapods (Minoli et al., 2009; Kaliontzopoulou et al., 2013). However, there are few cases of polydactyly described for lizards, especially for Gekkota (Bauer et al., 2009). Tail bifurcation in lizards is a relatively well-understood abnormality being closely related to variations in the process of caudal autotomy, which is widely used by lizards in response to the attack of predators (Meyer et al., 2002). This morphological change occurs when there is mechanical damage that does not result in complete loss of the tail (Arnold, 1988), thereby allowing the formation of an additional tail during the regeneration of the affected portion (e.g. Gogliath et al., 2012). This work describes instances of polydactyly and tail bifurcation in *Hemidactylus agrius* Vanzolini, 1978, a nocturnal gecko from Brazil that occurs in areas with rocky outcrops in the Caatinga Domain.

During a study on the population ecology of *H. agrius* between August 2012 and August 2013 in the Ecological Station of the Seridó (ESEC Seridó), a protected area located in the Caatinga Domain, municipality of Serra Negra do Norte, Rio Grande do Norte, Brazil (06°34'36.2"S, 37°15'20.7"W), 62 individuals, two of them with morphological abnormalities (polydactyly and tail bifurcation) were collected. In the laboratory these specimens were photographed, measured (snout-vent length – SVL) and analyzed under stereomicroscope. Both specimens were fixed in 10% formalin, preserved in 70% ethanol, and deposited in the Herpetological Collection of the Universidade Federal do Rio Grande do Norte (Voucher numbers: UFRN 4032 and 4055). The specimen with polydactyly was a female (SVL = 47.4 mm) collected on March 19, 2013, showing six digits in the left hind limb (Fig. 1A). The additional digit was near the toe V posteriorly located at 1 mm. This digit measured 1.4 mm, with in vivo colouration similar to that of the other digits, but there were no nail or infra-digital lamellae, suggesting a non-functional digit (Fig. 1B, C). The specimen tail bifurcation was a female (SVL = 50.6 mm), collected on October 23, 2012 (Fig. 2). The additional tail was 12.6 mm shorter than the main tail (22.6 mm). The additional tail had in vivo colouration similar to normal tails after regeneration, and developed in the individual's body medial plane (Fig. 2). Besides this collected

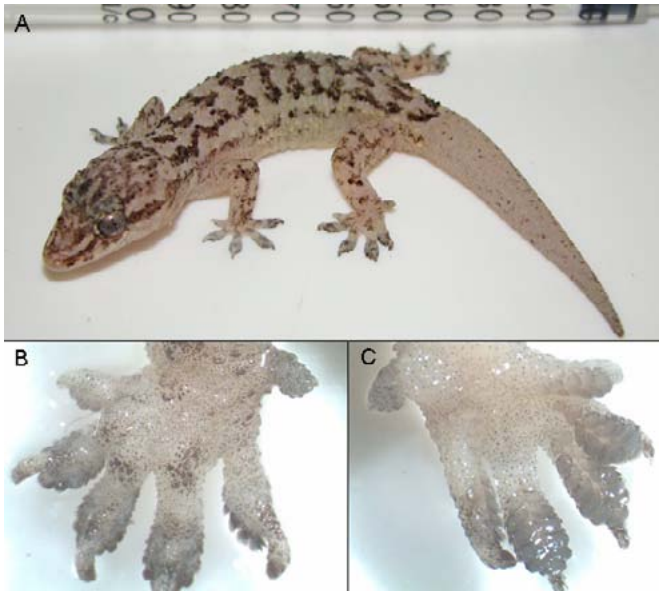


Figure 1. A) Female *H. agrius* (SVL = 47.4 mm) with polydactyly (six fingers) on a hindlimb, collected during March 2013 in the Ecological Station of the Seridó. B) Dorsal view of the left hind foot highlighting the absence of nail in the additional digit, inserted after the toe V. C) Ventral view of the left hind foot demonstrating the absence of plates in the additional digit, inserted after the toe V.

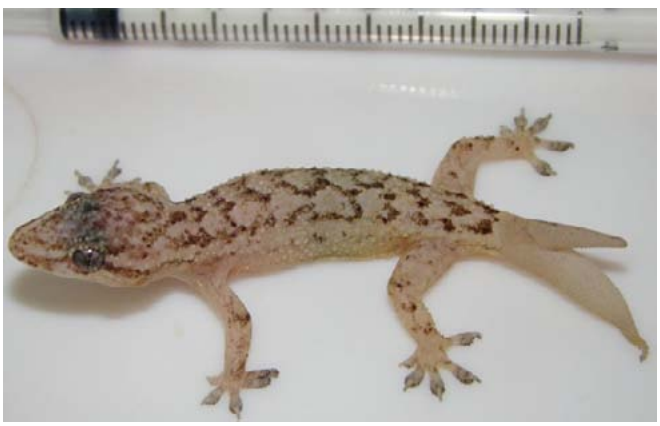


Figure 2. *H. agrius* (SVL = 50.6mm) with tail bifurcation in the medial body plane, collected in October 2013 in the Ecological Station of the Seridó. Dorsal portion length (additional) of 12.6 mm; ventral portion length (inset) of 22.6 mm.

specimen, a second record of tail bifurcation was observed in the field, but the lizard escaped the capture; in this instance the additional tail had developed in the individual's body lateral plane and was similar in size to the main tail.

The presence of a polydactyly in the *H. agrius* population of ESEC Seridó corresponds to a frequency of occurrence of 1.6%, a high estimate when compared to the rates reported in the literature for other lizards, for example, 0.5% in *Lacerta schreiberi* (Megia, 2012) and 0.6% in *Tropidurus etheridgei* (Pelegrin, 2007). Similar cases have been recorded for other lizards including *Podarcis muralis* (Lazić & Crnobrnja-Isailović, 2012) and *Liolaemus petrophilus* (Minoli et al., 2009).

The presence of two cases of tail bifurcation within the population of *H. agrius* of ESEC Seridó corresponds to a frequency of occurrence of 3.1%, suggesting this type of morphological change is relatively common in lizard populations. The injuries resulted in lateral and medial bifurcations to the body and represent additions to the two types of bifurcations described in the literature (McKelvy & Stark, 2012). This is the first record of polydactyly and tail bifurcation for *H. agrius*.

ACKNOWLEDGMENTS

We thank L. Anjos and M. Pichorim for helpful comments on a first draft of the manuscript, and the staff of Ecological Station of the Seridó logistical support on the field activities. This study was supported by research grants from the Coordernação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to MJMA, and from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to EMXF (process 304077/200819). The permit for collecting the lizards was granted by ICMBio/Sisbio (Permit 36189-1).

REFERENCES

Arnold, E.N. (1988). Caudal autotomy as a defence. In, *Biology of the Reptilia*, pp.235-273. Gans, C. & Huey, R.B. (Eds.). New York: Liss Inc.

Bauer, A.M., Hathaway, S.A. & Fisher, R.N. (2009). Polydactyly in the Central Pacific Gecko, *Lepidodactylus* sp. (Squamata: Gekkonidae). *Herpetology Notes* 2: 243-246.

Gogliath, M., Pereira, L.C.M., Nicola, P.A. & Ribeiro, L.B. (2012) *Ameiva ameiva* (Giant Ameiva). Bifurcation. *Herpetological Review* 43: 129.

Kaliontzopoulou, A., Salvi, D., Gomes, V., Maia, J.P.M.C. & Kaliontzopoulos, P. (2013). Polydactyly in the Tyrrhenian wall lizard (*Podarcis tiliguerta*). *Acta Herpetologica* 8: 75-78.

Lazić, M.M. & Crnobrnja-Isailović, J. (2012). Polydactyly in the Common Wall Lizard *Podarcis muralis* (Squamata: Lacertidae). *Herpetology Notes* 5: 277-279.

McKelvy, A.D. & Stark, C. (2012). *Plestiodon fasciatus* (Common Five-lined Skink). Bifurcation. *Herpetological Review* 43: 138.

Megía, R. (2012). Un caso de polidactilia en *Lacerta schreiberi* en el Sistema Central. *Boletin de la Asociacion Herpetologica Española* 23: 54-57.

Meyer, V., Preest, M.R. & Lochetto, S.M. (2002). Physiology of original and regenerated lizard tails. *Herpetologica* 58: 75-86.

Minoli, I., Feltrin, N. & Ávila, L.J. (2009). Un caso de polidactilia en *Liolaemus petrophilus* (Iguania: Squamata: Liolaemini). *Cuadernos de Herpetologia* 23: 89-92.

Pelegrin, N. (2007). Presence of a polydactylous *Tropidurus etheridgei* (Squamata: Iguanidae: Tropidurinae) in the Dry Chaco of Córdoba Province, Argentina. *Cuadernos de Herpetologia* 21: 115-116.

Accepted: 10 February 2015

Marine sightings of grass snakes *Natrix natrix*

JOHN M. R. BAKER

Amphibian and Reptile Recorder for Suffolk
Email: johninhalesworth@aol.com

The grass snake *Natrix natrix* frequents fresh water in search of prey (amphibians and fish) but there is also a long history of accounts of this species in sea water, although it is unclear whether such incidents resulted from accidental or active entry (Del Canto & Busack, 2011). In an early natural history volume Wood (1863) wrote 'This reptile will even take to the sea, and has been noticed swimming between Wales and Anglesea'. One of the earliest published first-hand observations was made by Böse (1897) who captured a grass snake approximately 1 km from land in Flensburg Fjord, Denmark, and reported that grass snakes were known to travel 3-4 km (1.9-2.5 miles) across sea water. Guérin-Ganivet (1909) reported a grass snake found swimming between the Îles de Glénan and the French mainland, about six miles (10 km) from the coast, or four miles (6 km) from the Île de Penfret. This may be the origin of the later account in Smith (1951) of a grass snake captured in the Bay of Biscay (the Îles de Glénan lie in the Bay of Biscay).

Boulenger (1913) also wrote that grass snakes were sometimes found in the sea and gave an example of one 25 miles (40 km) from land but with no specific details of location. Hecht (1930) catalogued several sightings of grass snakes in waters off the coasts of Denmark, Germany, Norway and Sweden from literature dating from 1873 to 1918. The furthest from land was approximately 25 nautical miles (29 miles/46 km). The coincidence of 25 (nautical miles) and 25 (miles) in Boulenger's text raises the possibility that these accounts stemmed from the same observation, albeit with inconsistent units of distance.

A more recent sighting was reported on the online forum Reptiles and Amphibians of the UK (RAUK) (www.herpetofauna.co.uk) in 2012; a grass snake was photographed off the Pembrokeshire coast of Wales, swimming between the mainland and an offshore island. The RAUK forum also includes two observations of unidentified snakes swimming off the eastern coast of Norfolk. These could be either grass snakes or adders. A more specific sighting, on the same forum, reported by the late Peter Stafford, relays a colleague's account of a young grass snake moving across the beach, towards (but not entering) the sea, at Minsmere, Suffolk, in the summer of 2005.

Suffolk appears to be a hotspot for grass snakes either in the sea or on the beach. Information held by Suffolk Biological Records Centre includes a juvenile grass snake seen on the beach at Minsmere, heading for the dunes, in June 2003, and a grass snake seen swimming in the sea and coming ashore at Fort Green, Aldeburgh, in July 2007. A description fitting that of a grass snake moving along the water's edge on a shingle beach, then swimming out to sea at Dunwich in September 2007 was sent to New Scientist magazine's The Last Word question and answer column (Parker, 2008). This prompted several comments including one from John Annis, who reported that 'it was not unusual to see grass snakes by the sea's edge' along the Suffolk coast 55 years previously (i.e. the 1950s), and a more specific, but anonymous, observation of a young grass snake entering the water from Dunwich Beach and swimming out to sea in July 2008.

Two further sightings have been reported to Suffolk Wildlife Trust (Baker & Simnett, 2013). In June 2010 Sue Thompson saw a grass snake at Sizewell crossing the shingle and swimming out to sea and in September 2013 Eve Simnett photographed a grass snake swimming about two metres from the shore at Walberswick harbour before making landfall on the sea defences.

In three of the eight aforementioned cases from Suffolk grass snakes were seen actually entering the water, showing that, sometimes at least, they actively enter the sea rather than being transported by some other means (for example stowing away on a boat, or being dropped by a bird during a predation attempt, or being carried out to sea by flooding). Grass snakes are relatively mobile reptiles so entering the sea may be a consequence of their natural wandering behaviour. Swimming in sea water, however, is presumably hazardous, risking predation and, eventually, dehydration. Nevertheless, marine dispersal may enable grass snakes to colonise offshore islands and maintain population exchange between these and the mainland.

ACKNOWLEDGEMENTS

I am grateful to Stephen Busack and Rosie Norton for supplying historical literature, Robert Jehle for translation and Martin Sanford for data from Suffolk Biological Records Centre. I also thank Sarah Colnet and Oka Last (Suffolk Wildlife Trust) and Caroline Robinson (The Wildlife Trusts) for relaying information.

REFERENCES

Baker, J. & Simnett, E. (2013). Grass snakes at sea. *White Admiral* 86: 7-8.

Böse (1897). *Tropidonotus* in Meerwasser. *Zoologischer Anzeiger* 20: 255.

Boulenger, G.A. (1913). *The Snakes of Europe*. London: Methusen & Co. Ltd.

Del Canto, R. & Busack, S.D. (2011). *Natrix maura* (Viperine Snake). Non-accidental salt water activity. *Herpetological Review* 42: 295-296.

Guérin-Ganivet, J. (1909). *Bulletin de L'Institut Océanographique* 132: 1-2.

Hecht, G. (1930). Mitteilungen aus dem Zoologischen Museum in Berlin 16: 345-346.

Parker, D. (2008). The Last Word: snakes alive. *New Scientist* 2637: 65.

Smith, M. (1951). *The British Amphibians and Reptiles*. London: Collins.

Wood, J.G. (1863). *The Illustrated Natural History*. Reptiles, Fishes, Molluscs. London: Routledge, Warne and Routledge.

Accepted: 27 January 2015

Lygodactylus conraui (Cameroon or Conrau's dwarf gecko): Use of edificarian habitat and anthropochory in Benin

GUY R. MANNERS[1*] & GEORG GOERGEN[2].

[1]Foxton, Cambridge, UK.
[2]International Institute of Tropical Agriculture (IITA), 08 BP 0932 Tri Postal, Cotonou, Benin.
[*]Corresponding author email: guy@grmanners.com.

On 7 February 2014, GRM observed and photographed an adult *Lygodactylus* sp. (Fig. 1) on the outside wall of a building of the research station of the International Institute of Tropical Agriculture (IITA) and Africa Rice Center (AfricaRice) in Godomey, Cotonou, Benin (6.42°N 2.33°E). This or another adult was again observed in the same place on 10 February and 14 March. On the first of these two later dates, it was seen in close proximity to a phenotypically rather different individual (Fig. 2). The first animal was originally tentatively identified (by GRM) as *L. grotei* Sternfeld, 1911 on the basis of colouration, and this was 'confirmed' by owners of pet *Lygodactylus* in Europe; subsequently, however, all photographed individuals have been identified as within the natural phenotypic variation of *L. conraui* Tornier, 1902 in West Africa (J.-F. Trape, personal communication).

Lygodactylus conraui is widespread from Sierra Leone to Cameroon in dense forest areas (Trape et al., 2012) and may also occur in Equatorial Guinea (Bauer et al., 2006). Although considered a forest species (Bauer et al., 2006), Trape et al. (2012) say "it is sometimes locally abundant in anthropogenic environments – especially in Accra, Ghana – where it can be found in large numbers in gardens, plantations and on various constructions". The species first appeared in and around residences in Cotonou (Les Cocotiers area, 6.21°N 2.23°E) in the late 1990s (GG personal observation), and has apparently become established in and around at least two office buildings at the IITA–AfricaRice campus.

Meanwhile, *L. conraui* has also been recorded (by GG) on *Acacia auriculiformis* A.Cunn. ex Benth., 1842 in an area used for car-parking adjacent to one of the offices where the species has taken up residence at IITA–AfricaRice. Thus, the species is not restricted to the buildings once it takes up residence there.

One of us (GG) has observed *L. conraui* several times on his car during the journey between his residence and the research station. The surprising behaviour was that the geckos did not seem to be particularly concerned about being on a moving object. They did not attempt to leave the car and remained 'calm' during short bursts of speed; they always arrived safely at the research station. Thus, at least for short distances, anthropochory (dispersal via human intervention, albeit unwittingly) may be a practical mode of dispersal for this species.

This anthropochory is of particular interest given the history of the IITA–AfricaRice site. The first buildings of the research station were built by IITA in Godomey in the 1980s, when Godomey was very much an isolated area of farmland well outside of the city of Cotonou. The first staff moved in in 1987 when there was just one house and a water-pumping station between the research station and the nearest main road (a little over 2 km away). Since that time, like so many other African cities, Cotonou has expanded considerably and engulfed a good number of outlying 'villages', which are now effectively quartiers of 'Greater Cotonou'. By about 1991, the

Figure 1. *L. conraui*, IITA–AfricaRice research complex, February 2014.

Figure 2. *L. conraui,* IITA–AfricaRice research complex, February 2014.

main access road to the station was half built up and local urbanisation was complete by the mid-2000s. It is perhaps surprising that the dwarf gecko has apparently moved from the city to the research station (assuming that ad-hoc observations are to be believed) rather than the other way around. But then, Godomey has not been forested for many decades.

Very young offspring of *L. conraui* have been observed inside GG's house, indicating that this species can both breed within houses and find enough food there to survive. Although we have been unable to find reports of any studies on the natural diet of this species, Trape et al. (2012) state that it feeds on small insects; moreover, both *L. chobiensis* FitzSimmons, 1932 and *L. capensis* (A. Smith, 1849) feed almost entirely on arthropods in Kafue Flats, "a maze of swampy channels and lagoons" (Wikipedia contributors, 2014), Zambia (Simbotwe, 1983). The most abundant food source inside GG's house is ants (Hymenoptera: Formicidae). At this site, the dwarf gecko species cohabits with house gecko (*Hemidactylus* sp., most likely *H. angulatus* Hallowell, 1852, *H. albivertebralis* Trape & Böhme, 2012 or *H. mabouia* (Moreau de Jonnes, 1818), but potentially *H. albituberculatus* Trape, 2012 or *H. fasciatus* Gray, 1842; see Trape et al., 2012; Bauer et al., 2006). *Hemidactylus* is a potential predator of *L. conraui*: although *Hemidactylus* typically prey almost entirely on arthropods (e.g. Avery, 1980; Rocha & Anjos, 2007), *H. frenatus* is known to practise cannibalism (Galina-Tessaro et al., 1999); whether this is simply cashing in on an abundant food source or protection of hunting territory against conspecifics is not known. Either way, from cannibalism it is only a short step to predation on a smaller similar species, especially given the size difference between *Hemidactylus* and *L. conraui* (the former full-grown snout-to-vent 6–19 cm depending on species; the latter just 4 cm, and a small fraction of that as neonate; Trape et al., 2012). The fact that the two species can coexist sympatrically confined in the same building suggests that they exhibit niche partitioning. It seems likely that partitioning is involved because (typical of their respective genera) *L. conraui* is definitively diurnal (under normal conditions), whereas *Hemidactylus* is exclusively nocturnal. This requires further investigation.

There is a question as to why a forest species is present in the Dahomey gap, an area known to lack any continuous forest cover as compared to the remaining countries where it occurs (Neuenschwander et al., 2011). Whether the adaptation to edificarian habitat is comparatively more often observed in Benin than in countries with a higher prevalence of dense forest areas would perhaps be worth pursuing.

ACKNOWLEDGEMENTS

We thank Jean-François Trape for reviewing all the photographs and confirming that the geckos were all within the phenotypic range of *L. conraui*. We also thank Simon Chater, Peter Neuenschwander and two anonymous reviewers for commenting on earlier drafts of this paper.

REFERENCES

Avery, R.A. (1980). Feeding ecology of the nocturnal gecko *Hemidactylus brookii* in Ghana. *Amphibia-Reptilia* 1: 269–276.

Bauer, A.M., Tchibozo S., Pauwels, O.S.G. & Lenglet, G. (2006). A review of the gekkotan lizards of Bénin, with the description of a new species of *Hemidactylus* (Squamata: Gekkonidae). *Zootaxa* 1242: 1–20.

Galina-Tessaro, P., Ortega-Rubio, A., AlvarezCárdenas, S. & Arnaud, G. (1999). Colonization of Socorro Island (Mexico), by the tropical house gecko *Hemidactylus frenatus* (Squamata: Gekkonidae). *Revista De Biologia Tropical* 47: 237–738.

Neuenschwander, P., Sinsin, B. & Goergen, G. (Eds) (2011). Protection de la Nature en Afrique de l'Ouest : Une Liste Rouge pour le Bénin / Nature Conservation in West Africa: Red List for Benin. Ibadan, Nigeria: International Institute of Tropical Agriculture. 365 pp.

Rocha, C.F.D. & Anjos, L.A. (2007). Feeding ecology of a nocturnal invasive alien lizard species, *Hemidactylus mabouia* Moreau de Jonnès, 1818 (Gekkonidae), living in an outcrop rocky area in southeastern Brazil. *Brazilian Journal of Biology* 67: 485–491.

Simbotwe, M.P. (1983). Comparative ecology of diurnal geckos (*Lygodactylus*) in Kafue flats, Zambia. *African Journal of Ecology* 21: 143–153.

Trape, J.-F., Trape, S. & Chirio, L. (2012). *Lézards, Crocodiles et Tortues d'Afrique Occidentale et du Sahara*. Montpellier, France: IRD Éditions. 503 pp.

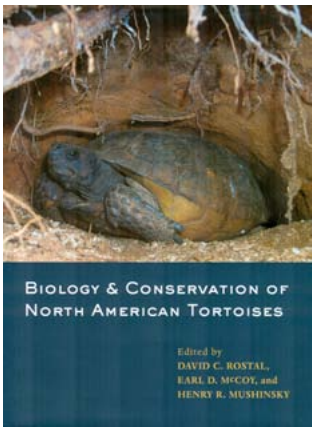
Wikipedia contributors (2014). Kafue River – Kafue Flats. In: Wikipedia, The Free Encyclopedia. http://en.wikipedia.org/w/index.php?title=Kafue_River&oldid=608381125 (last accessed 17 May 2014).

Accepted: 1 February 2015

Biology & Conservation of North American Tortoises

David C. Rostral, Earl D. McCoy & Henry R. Mushinsky, Editors, 2014

Publisher: The Johns Hopkins University Press 190 p ISBN: 9781421413778



This contribution is by no less than 33 North American tortoise researchers. The book is the latest in a recent series of monographs on the biology of specific North American turtle groups. It presents an up-to-date summary of the life history of the gopher tortoise genus *Gopherus*, and continues with more extensive reviews of the five extant species *G. agassizii* (desert tortoise, Mojave [Mohave] desert tortoise, Agassiz's desert tortoise, Agassiz land-tortoise), *G. berlandieri* (Berlandier's tortoise or Texas tortoise), *G. flavomarginatus* (Bolsón tortoise, Mexican giant tortoise, or yellow-bordered tortoise), *G. morafkai* (Morafka's desert tortoise, Sonoran desert tortoise), and *G. polyphemus* (gopher tortoise) than those of the "three" United States species, *G. agassizii*, *G. berlandieri*, and *G. polyphemus*, previously presented in more condensed accounts by Ernst & Lovich (2009).

A preface explaining the reason for the book, the binomial nomenclature, and common names used is followed by 18 chapters. These can be separated into the following broad categories: 1). Morphology, fossil record, evolution, and systematics (3 chapters); 2). Thermal and reproductive physiology (2 chapters); 3). Embryology, temperature-dependent sex determination, and growth patterns (2 chapters); 4). Health problems (1 chapter) 5); Habitat characteristics and ecology (2 chapters); 6). Behaviour (3 chapters); 7). Population characteristics, status, and genetics (3 chapters); and 8). Interactions with humans and conservation (2 chapters). These are followed by a current list of references, and an adequate index.

Chapter 1 basically covers the morphology, taxonomy & distribution of gopher tortoises. Unfortunately, the photos in the chapter of the five living species are not the best, and would have been greatly enhanced if in colour. The origins, fossil record (including the extinct genus *Oligopherus*) are described and diagramed in Chapter 2. Photos of the carapacial bones of a new fossil *Gopherus* from South Carolina are presented and compared to photos of bones from G. polyphemus. It is in these first two chapters that the greatest controversy occurs; the use of the genus *Xerobates* Agassiz, 1857 for the extant species *agassizii*, *berlandieri* and *morafkai*, instead of the more familiar and precedential *Gopherus* Rafinesque, 1832, which is used for flavomarginatus and polyphemus. Use of *Xerobates* for the three western species is explained in the first chapter as due to osteological differences, especially skull and inner ear features, a more specialised hand and more robust cervical vertebrae in *Gopherus*, and molecular differences. Surprisingly, Chapter 3 covering the systematics of these tortoises still refers them to the genus *Gopherus*; and, in spite of these differences, authors of the remaining 16 chapters continue to use the name *Gopherus*. Use of *Xerobates* will probably be a "hard sell" among turtle taxonomists.

Chapters 4 & 5 give excellent reviews of the thermal energetics, including the possible role of future climate change; and reproductive cycles, physiology (including hormonal), oviductal egg development, and the effects of upper respiratory disease on reproduction. These are followed by a well illustrated Chapter 6 covering embryology, temperature sex determination, incubation temperatures, and the possible detriments of climate change. Growth patterns are covered in Chapter 7, including the allometric changes leading to sexual dimorphism, and the effects of climate and habitat quality, especially food availability. A good discussion of aging tortoises using growth rings is presented.

Various health issues (infectious diseases, noninfectious diseases, injuries, and trauma), as well as how to assess the health of a tortoise including a recommended extensive set of field data sheets, are discussed in detail in Chapter 8 by the leading North American expert on turtle diseases, Elliot Jacobson. Also, included is a helpful glossary of important definitions regarding health & disease terms. This chapter will be of interest to all researchers of turtles, and especially those keeping them in captivity.

Chapters 9 & 10 are two very good chapters covering the habitat differences of the species of *Gopherus*. The first covers the physiography, geology, plants present, rain fall, temperature regimes, availability of burrow sites, and present threats to tortoise' habitats. Chapter 10 covers the behaviour necessary to acquire food and water (including the needs of juveniles); and contains an informative diagram illustrating when during the annual cycle the various necessary behaviours (foraging, reproductive & seasonal inactivity) of five species most frequently occur. This is followed by a table listing percentages of dietary plant growth forms (annual & perennial forbs & grasses, woody plants & succulents) in the diet of *agassizii*, *berlandieri*, *morafkai*, and polyphemus.

Home range, including typical sizes and distances moved, daily activity cycles, seasonality of movement activity, burrow/shelter numbers and use, are specifically compared in chapter 11. This chapter should be of especial use to field researchers.

Chapter 12 relates the typical social behaviours of tortoises. Male-male and female-female aggression encounters are described in detail, as is female choice of mating partners. The distinctive behaviours evolved for social communications and dominance positions are discussed and illustrated; and

dispersal of individuals from their natal social group to others and its important role in genetic exchange is stressed. Observations of interspecific aggressive interactions among captives indicate that the size of the individual is important in dominance determination, with the two largest species, *flavomargiantus* and *agassizii*, being the most dominant, and the smallest, *berlandieri* and *polyphemus*, less so. The social systems of North American tortoises and ground squirrels are compared in a table.

Chapter 13 covers reproductive behaviour in *Gopherus*. It discusses reproductive output (clutch size) and its relation to female shell length and volume, choice of nest sites; and the nesting act is diagramed.

Chapters 14-16 deal with the aspects and importance of population ecology. The first chapter covers tortoise abundance and the various methods, including mathematical, of determining population size and species density, and relates current population trends and concerns to tortoise abundance. The following chapter discusses present knowledge of population genetics of *Gopherus*, and its implications for future conservation of its species. Along with a lengthy discussion of the mtDNA of each the five species is a summary table of the genetic statistics of those populations previously studied. Data are most lacking for the two Mexican species. To illustrate the use of genetic data in conservation recovery plans, a map is presented delineating the genetic recovery units of various populations of G. *agassizii*. Discussed is how habitat fragmentation by humans is detrimental to overall genetic diversity of tortoise populations, especially G. polyphemus and G. *morafkai*; and that such diversity must be maintained to ensure no further losses of population sizes. Interestingly, a genetic study by Edwards et al. (2010) of captives in the Phoenix, Arizona, area indicates tortoises there are a hybrid swarm of *agassizii*, *agassizii x morafkai* or even *berlandieri* x *morafkai* instead of the expected pure *morafkai*. Could this indicate that these taxa are merely subspecies instead of species? Chapter 16 summarises the demography of each of the five species covering population age distribution, body size and mass; sexual maturity and reproductive capacity; growth in relation to diet, the biomass of grass and forbs in their habitat; and survivorship.

A narrative of tortoise/human interactions and other threats from the Paleo-Indian and early Archaic Periods to recent; along with the current USA Federal and State, Cites and IUCN Red List status of each species is found in Chapter 17. Chapter 18 is composed of discussions of each of the present threats to the survival of *Gopherus* populations. Tables summarize these threats and assess their levels of importance to conservation of *agassizii*, *berlandieri*, *morafkai* and *polyphemus*. Following these are suggestions of how to alleviate threats by governmental protection (Federal and State), protection of habitat and migration routes, head-starting, and the role of non-governmental conservation groups.

This book is an excellent well-illustrated review of our current biological knowledge of North American tortoises, and should be a valuable addition to the libraries of all persons interested in these animals. I highly recommend it.

Agassiz, L. (1857). *Contributions to the Natural History of the United States of America*. Vols. 1&2. Boston, MA.; Little, Brown and Company.

Edwards, T., Jarchow, C.J., Jones, C.A. & Bonine, K.E. (2010). Tracing genetic lineages of captive desert tortoises in Arizona. *Journal of Wildlife Management* 74:801-807.

Ernst, C.H. & Lovich, J.E. (2009). *Turtles of the United States and Canada*. 2nd ed. Baltimore, MD; Johns Hopkins University Press.

Rafinesque, C.S. (1832). Descriptions of two new genera of soft-shell turtles of North America. *Atlantic Journal and Friend of Knowledge*. 1:64-65.

CARL H. ERNST

Division of Amphibians & Reptiles, mrc 162
Smithsonian Institution, P.O. Box 37012,
Washington, D.C. 20013, USA.
Email: chernst@frontiernet.net.

Review received: 7 October 2014

HERPETOLOGICAL BULLETIN REPORT

January 2015

All four 2014 issues of Herpetological Bulletin were published during 2014, mostly due to the efforts of the new Managing Editor, Sarah Berry. This has resulted in a significant shortening of the time between manuscript acceptance and publication. To make Herpetological Bulletin more attractive to authors it is important that manuscript processing is speeded up. In this respect the new layout for Herpetological Bulletin, designed by Sarah Berry, with improved paper presentation and eye-catching cover design will hopefully increase the Bulletin's appeal. Additionally we have set a target for 2015 of publication of papers in the next available issue once they have been accepted

Due to heavy work commitments at a new post Laurence Jarvis had to stand down as Managing Editor. We are grateful to Laurence for his efforts in Bulletin production during an initial difficult period and also his contribution in making the change from A5 to A4 layout. Stuart Graham continues as Associate Editor and has been of valued assistance in, among other things, proof reading but also by assisting new authors in manuscript preparation. It will be apparent from the above comments that producing Herpetological Bulletin has been a team effort.

During 2014, 53 manuscripts were submitted to Herpetological Bulletin with an additional 4 (not included in the table below) rejected without review as inappropriate material. This represents a slight increase in submissions compared to 2014 but there was a lower acceptance rate. The Table below provides the details with comparable figures for 2014 shown in parentheses.

	Submitted	Accepted	Percent accepted
Full papers	20 (17)	11 (12)	55.0 (70.1)
Short Notes	11 (6)	7 (5)	63.6 (83.3)
Natural History Notes	22 (27)	12 (17)	54.5 (62.9)
Total	53 (50)	34 (30)	56.6 (68.0)

Two review papers were published during 2104. Allison Hegan reviewed the problems of Alien Herpetofauna in the USA and Christine Tilley Herpetological Activities of the British Chelonia Group. We will continue soliciting additional material of this kind for 2015.

The following people reviewed manuscripts for Herpetological Bulletin during 2014: Roger Avery; John Baker; Chris Barratt; Aaron Bauer; Trevor Beebee; Daniel Bennett; Rebecca Cattell; John E. Cooper; Chris Gleed-Owen; Richard Griffiths; Stuart Graham; Rick Hodges; Robert Jehle; Todd Lewis; Simon Maddock; Roger Meek; Declan Quigley; ALan Rees; Steve Richards; David Sewell; Mark Stidworthy; Christine Tilley; Eleanor Warren-Thomas; John Wilkinson: Mark Wilkinson.

Roger Meek, Roger Avery
Editors

BRITISH HERPETOLOGICAL SOCIETY COUNCIL 2014/2015

Society address: c/o Zoological Society of London, Regent's Park, London, NW1 4RY

Website: **www.thebhs.org**

President	Prof. R.A. Griffiths	Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Marlowe Building, Canterbury, Kent, CT2 7NR. Tel: +44 (0)1227 823434. r.a.griffiths@kent.ac.uk
Chairman:	Dr. C. Gleed-Owen	CGO Ecology Limited, 5 Cranbourne House, 12 Knole Road, Bournemouth, Dorset, BH1 4DQ. chair@thebhs.org
Treasurer:	Mr M. Wise (co-opted)	Tel. +44 (0)7531 336995. treasurer@thebhs.org
Secretary:	Mr. T. Rose	11 Strathmore Place, Montrose, Angus DD10 8LQ. Tel +44 (0)1674 671676; Mob: +44 (0)7778 830192. secretary@thebhs.org
The Herpetological Journal		
Receiving Editors:	Dr. R. Jehle (co-opted)	Salford University, School of Environment & Life Sciences, Peel Building, Salford Crescent, Salford, Greater Manchester, M5 4WT. Tel: +44 (0)161 295 2146. herpjournal@thebhs.org or r.jehle@salford.ac.uk
Managing Editor:	Dr. C. Barratt	herpjournal@thebhs.org
The Herpetological Bulletin		
Receiving Editors:	Mr. R. Meek Dr. R. Avery	herpbulletin@thebhs.org
Managing Editors:	Mrs. S. Berry	info@sarahberryonline.com
Associate Editors:	Mr. S. Graham Dr. L. Jarvis	stuartgrahamuk@hotmail.co.uk lawrence@hotmail.co.uk
The NatterJack		
Editor:	Mr. D. Willis	davewillisbhs@yahoo.co.uk
Librarian:	Mr. D. Bird	Jacaranda Cottage, New Buildings, Spetisbury, Blandford Forum, Dorset, DT11 9EE. drbird.herp1@virgin.net
Development Officer:	Mr. M. Hollowell	mark22@btinternet.com
Website and Communications Officer:	Mr. A. Plettenberg Laing	07810 043520. avonplettenberglaing@gmail.com
Conservation Officer:	Mrs. J. Clemons	8 Carthusian Road, Coventry, CV3 6HA. clemons@btinternet.com
Trade Officer:	Mr. J. Coote	tradeofficer@thebhs.org
Meetings Organiser:	Vacant	
Captive Breeding Committee		
Chair:	Dr. S. Townson	Tel: +44 (0)1438 880039. s.townson@imperial.ac.uk
Education Committee		
Chair	Ms. K. Le Breuilly	9 Anvil Close, Streatham, London SW16 6YA. Tel: +44 (0)208 6969733; Mob: +44 (0)7732 949344. kim.lebreuilly@o2.co.uk
Research Committee		
Chair:	Dr. C. Gleed-Owen	CGO Ecology Limited, 5 Cranbourne House, 12 Knole Road, Bournemouth, Dorset, BH1 4DQ. chair@thebhs.org

Ordinary Members

Mr. I. Gill	ZSL London Zoo, Regents Park, London, NW1 4RY. iri.gill@zsl.org (1st year)
Dr. I. Stephen	ianstephen@gmail.com (1st year)
Ordinary Member 3	Vacant

Fellows of the British Herpetological Society

Prof. T.J.C. Beebee, Mr D. Bird, Mr F. Bowles, Prof. J.E. Cooper, Mr. J. Coote, Mrs M. Green, Prof. R.A. Griffiths, Mrs E. Haslewood, Mr T. A. Rose, Mr V. F. Taylor, Dr. S. Townson

Past Presidents (retiring date)

Dr M. A. Smith (1956), Dr J.F.D. Frazer (1981), The Earl of Cranbrook (1990), Prof. J.L. Cloudsley-Thompson (1996), Dr. R. Avery (1997), Dr. H. Robert Bustard (2005), Prof T.J.C. Beebee (2013)

THE HERPETOLOGICAL BULLETIN

Contents: Issue 131, Spring 2015

Registered Charity No. 205666