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EDITORIAL

The conservation of amphibian and reptiles in Scotland – the role of publishing

C.J. McInerny

University of Glasgow, President Glasgow Natural History Society (GNHS), Chairman Biological Recording in Scotland (BRISC), and Clyde Amphibian and Reptile Group (CARG)
School of Life Sciences, University of Glasgow, Glasgow G12 8QQ

E-mail: Chris.McInerny@glasgow.ac.uk

Scotland contains rich populations of amphibians and reptiles, some of which are important in a U.K. context. In other parts of the U.K. amphibian and reptile populations and ranges have declined and contracted due to a combination of urbanisation, fragmentation of habitats, and pollution. While Scotland has rich populations it is crucial that we do all we can to protect these animals which make an essential contribution to Scottish ecosystems. To inform such conservation approaches, baseline information is required on numbers, distribution, annual cycles and habitat requirements.

A step forward in this process was the publication in 2016 of *The Amphibians and Reptiles of Scotland* (McInerny & Minting, 2016) by the Glasgow Natural History Society (GNHS). This book attempted to draw together all known Scottish information at the time. By doing so, it also revealed many gaps in the knowledge. These gaps were highlighted, and in some cases formed the basis for subsequent research.

The Proceedings of the June 2018 conference *The Amphibians and Reptiles of Scotland: current research and future challenges* published in this supplementary issue of *The Glasgow Naturalist* are the next step in the process. They show the range of research being done to conserve and protect amphibians and reptiles, with much directly relevant to Scotland. This information will, we hope, inform conservation approaches including those supported by government through Scottish Natural Heritage.

In conclusion, it is only through published research informing conservation practices and approaches that we can ensure the long-term persistence of these wonderful creatures in Scotland for future generations.

ACKNOWLEDGEMENTS
I thank Iain Wilkie for co-editing this supplementary issue of *The Glasgow Naturalist.* I thank Roger Downie for his encouragement as the then GNHS President for supporting publication of *The Amphibians and Reptiles of Scotland*. And I thank my co-author Peter Minting for helping to produce the book, and Ian Andrews, Chris Cathrine and David O'Brien for their contributions.

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Amphibians and Reptiles of Scotland: current research and future challenges

University of Glasgow, Graham Kerr Building,
Saturday 9th June 2018

Arrival time 9.00am, start 9.30am, close 4.30pm.

Register for your free tickets online with the Glasgow Science Festival

With thanks to our conference organising team

Speakers	Talks	Time
Roger Downie University of Glasgow, GNHS, Clyde ARG, Froglife	**Opening and welcome; messages from MSP species champions**	09.30-09.35
Chair: Roger Downie		
Silviu Petrovan University of Cambridge Froglife	**Using an evidence-based approach to improve the understanding and effectiveness of road mitigation schemes for amphibians and reptiles** Roads fragment habitats and endanger wildlife across the globe. We review multiple projects undertaken in Scotland and England since 2013. We discuss the results using an evidence-based approach to highlight success stories, knowledge gaps and areas of concern, related to species ecology and chemical pollution.	09.35-10.05
Nigel Hand Central Ecology	**The vanishing viper: using radiotelemetry to unlock the secret life of the adder** We pioneered the use of telemetry in the UK to better understand adder spatial ecology, often revealing unexpected results and allowing management plans to be better targeted.	10.05-10.25
Lynsey Harper University of Hull	**Enhancing understanding of great crested newt habitat and environmental influences in Scotland** Threatened by industrial development, Scotland's largest great crested newt population was relocated to Gartcosh Nature Reserve, North Lanarkshire, in Scotland's first ex-situ conservation-based translocation. We report on changes in Habitat Suitability Index (HSI) post-translocation, and viability of this index. We also report on abiotic determinants of this Scottish population and provide guidance for future conservation management.	10.25-10.35
David O'Brien Highland Biological Recording Group	**SuDS and amphibians - are constructed wetlands really benefitting nature and people?** Sustainable Drainage Systems (SuDS) have potential as amphibian habitats, as part of habitat networks and as places where urban people can experience nature. Our eight-year study suggests ways to improve the design and management of SuDS for people and nature, making access to high quality ponds available to all social groups.	10.35-10.50
Chloe Rossi & Iain Hill University of Glasgow	**Under-road tunnel mitigation at Frankfield Loch, Glasgow** This study assessed the efficacy of three amphibian tunnels in a recently developed area at Frankfield Loch, Stepps, Glasgow. In 2016 and 2015 an assessment was conducted on the success of wildlife passage through these tunnels using video recordings. The results can contribute in showing the significance of mitigation measures.	10.50-11.00
Matthew Witt University of Exeter	**Waifs and strays or seasonally resident? Marine turtles in the British Isles** Although not typically considered part of British biodiversity, marine turtles have appeared in records of fauna around our coastal seas for hundreds of years. In this talk we will investigate patterns of sightings and strandings of this taxon demonstrating their regular occurrence, highlighting these animals are indeed part of our maritime natural heritage**.**	11.00-11.20
COFFEE BREAK		11.20-11.40
Chair: Chris McInerny		
Rick Hodges Kent ARG	**Long-term monitoring for adders** Long-term monitoring of adders offers prospects for understanding the impacts of habitat management and climate change, as well as providing life-history details. An approach to long-term monitoring will be presented, as work in progress, based on experiences gained on a chalk grassland reserve.	11.40-11.55
Pete Minting ARC	**Great crested newt detectives; citizen science, education and DNA technology** An innovative, Scotland-wide project. Volunteers were trained in amphibian identification and surveying and provided with eDNA sampling kits to test for the presence of great crested newts in ponds. Children also took part, in education sessions at schools and by entering a competition to help create a new, free publication called *Amazing Animals, Brilliant Science; how DNA technology is helping to save Scotland's wildlife*	11.55-12.10
James Stead & Louise Smith Froglife	**Froglife in Scotland** Froglife's Scottish projects to protect and conserve amphibians and reptiles include the Scottish Dragon Finder Project, a 4.5 year project, beginning in 2014, bringing together practical conservation, educational activities and data collection, and the Glasgow Green Pathways, working with vulnerable and disadvantaged young people on practical activities to improve local greenspaces for wildlife.	12.10-12.25
Chris Cathrine Caledonian Conservation	**A novel approach to reptile mitigation in peatland habitats for an underground power line development in Kintyre** Caledonian Conservation Ltd was contracted by Renewable Energy Systems Ltd to undertake ecological mitigation for an underground power line in Kintyre, to connect Freasdail Wind Farm to the National Grid. The site crossed 10 km of remote peatland occupied by reptiles. Mitigation approaches will be discussed in detail, as well as results	12.25-12.40

LUNCH BREAK		12.40-13.45
Chair: Deborah McNeill		
Erik Paterson & Ryan Bird University of Glasgow Clyde ARG	**Amphibians and water quality in East Kilbride** We will present the results of long-term citizen science amphibian monitoring study in East Kilbride. Results will be related to recent work examining water quality determinants of amphibian species presence and abundance within a variety of pond types around the town.	13.45-14.00
Chris McInerny University of Glasgow GNHS, BRISC, Clyde ARG	**The study and conservation of adders in Scotland** Adders remain widespread and reasonably common in parts of Scotland, but there remains much work to conserve and protect the species. This talk describes two conservation projects that have revealed information about adder biology in Scotland and how the species can live alongside humans and human development.	14.00-14.15
Kathleen McMillan Clyde Muirshiel Regional Park	**A study of the reptile populations at the Greenock Cut and management plan** Since 2013 we have surveyed reptile species and populations present at the Greenock Cut. We describe the creation of a habitat management plan to ensure the long-term presence of these animals at the site.	14.15-14.25
Rob Raynor Scottish Natural Heritage	**An amphibian and reptile strategy for Scotland: talk followed by discussion** A strategic approach to Scottish Herpetological conservation is presented, describing the threats and opportunities, the reasons why action is urgent and the challenges we need to meet for their conservation. The strategy recognises that a partnership approach between the relevant organisations is required if we are to achieve our objectives.	14.25-15.25
COFFEE BREAK		15.25-15.40
Chair: Roger Downie		
Andrew Cunningham Institute of Zoology	**Infectious disease threats to amphibian conservation** Only since the discovery of amphibian chytridiomycosis has infectious disease been considered a threat to amphibian conservation. While a growing number of infectious diseases of amphibians are recognised, only a small number threaten populations or species; however their mitigation is more difficult than for other threats such as habitat loss.	15.40-16.10
CONFERENCE CLOSE		16.10-16.30

With thanks to our conference supporters

And Friends of Angus Herpetofauna

The Glasgow Naturalist (2019) Volume 27, Supplement. *The Amphibians and Reptiles of Scotland*, 7-8

FULL PAPERS

Conference: *The Amphibians and Reptiles of Scotland: current research and future challenges*

J.R. Downie

University of Glasgow, Glasgow Natural History Society (GNHS), Froglife, and Clyde Amphibian and Reptile Group (CARG)
School of Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ

E-mail: roger.downie@glasgow.ac.uk

OPENING REMARKS

Welcome to the conference, organised as a contribution to Glasgow Science Festival, 2018. I am delighted that the conference is supported by so many organisations: Glasgow Natural History Society, Scottish Natural Heritage (SNH), Froglife, Amphibian and Reptile Groups-UK, Amphibian and Reptile Conservation, British Herpetological Society, and Friends of Angus Herpetofauna. Their support allowed attendance at this conference to be free of charge. Please visit their stalls during the breaks.

To my knowledge, this is the third such conference, following Glasgow in November, 2011 and Edinburgh in October, 2014. Previous meetings in Scotland were part of the series of Herpetofauna Workers meetings which occur annually at different venues around the country.

The immediate stimulus for us in organising this conference was the publication, in 2016, of Chris McInerny and Pete Minting's *The Amphibians and Reptiles of Scotland* (McInerny & Minting, 2016). As far as I know, this was the first attempt to put together a comprehensive account of Scotland's herpetofauna. GNHS acted as the book's publisher, and some of us felt that it could act as a stimulus for further work, since, as well as collating a large amount of information, the book drew attention to gaps in our knowledge; hence the subtitle of the meeting: *current research and future challenges.* One obvious gap is the lack of recording in some parts of Scotland, contributing to some big blanks on the distribution maps.

To make the conference of enduring value, the Proceedings will be published in *The Glasgow Naturalist*, Scotland's leading natural history journal, now freely available on-line. In addition to the exciting range of talks on the programme, we have a fine set of posters for you to browse during the breaks.

The Scottish Government has designated 2018 as *Year of Young People* with activities under six themes: participation, education, health and wellbeing, equality and discrimination, enterprise and regeneration, and culture. As our contribution, several of the talks are being given by younger researchers, and the poster display includes work done by young people as part of Froglife's *Glasgow Green Pathways* project which engages with vulnerable and disadvantaged young people. In addition, as a follow-up to the conference, we have a stall at Science Sunday, Glasgow Science Festival's family day which engages parents and children in the exciting science being done in the city.

In an effort to enlist the support of our politicians, Scottish Environment Link has developed a Species Champion scheme, whereby Members of the Scottish Parliament (MSPs) are encouraged to sign up to champion a particular species, possibly one of particular importance to their constituency. By April 2018, 103 species had champions. Interestingly, reptiles and amphibians are popular with our MSPs with three reptiles [common lizard (*Zootoca vivipara*), leatherback turtle (*Dermochelys coriacea*) and slow-worm (*Anguis fragilis*)] and four amphibians [common toad (*Bufo bufo*), great crested newt (*Triturus cristatus*), natterjack toad (*Bufo/Epidalea calamita*) and smooth newt (*Lissotriton vulgaris*)] championed. Although no MSP was able to attend the conference, we have received messages of support from Jackie Baillie (Dumbarton), Emma Harper (Dumfries) and Bruce Crawford (Stirling).

Jackie Baillie wrote: "I am happy to support Scottish Environment Link's Species Champion programme, working in partnership with Amphibian and Reptile Conservation to promote the smooth newt. In Scotland, smooth newt populations are in the southern half of the country, where natural pond

chemistry is more suitable and industrial development often results in the destruction of amphibian habitat. This makes my constituency, on the banks of Loch Lomond and subject to the National Park's rules on planning and development, the perfect home for the smooth newt. Nevertheless the species still faces a number of challenges, including destruction of breeding ponds, introduction of non-native species and pollution. I welcome all speakers and attendees to the Scottish Herpetological Conference and I look forward to future discussions on a national strategy for amphibians and reptiles".

Emma Harper wrote: "I fully support the work of the Glasgow Natural History Society to draw attention to Scotland's fauna, and the plight of many of our species and the threats they face. As species champion for the natterjack toad, I have been following progress for increasing numbers and protecting their habitat on the Solway Coast, which is the area I represent as a South Scotland MSP. I commend the work that the RSPB are doing to preserve and protect the habitats of the natterjack toad. I look forward to supporting RSPB, and I hope to attend future events and work with RSPB and other organisations on this very important matter."

Jackie Baillie's reference to a "national strategy for amphibians and reptiles" highlights another of the stimuli for this conference. Two to three years ago, several members of the Scottish herpetological community discussed ideas for a national strategy with SNH's David O'Brien. We are delighted that a discussion session on SNH's draft strategy forms a key part of the conference, allowing attendees to provide feedback on the strategy's main features.

Finally in these opening remarks, I wish to thank all members of our organising team; in addition to myself, Chris McInerny, Debbie McNeill, Erik Paterson and Louise Smith.

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Towards an evidence-based approach for road mitigation schemes for amphibians and reptiles in the U.K. – a review

S.O. Petrovan[1,2]

[1]Conservation Science Group, Department of Zoology, The David Attenborough Building, University of Cambridge, Cambridge CB2 3QZ
[2]Froglife, 1 Loxley, Werrington, Peterborough PE4 5BW

E-mail: sop21@cam.ac.uk

ABSTRACT
Road networks have substantial and diverse impacts on wildlife, including amphibians and reptiles. However, despite significant progress, ecological mitigation measures designed to reduce such impacts are often insufficiently tested and described in terms of their efficiency for a range of species. Incorporating a solid evidence-based approach could greatly benefit the sector as a whole, but would require increased and adequate monitoring effort of implemented mitigation schemes, as well as a requirement to make the results available, to ensure practitioners use and regulators validate the evidence. To this goal *The Conservation Evidence* project (www.conservationevidence.com) brings together and evaluates conservation actions to make them freely accessible and directly comparable for practitioners.

REVIEW
Roads and road traffic have been known to represent threats to wildlife and habitats for almost 100 years (Forman, 1998; Van der Ree *et al.,* 2011), but proactive measures to reduce such impacts, broadly known as ecological road mitigation measures, have become widely adopted only in recent decades. At the same time, the study of the impacts of transport infrastructure and traffic on the wider environment including wildlife and habitats, now referred to as "Road ecology", has become a fully-fledged branch of ecological research, with rapidly growing numbers of targeted scientific papers, books, conferences (e.g. IENE in Europe www.iene.info/, or ICOET in North America www.icoet.net/ICOET_2019/index.asp), and best practice guidelines.

Huge progress has been achieved in understanding and documenting the extensive ecological effects of road networks (Coffin, 2007). These effects range from barriers to movement and enormous sources of wildlife-traffic collisions, to acting as dispersal corridors, including for invasive species. Roads also play a role in promoting habitat loss and land use changes (e.g. from forest to agricultural land), and in causing alterations to the hydrology, sedimentation rates, water and air chemistry, as well as substantial sound and light pollution. Each of these factors can potentially transform the fate of local wildlife populations; additionally they typically act together. As such, the task of reducing or mitigating such factors is daunting and inherently complex.

For many of the road-associated ecological effects mitigation measures, guidelines and options designed to minimize impact have been published, such as those described in the *COST 341* handbook (Trocmé *et al.,* 2003). These guidelines start from the planning stage (e.g. via avoidance of road construction in ecologically valuable habitat, proposing mitigation schemes etc.), to the construction stage, both for the impact during construction as well as including specific infrastructure (e.g. wildlife passages, attenuation ponds to collect water runoff, noise-absorbent panels, modified spectrum streetlights etc.). Post-construction, there are numerous other measures aimed at reducing negative impacts on the environment, from using less polluting fuels, to traffic speed reductions in particular road sectors, and automated systems for detecting the presence of wildlife near roads and alerting the drivers via traffic warnings.

Greater understanding of road mitigation measures has allowed landscape scale planning of green corridors, combining habitat creation and restoration with underpasses or overpasses in a way which would have been impossible 20-30 years ago (Langton, 2015). Yet numerous challenges and questions of mitigation effectiveness remain, perhaps unsurprisingly given the complexity of the task.

There have been well-publicised cases of mitigation failure and "evidence complacency" perhaps most notably shown in the case of road mitigation measures for bats. Here bat gantries designed to provide a safe passage for bats over the roads have continued to be implemented even after they were shown to be ineffective (Berthinussen & Altringham,

2012; Sutherland & Wordley, 2017). Conservation practitioners or consultant ecologists often do not have the time or access to consult scientific literature, mitigation monitoring results generally remain hidden in reports, and a substantial amount of conservation actions remains untested.

However, financial resources for conservation measures are scarce and numerous species are rapidly declining, including in the U.K. Thus it is vital for implemented mitigation projects to fulfill their roles. For road mitigation, this is especially relevant given the seemingly inexorable demand for road construction and increases in traffic volumes.

In the U.K., which has one of the densest road networks in the world, traffic volumes and the numbers of vehicles have almost doubled over the past 35-40 years, and new road construction projects inherently clash with the need to protect remnant fragments of habitat (Department for Transport, 2014). Construction sites across south-east England are a visible testament to this, with miles of "newt fencing" designed to prevent accidental killing or injuring of the protected great crested newt (*Triturus cristatus*) during road building and road widening projects.

While many aspects of road mitigation for species such as deer (Cervidae) or large carnivores are now well studied and robustly implemented, there is a general lack of targeted evidence-based support for road mitigation for a wide range of other vertebrates including smaller mammals, reptiles and amphibians (Lesbarreres & Fahrig, 2012; Beebee, 2013). This is despite the fact that there is growing awareness that roads are severely affecting such species, most likely contributing to their declines such as those apparent in two widespread and previously common species, the common toad (*Bufo bufo*) and the European hedgehog (*Erinaceus europaeus*) (Huijser & Bergers, 2000; Petrovan & Schmidt, 2016).

The great crested newt provides an example of the lack of mitigation implementation analysis. Despite numerous projects that have incorporated road mitigation measures for this species since the 1990s, the first scientific paper describing the usage by newts of one such road mitigation was published only in 2017 (Matos *et al.*, 2017). This study indicated that the mitigation measures were used differently by newts compared with other amphibian species, as most newt road crossing through tunnels took place during late autumn-winter and not the spring (Fig. 1).

Such findings are important as they demonstrate that mitigation measures should be tailored for the species ecology rather than for broad groups (e.g. amphibians), and that once implemented they need careful monitoring to evaluate their usage and effectiveness. Implementing road tunnels for newts in a typical amphibian scenario, by connecting terrestrial and aquatic habitat using tunnels and fences, instead of connecting areas with breeding ponds on both sides of the road, could be damaging for populations, as encouraging dispersal movements into areas with no breeding habitat could result in newts being stranded.

Fig. 1. Female great crested newt (*Triturus cristatus*) crossing an ACO amphibian road tunnel in England as recorded by Froglife custom-made cameras. Note the date of 25th December 2015. Collecting information using novel technology allows data gathering over long periods and can reveal interesting species ecology. In this case, the mild winter weather in 2015/16 meant that newts continued to be active throughout the winter months. (Photo: Froglife)

Evidence-based evaluations, such as the *Cochrane Collaboration* (http://www.cochrane.org) operate by bringing together and reviewing the evidence on scientific trials of medical treatments. *The Conservation Evidence* project (www.conservationevidence.com) has a similar purpose applied to conservation, through collating and synthesizing information on the success of conservation interventions, ultimately for all species groups and habitats. It provides a free, authoritative and user-friendly resource aiming to facilitate effective decision-making by conservation practitioners and policy makers.

While *Reptile Conservation* and *Mammal Conservation* are currently in preparation, the *Amphibian Conservation* synopsis was published and has been evaluated by a group of experts from across the globe (Smith *et al.,* 2018). What can it teach us about road mitigation for amphibians? One example is the evaluation of volunteers used to move amphibians across the road, known in the U.K. as "Toads on Roads". Rather worryingly it was found to be "Unlikely to be beneficial". While this might dismay the extremely dedicated volunteers that go out night after night and move amphibians to protect them from cars, a recent analysis of the 30-year data collected in the U.K. and Switzerland showed that substantial toad declines have continued even in sites with toad patrols (Petrovan & Schmidt, 2016). The work from volunteers, while invaluable as citizen science to estimate long-term trends, appears unable on its own to halt population declines. However, more positively, the volunteer work is likely to be significantly slowing the rate of declines, and thus allowing more time to search for additional solutions.

In contrast, the effectiveness of "Install culverts or tunnels as road crossings" is relatively well supported in the *Amphibian Conservation* synopsis, with 32 studies. But even here, there is evidence that some designs are not effective, or that high levels of mortality continued at some sites (Smith *et al.,* 2018). As mentioned, at the time of data collection in 2014, there were no studies on great crested newts. Additionally, almost no studies addressed the usage of such tunnels or underpasses by juveniles, instead focusing on adult migrations in spring to the breeding areas.

The effectiveness of road signs warning motorists to protect amphibians appeared to be poorly supported by evidence. However, the main reason why many volunteers install traffic warning signs is not to protect amphibians but rather to indicate to motorists that volunteers are on roads rescuing toads, and thus to be vigilant and drive slowly. Installing barrier fencing along roads was much better supported, but the outcome was assessed as "Trade-off between benefit and harms". Effective fencing can rapidly reduce mortality and thus eliminate the obvious issue on mass mortalities on the roads. Even so, unless they are combined with effective tunnels, the whole system acts as a barrier and can result in a substantial reduction in breeding activity, lack of recruitment and ultimately the extinction of populations.

CONCLUSIONS

Considering the large volume of literature included as part of the *Amphibian Conservation* synopsis, with 49 systematically searched journals (https://www.conservationevidence.com/journalsearcher/index), the evidence remains limited for a range of important conservation actions, including road mitigation, highlighting the need for targeted research. A participative effort, with conservation practitioners examining, testing and publishing the evidence of effectiveness of success is an important step in improving the outlook for a range of conservation actions, and has the potential to create a fundamental change in the way to do conservation. Though road mitigation is often expensive, it is directly relevant and important for the conservation of wildlife, including smaller vertebrates, and so it is vital that it is correctly implemented. Strategic research, participative monitoring efforts and publication of the results could substantially improve the current situation.

ACKNOWLEDGEMENTS

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The Glasgow Naturalist (2019) Volume 27, Supplement. *The Amphibians and Reptiles of Scotland,* 13-18

The secret life of the adder (*Vipera berus*) revealed through telemetry

N. Hand

Central Ecology, 45 Albert Road, Ledbury HR8 2DN

E-mail: nigel@centralecology.co.uk

INTRODUCTION

I have radio-tracked the movements of European adder (*Vipera berus*) populations on six sites in central and southern England since 2010, using telemetry of tagged snakes (Fig. 1A). During eight years of tracking projects, 75 snakes have been successfully fitted with external tags and their movements mapped.

Fig. 1. Telemetry tags used to monitor the movements of adders (*Vipera berus*). Position of a tag on an adult female (A). Tags are fitted with surgical tape low down the body, avoiding the widest body area. The tape does not go entirely around the body but only a small section applied to the flank. Tags are typically sloughed off on cast skin and retrieved whilst still transmitting (B). (Photos: N. Hand)

The advantages of telemetry are:

1. Snakes have their own tag signal pinpointing the movements of individuals.
2. The movements of snakes are tracked and mapped revealing home ranges.
3. The method allows locating adders which are hidden from view or underground.
4. The data inform landscape management beneficial to snakes.

RESULTS

I first tested whether externally attached telemetry tags would affect snake behaviour. In 2010 a tag was tested on an adult male for 15 days and activity monitored. In this time the adder was observed basking and moving as expected, with the tag not impeding progress. Tagged snakes have subsequently been recorded in combat, courtship, copulation, basking, and exhibiting evidence of prey ingestion (Fig. 2).

Fig. 2. Adder (*Vipera berus*) behaviour was unaffected by telemetry tags. A number of tagged snakes have been observed having caught and ingested prey items (A). Tagged snakes have been observed in combat (B), courtship, and mating. (Photos: N. Hand and N. Saunders)

Tags are shed during normal skin sloughing (Fig. 1B), and so tag attachment has to be timed to fit between repeated sloughing cycles. In the Midlands and southern England male adders first slough around

the second to third week of April, followed by the next slough at the end of May and early June. Most females instead slough for the first time later at the end of May or early June. There are further sloughs for both sexes between July and September, with up to four sloughs per year for an adult adder.

The capture and tag fitting sites were usually close to hibernation sites (hibernacula). Males were also captured searching for mates. Telemetry tags have had a lifespan of 80-90 days, but their project tracking time may be reduced by being cast off during sloughing cycles. The longest continuous tracking of a snake was 101 days, with the tag re-applied to the same snake after sloughing, but the average time was 49 days. The main tracking period is from April to June, covering a mix of breeding and non-breeding adult animals. Tags were not placed on snakes below 35 cm in length as there is a risk that the tag could impede a snake below this size, so very few juveniles have been monitored.

Fossorial behaviour was evident with all tracked adders. After breeding in mid-May, males became less conspicuous, but were tracked sheltering beneath bracken humus (*Pteridium* spp.), and below ground in rodent burrows (Fig. 3). Non-breeding females and juveniles also followed this pattern, but gravid females tended to remain above ground basking. Often snakes below ground were found with prey bulges indicating that they were feeding (Fig. 2A). Many snakes appeared to retreat into humid areas when going through the sloughing cycle during late May.

Males were observed mate searching, and with radio telemetry their interactions with other snakes monitored (Figs. 4, 5). Males were mapped travelling to breeding sites, with some arrivals leading to other males suddenly deserting breeding areas. In contrast, females were mapped remaining close to capture sites or near to hibernacula. Females traveled on average 158 m from site of capture to the June skin shed tag location, whereas average male movements were 570 m from site of capture to shed tag location.

When mapping adder movements through habitats, telemetry revealed vegetation and topography that allowed movements, along with barriers (Figs. 4, 5). It is noteworthy how far males searched for mates, and the variety of habitats that they passed through. Males moved through woodland in April and May when the canopy growth was sparse, and sunlight could reach the ground. They also moved along ride edges during this period. Tracking revealed that power cable wayleave corridors are used as adder routes. Similarly, unmade roads and open mown areas up to 30 m in width were crossed to reach females or further habitat. Mapping revealed adders could move through mature deciduous woodland. However, adders were not encountered in heavily grazed or short grassland. Movements could be tracked up to the habitat boundary edge, fence line or open area, with snakes then turning back or remaining at the edge.

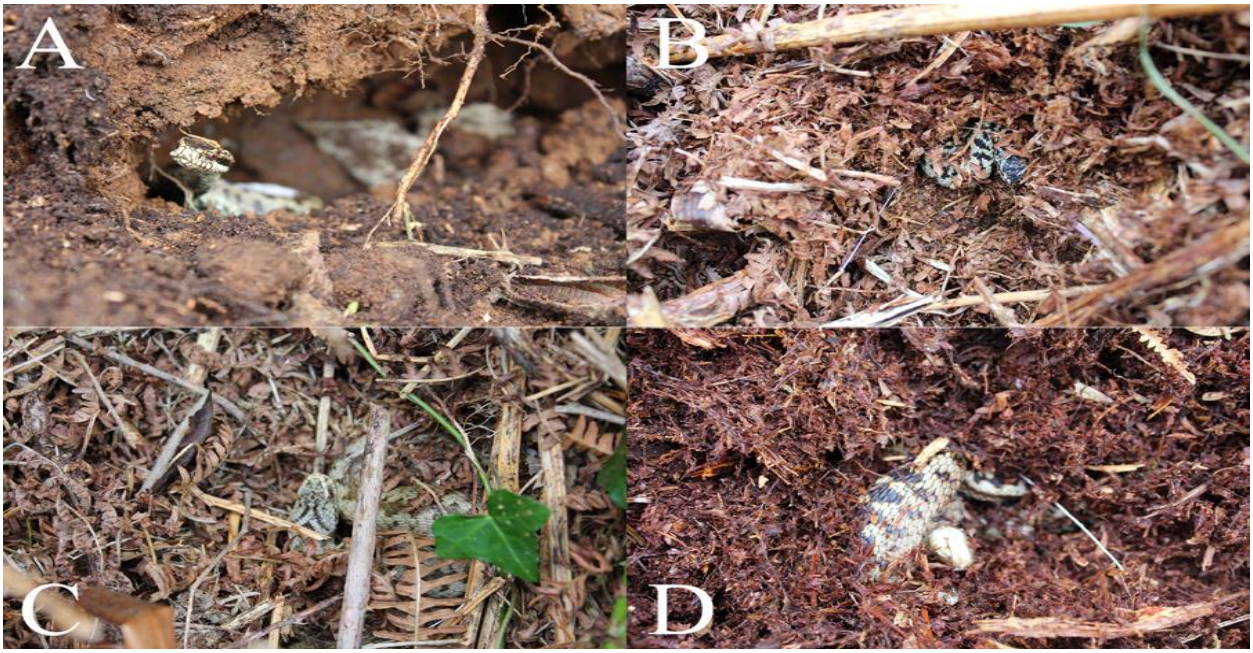

Fig. 3. Tagged adders (*Vipera berus*) as found in habitat. Males and females spend long periods underneath vegetation (B-D), or in rodent runs and burrows (A). This was either to avoid inclement weather, for hunting prey, or as overnight refuge areas. The images show snakes in habitats dominated by bracken (*Pteridium* spp.) where they hide underneath bracken thatch. At other sites adders were found underneath gorse (*Ulex* spp.) needle humus, heather (*Calluna vulgaris*) roots or moss layers. There are important implications for habitat management due to this behaviour: heavy machinery cutting and collecting could result in snakes being entombed and crushed underground. (Photos: N. Hand)

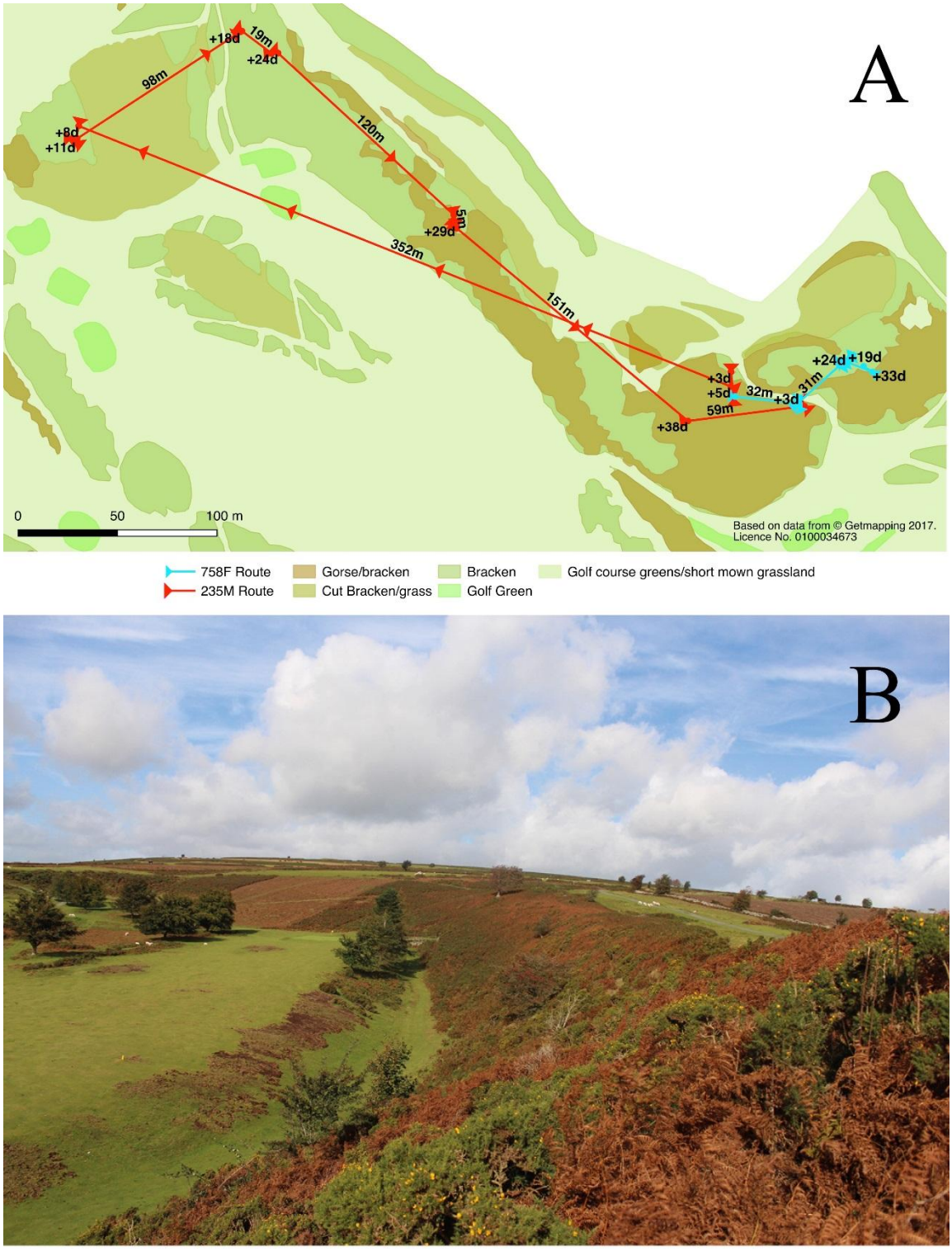

Fig. 4. Adder (*Vipera berus*) movements of two tracked snakes at a Herefordshire golf course and common, from April to early June 2017. A map (A) and photograph (B) of the study site. Snakes were tracked every two days with their position plotted using a GPS device. The routes of the male 235M (red line) and the female 758F (blue line) are shown. Male 235M tracked for 47 days, used a gorse (*Ulex* spp.) and bracken (*Pteridium* spp.) bank during the mate-searching period from April to early June, and was not recorded on short mown golf course fairways or greens. This snake found two females around day eight and day 11 (+8d, +11d) and on the return journey crossed a cut bracken/grassland area approximately 30 m wide, and is suspected to have attempted crossing this area twice. This snake moved 350 m over +5d to +8d, this outward move over three days to locate females was between tracking visits. This snake was not seen on the mown greens during tracking and likely travelled within cover. Notably the recorded return back to the initial overwintering area revealed a route through bankside cover. In contrast, female 758F movements were within a small area moving 109 m from tagging to tag sloughing, in a tracked time of 39 days, behaviour typical of females. 758F was thought to have been captured by a common buzzard (*Buteo buteo*) after the tag had been shed. These observations reveal the importance of suitable habitat to create safe dispersal corridors for snakes: overzealous management could result in isolating individual snakes and groups (Photo: N. Hand)

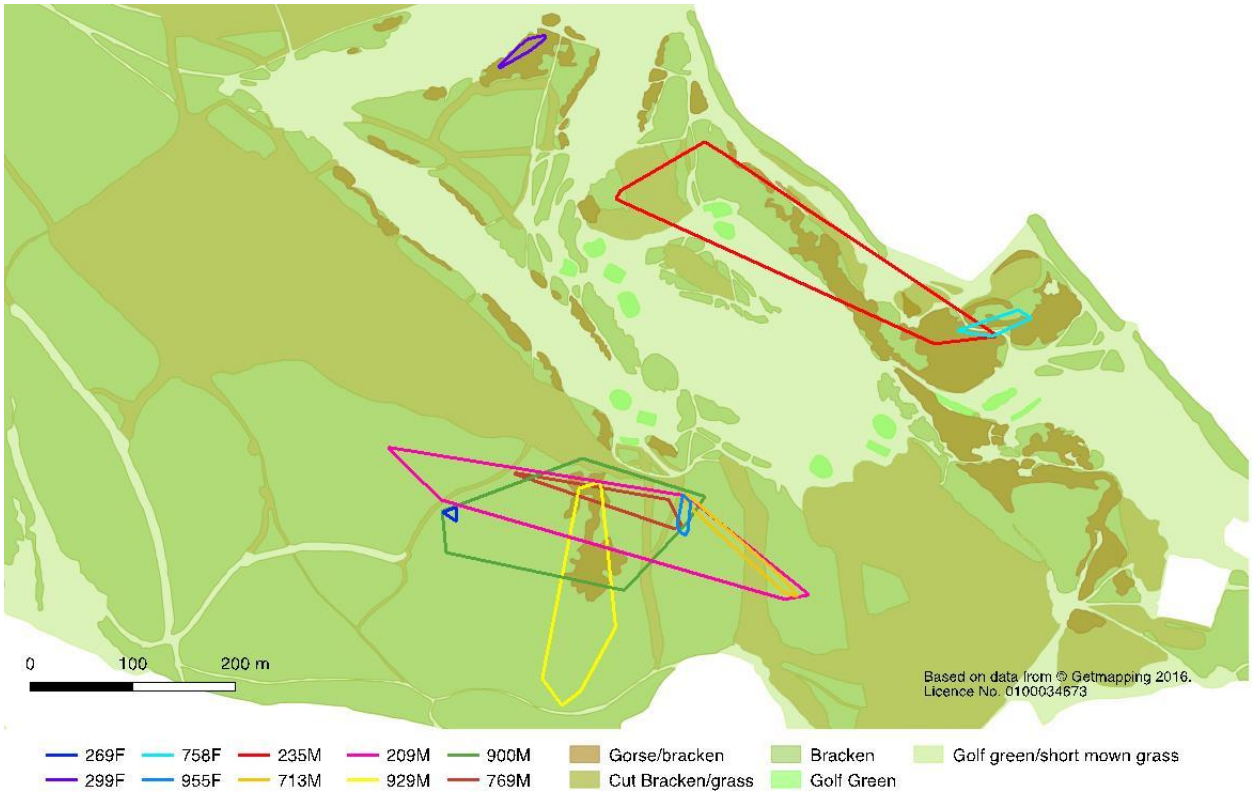

Fig. 5. Home ranges of ten adders (*Vipera berus*) followed by telemetry tags at a Herefordshire golf course and common, from April to early June 2017. Males moved large distances searching for mates with activity centered on the location of females. Male movements slowed from the end of May and into June, when they exhibited fossorial behaviour as mating declined and feeding commenced. Within the larger group of snakes, male 900M (length 560 mm) was dominant. Originally he was located near female 269F (450 mm), but this female was suspected as non-breeding in 2017 and 900M moved to female 955F where three other tagged males were already gathered, as well as two non-tagged males. On arrival the other males moved away, including the previously dominant male 209M (535 mm). Male 235M (500 mm) travelled the furthest distance of 893 m. Male 209M travelled 743 m and male 900M 642 m. Male 929M (435 mm) descended the slope and did not compete over females, feeding in lower parts of the site. Females remained in small home ranges. The longest female movement of 109 m was by 758F (570 mm); this female was thought to have been taken by a common buzzard (*Buteo buteo*). The distribution of snakes at this site reflects habitat management, with the larger group of snakes able to move through extensive undisturbed suitable habitat on the common. In contrast snakes on the golf course were within fragmented and disturbed habitat where large areas of bracken were cut providing areas of short grassland for sheep grazing. Such wide expanses of short vegetation result in isolating adders. Thus female 299F (495 mm) appeared to be isolated from other snakes. Following these observations habitat management at the site is attempting to address connectivity problems. Similar connectivity issues have been noted on other telemetry sites and likely to be the case at other U.K. adder sites.

Short grassland thus acted as a barrier to adder movement. One male was tracked from a Corsican pine (*Pinus nigra*) plantation hibernacula, through an old fruit orchard, to an unimproved rough grassland meadow with abundant field vole (*Microtus agrestis*) burrows and runs. This snake spent much of the remaining tracking time, from May to June, moving within vole runs. Other males were tracked moving from lowland heath, through woodland of mature oak (*Quercus* spp.) and into woodland glade edge habitat, where a small group of females and juveniles was located. In 2018 a large adult male (length 600 mm) moved from a small population of fewer than ten snakes, to a much larger population, travelling through deciduous woodland, ascending a steep 150 m high sandstone escarpment, and onto lowland heath, a distance of nearly 700 m. It is possible that this male had originated from the larger population, and so this may have been a return visit.

A few snakes and their tags were lost during tracking. There have been two confirmed predations. A female adder from a hillside summit with its tag tracked and retrieved beneath an oak tree in a deciduous coppice 800 m away from the last recorded location. An active common buzzard (*Buteo buteo*) nest was noted in the tree above. It was concluded that the snake had been caught on the hillside by a buzzard and fed to its chicks in the nest.

CONCLUSIONS

Telemetry mapped movements

From April to June observations suggest that males move further and have greater home ranges than females. Males during this period moved an average distance of 570 m: these movements are typically males searching for mates. The longest recorded distance from capture to final recorded location was 2,207 m was by a melanistic male. However, not all males roamed widely. In contrast, females remained

within small areas usually near hibernacula with some remaining beneath a single gorse (*Ulex* spp.) or bramble (*Rubus fruticosus* agg.) patch.

Male adders possibly develop familiarity with landscapes learnt through mate searching over successive years, when they roam over wide areas and habitat types, finally returning to the original location areas. These observations have implications for habitat management or land change at adder sites. Furthermore, they suggest that translocation projects, moving snakes to new areas, could be disorientating to some snakes.

Mature deciduous woodlands are not necessarily a barrier to dispersal as snakes can move through them. Thus the recent conservation policy to create and manage woodlands as open wooded pasture may suit adders, particularly when these areas are close to existing populations, or form a corridor between areas of open habitat.

Snake breeding and combat does not necessarily occur in the same areas each year, but is dependent on the location of breeding females.

Adders were observed to cross footpaths, unmade roads and off-road tracks on all sites, sometimes repeatedly crossing the same tracks. Males searching for mates crossed mown tracks up to 30 m wide, but were never witnessed crossing tarmac roads. At one site, popular with dog walkers, two populations of adders were divided by a tarmac road. Here the snakes moved close to the road edges on both roadsides, but no snakes crossed the road during the tracking period.

Males are fossorial after the breeding period, from April to July, spending much time undercover or underground. This may be important for humidity, foraging, protection from predators, escape from inclement weather and overnight refuge. Snakes were regularly noted with prey bulges when found below vegetation or refuge piles. This observation has important implications for the timing of adder location and capture during surveys or translocation projects. This period of the year will be the least effective for observational survey or capture of snakes, particularly males. Such fossorial activity also has implications for site management. Cutting and scarifying bracken, gorse or rough grassland areas could lead to snake mortalities, compacted soil structure, loss of insulating thatch and damp humus, and a reduction in prey species.

Between May and June snakes move from spring habitats of open heath or hillside slopes to semi-shaded woodland edges, stands of trees, such as silver birch (*Betula pendula*) or within thicker gorse blocks. The ambient temperatures during this period of the year remain stable, allowing snakes to frequent such areas.

Snakes were noted regularly within woody brash piles created by habitat management work parties. On one site a number of woody piles had mossed over providing ideal snake cover, along with rodent runs and burrows.

Whilst tracking adders a diverse range of animal species were also found in bracken. These included:

1. Mammals, such as nesting dormice (*Muscardinus avellanarius*), nests of harvest mice (*Micromys minutus*), common shrews (*Sorex araneus*) and pygmy shrews (*Sorex minutus*). Field vole runs were also found in bracken humus.
2. Amphibians, such as toads (*Bufo bufo),* great crested newts (*Triturus cristatus*) and common frogs (*Rana temporaria*).
3. Birds, such as meadow pipits (*Anthus pratensis*), roosting short-eared owls (*Asio flammeus*), common snipes (*Gallinago gallinago*) and woodcocks (*Scolopax rusticola*).
4. Butterflies, such as the pearl-bordered fritillary (*Boloria euphrosyne*).

Implications for habitat management for adders

Michael *et al.* (2014) state that "although habitat management can be beneficial, herpetofaunal diversity may still be restricted by the presence of dispersal barriers. Dispersal barriers are not always as simplistic as the presence of a linear feature such as a road and can be difficult to detect by land managers who are not sufficiently experienced with the ecology of target species." Based on this statement and my telemetry work I suggest the following:

1. Greater awareness is needed of the significance of habitat corridors to protect adder populations. This requires avoiding excessively mowed wide expanses resulting in the isolation of snakes. Banks and bunds running across sites may form important linkage corridors.
2. Snakes can move through areas that may not be considered typical habitats, such as travelling between sites through woodland. Outlying areas may also be used, particularly if there is an abundance of prey.
3. Greater awareness is required of the importance of bracken and rough grassland for snakes. Adders may be present in an area but out of site underground particularly from April to July. This observation has important implications for the timing of adder surveys or translocation projects.

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Great crested newt (*Triturus cristatus*) populations are not one and the same: Scottish newts respond differently to Habitat Suitability Index (HSI) and abiotic factors

L.R. Harper[1,2], J.R. Downie[2] & D.C. McNeill[2]

[1]Biology Department, School of Environmental Sciences, University of Hull, Kingston upon Hull HU6 7RX
[2]School of Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ

E-mail: lynsey.harper2@gmail.com

BACKGROUND

McNeill *et al*. (2012) described the translocation of the great crested newt (*Triturus cristatus*) population at Gartcosh, North Lanarkshire, which is thought to be the largest Scottish population of this European-protected species. The translocation was phased over three years (2004-2006) from the original breeding site to a specially created nature reserve nearby, comprising four clusters of new ponds. In addition to over 1,000 adult great crested newts, the translocation involved thousands of common frogs (*Rana temporaria*), common toads (*Bufo bufo*), palmate newts (*Lissotriton helveticus*), and smooth newts (*L. vulgaris*). Harper *et al.* (2017) followed with annual monitoring results for the great crested newt population during the first ten years post-translocation. Using the standard torchlight survey method to generate peak adult counts, the great crested newt population appeared to have grown during the 10-year period, but some pond clusters had been more successful than others.

Despite the apparent success of the translocation on the whole, Harper *et al.* (2017) concluded that much needs to be learned about the overall habitat requirements of great crested newts in Scotland. For instance, since the new Gartcosh ponds were constructed according to best practice guidelines for habitat management (English Nature, 2001; Langton *et al*., 2001), why had some been more successful than others?

Most research on great crested newt habitat requirements has been carried out in England and in continental Europe. Scottish great crested newt populations are mostly small and scattered in the central belt, Dumfries and Galloway, the Borders, and a cluster of sites in the Inverness area (McInerny & Minting, 2016). Use of environmental DNA (eDNA) analysis to identify new sites has had some successes, but within areas that have existing distribution records (Minting, 2018). A widely used method for assessing potential suitability of ponds for great crested newts is the Habitat Suitability Index (HSI) devised by Oldham *et al.* (2000), but only one study has evaluated its usefulness in Scotland (O'Brien *et al*., 2017). Given the climatic differences between the Scottish, English, and continental ranges of the great crested newt, Scottish populations may exhibit local adaptations that are incompatible with conservation management and monitoring criteria derived from other areas. As an example, Paterson (2018) found that great crested newts at Gartcosh were active earlier in the year and at significantly lower temperatures than expected in England. Therefore, surveys of this Scottish population using recommended methodology (Langton *et al*., 2001) would have generated unreliable data.

RECENT RESULTS

Here, we provide a brief summary of HSI and abiotic data from Gartcosh. The detailed results are published elsewhere (Harper *et al.,* 2018). HSI scores for the Gartcosh ponds were largely consistent between 2006 and 2015, but some individual ponds had improved whereas others had deteriorated. In contrast to Oldham *et al*. (2000), we did not find a significant relationship between HSI scores and great crested newt peak or average adult counts. This would support abundance as a poor indicator of habitat quality (Unglaub *et al*., 2015) and the use of an adapted HSI for Scottish great crested newts (O'Brien *et al*., 2017). Consistent with studies on English or European great crested newt populations, we identified a positive correlation between pH and adult counts (Skei *et al*., 2006; Gustafson *et al*., 2009). Echoing Paterson (2018), we also found great crested newts were active at lower air temperatures than expected by current guidelines (English Nature, 2001; Langton *et al*., 2001). Furthermore, we uncovered a positive influence of moon visibility in combination with air temperature and moon phase on adult counts. However, further study of lunar periodicity in great crested newts in relation to breeding activity and reproduction is needed to understand this effect (Grant *et al*., 2012).

CONCLUSIONS

Our study provides evidence for conservation management of great crested newt populations according to geographic location. Blanket monitoring guidelines may not be applicable to all populations and produce misleading data on temporal trends. We advocate fresh consideration of survey and pond suitability criteria for great crested newts in Scotland. Specifically, air temperature at which surveys can be performed (currently 5°C) should be lowered to 3-4°C (Paterson, 2018; Harper *et al.,* 2018), and moon visibility and phase during survey recorded. Ultimately, the most appropriate temperature for survey must be determined by modelling of detection probability using data generated by multi-method surveys over several consecutive weeks in the breeding season. Further pond creation and management is required to improve habitat suitability for the great crested newts at Gartcosh. Continued monitoring of this population is also necessary to confirm the nature of the effects reported here, and post-translocation status.

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SuDS and amphibians - are constructed wetlands really benefitting nature and people?

D. O'Brien[1], J. Hall[1], A. Miró[2], M. Rae[3] & R. Jehle[4]

[1]Highland Biological Recording Group, c/o 7 Woodside of Culloden, Inverness IV2 5BP
[2]Centre for Advanced Studies of Blanes, Spanish Research Council (CEAB-CSIC), Accés a la Cala St. Francesc, 14, Blanes, 17300, Girona, Catalonia, Spain
[3]Highland Council, Glenurquhart Road, Inverness IV3 5NX
[4]University of Salford, School of Environment and Life Sciences, Manchester M5 4WT

E-mail: David.OBrien@nature.scot

ABSTRACT
While urbanisation is a major threat to global biodiversity, it also brings opportunities for some species. Sustainable Drainage Systems (SuDS) have been installed in all Scottish cities to reduce flood and pollution risk and they can also offer new habitats for wildlife. We studied SuDS in Inverness and the Scottish Central Belt to assess their value as amphibian breeding sites, habitats, and as places where urban people can experience nature. The nine-year study revealed that many SuDS were of similar ecological quality to wider countryside ponds but that the quality of ponds is not equitably distributed between neighbourhoods inhabited by different socio-economic classes. However, the findings suggest ways to improve the design and management of SuDS for people and nature, making access to high quality ponds available to all social groups.

INTRODUCTION
Urban expansion is a major threat to biodiversity (Beninde *et al.*, 2015). Expanding cities take away land from biodiversity, perturb "natural processes" such as flooding and nutrient cycling, and fragment habitats. Urban citizens are less likely to experience nature and the health and well-being effects it provides. The alienation of young people from nature has been termed "nature-deficit disorder" (Louv, 2005), and has been linked to physiological, emotional and social problems. However, when sympathetically managed, urban green (parks and gardens) and blue spaces (coast, ponds, lochs, canals and rivers) can provide valuable wildlife habitats (Hill *et al.*, 2016; Aronson *et al.*, 2017) especially when the surrounding countryside has been degraded by intensive agriculture (Deutschewitz *et al.*, 2003; Colding & Folke, 2009).

Cities can provide novel habitats for species. For example, two bryophytes, common liverwort (*Marchantia polymorpha*) and silver moss (*Bryum argentium*), thrive in pavement cracks (Atherton *et al.*, 2010), cliff-nesting birds such as peregrines (*Falco peregrinus*) nest on buildings, and scavenging species like foxes (*Vulpes vulpes*) and gulls (*Larus* spp.) exploit human rubbish. Some groups such as pollinators and passerine birds appear to be very successful in urban gardens.

However, not all species have benefitted from urbanisation. Amphibians are amongst the most threatened taxa. These threats come from climate change, introduced species, pathogens spread by humans, over-exploitation, chemical pollution, habitat loss, as well as urbanisation. Even so, some species, like common frog (*Rana temporaria*) have been quick to colonise urban gardens, often with the help of home-owners (Beebee & Griffiths, 2000). There is evidence that urban common frogs and common toads (*Bufo bufo*) have lower genetic diversity than their rural counterparts, perhaps because of population isolation (Hitchings & Beebee, 1997, 1998).

An increasing form of green and blue space in cities is SuDS. Whilst their primary purpose is water management, we postulated that SuDS ponds might also bring benefits to biodiversity. This could be through their role as breeding sites (Jones & Fermor, 2001), habitats for a range of species (Woods-Ballard *et al.*, 2015), and connecting otherwise isolated populations to form metapopulations as part of a habitat network. Furthermore they could bring urban-dwelling humans into contact with nature, with consequent health and well-being benefits (Hill *et al.,* 2016; O'Brien *et al.*, 2015; Parris, 2016; Woods-Ballard *et al.,* 2015). Amphibians were chosen as a model taxon as they are relatively easy to find and survey, disperse over a comparatively small area, and are sensitive to pollution. The study areas include Inverness, one of the fastest growing settlements in Europe since 2000, and the Scottish Central Belt, an area with a long history of development for housing and industry.

METHODS

We carried out amphibian breeding surveys initially in 12 ponds in Inverness during 2010-2013 (O'Brien, 2015), and then in all 40 Inverness SuDS during 2014-2017 (Miró *et al.*, 2018) following the National Amphibian and Reptile Recording Scheme protocol (ARG-UK, 2013). We also carried out freshwater invertebrate surveys in the 40 SuDS following the OPAL protocol (Davies *et al.*, 2011) in 2014. In 2017 we extended the project to 38 SuDS ponds in Central Scotland, surveying for invertebrates and amphibians (Rae *et al.*, 2019).

For all 78 ponds, we carried out detailed habitat assessments following the protocols developed by one of the authors (AM), initially for use in the Pyrenees but modified for use in Scotland (Miró *et al.*, 2017), including assessing ten designable or manageable characteristics previously linked to SuDS ecological quality (O'Brien, 2015; Woods-Ballard *et al.*, 2015).

To assess equality of access to these ponds, we used data from the most recent Scottish census in 2011 (National Records of Scotland, 2016) to assess the comparative population economic wealth surrounding SuDS, and considered the relationship between these data and the findings on the ecological quality of neighbouring SuDS. We restricted this part of the study to Inverness to remove the influence of other socio-economic impacts such as demographic history. Detailed methods can be found in O'Brien (2015) and Miró *et al.* (2018).

RESULTS

Do amphibians breed in SuDS ponds?

Over the nine-year study, we found that common local native amphibians, common frog, common toad and palmate newt (*Lissotriton helveticus*) bred successfully in at least 21 out of 23 SuDS ponds. When we extended the study to the Scottish Central Belt, we found evidence of breeding by two additional native newt species, the smooth newt (*L. vulgaris*) and the European protected great crested newt (*Triturus cristatus*), as well as the introduced Alpine newt (*Ichthyosaura alpestris*). Thus all of Scotland's native and introduced amphibians, with the exception of the natterjack toad (*Bufo/Epidalea calamita*), were shown to breed in SuDS.

Do SuDS offer good habitats?

Five biotic and abiotic ecosystem components were highly correlated and accurately described SuDS ecological quality: amphibian richness, macro-invertebrate richness, macrophyte richness, terrestrial habitat richness and urbanisation (Miró *et al.*, 2018). Chemical analysis (pH, ammonia, nitrate, nitrite, phosphate and chloride ions) showed that none of the ponds contained pollutants at levels known to have adverse effects on amphibians. Nutrient levels in Inverness SuDS are lower than those found in a previously published sample of lowland British ponds: six of the 12 SuDS ponds had NO_3 concentrations <0.5 mg l^{-1} N and phosphate <0.05 mg l^{-1} P (i.e. below levels which would normally be considered eutrophic) (O'Brien, 2015). This was reflected by the diversity of invertebrate taxa found, which included groups that are characteristic of unpolluted water such as Odonata, Ephemeroptera and Trichoptera (Rae *et al.*, 2019). Ecological quality of many ponds was comparable with wider countryside ponds, though some showed a legacy of nearby industrial contamination (e.g. Glenrothes, Fife).

Can SuDS ponds bring urban-dwellers into contact with nature?

While public events held as part of the project showed that SuDS can bring local people into contact with nature, the wide variation in ecological quality means this potential benefit is not equitably available to all those who live near SuDS (Miró *et al.*, 2018). Furthermore, these findings suggest that those from the poorest social backgrounds are more likely to live near SuDS of poor ecological quality (Miró *et al.*, 2018).

DISCUSSION

These findings support the hypothesis that SuDS ponds might bring benefits to biodiversity and people: as breeding sites; by connecting otherwise isolated populations to form metapopulations as part of a habitat network; and by bringing urban-dwellers into contact with nature, with consequent health and well-being benefits (Fig. 1).

Fig. 1. SuDS swale with high macrophyte diversity adjacent to a green path, with cycling and pedestrian access at the back left of the image. Note the lack of barriers, other than where the path is immediately next to the SuDS. This site is used by local families for recreation and holds large breeding populations of common frogs (*Rana temporaria*) and palmate newts (*Lissotriton helveticus*). (Photo: D. O'Brien)

The frequency with which we found metamorphosed amphibians at SuDS suggests that they are most likely acting as source, rather than sink populations, though we have not undertaken the detailed population studies required to confirm this.

As part of the project, we carried out a public event at one of the ponds, which attracted over 100 people (Rae *et al.*, 2019). Most of these were local families with children, suggesting that when made aware of the value of SuDS, people are genuinely enthusiastic. For children, the opportunity to grow up in contact with nature has also been linked to improved mental health and educational outcomes (Bingley & Milligan, 2004). However, we found that these benefits are not equitably distributed: poorer neighbourhoods have SuDS of lower ecological quality, though this can be improved by design and management (Miró *et al.*, 2018). Ponds offer greater diversity of wildlife than other types of SuDS, such as swales and detention basins.

How can we use these findings?

In both a fast-growing city and in long-established towns and cities, SuDS are generating multiple benefits for amphibians and humans, whilst also serving an important role in the drainage system. These could be further increased through improved design and management (Fig. 2). We are now extending these findings to some of the most deprived urban areas of Europe, through links with the Green Infrastructure Strategic Intervention managed by Scottish Natural Heritage and part of the 2014-2020 European Regional Development Fund (ERDF) programme.

Fig. 2. Influence of the mowing regime on macrophytes at SuDS. Note the large area of cover plants in the right image compared with the left image where mowing has occurred. Such habitat supports grasshopper warblers (*Locustella naevia*) and water voles (*Arvicola amphibius*), as well as foraging habitat for common frogs (*Rana temporaria*), common toads (*Bufo bufo*) and palmate newts (*Lissotriton helveticus*). (Photos: D. O'Brien)

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Mitigating the effects of a road on amphibian migrations: a Scottish case study of road tunnels

I.D.C. Hill[1], C.A. Rossi[1], S.O. Petrovan[2,3], M. Hartup[2], F. Clark[2] & J.R. Downie[1]

[1] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ
[2] Froglife, 1 Loxley, Werrington, Peterborough PE4 5BW
[3] Conservation Science Group, Department of Zoology, The David Attenborough Building, University of Cambridge, Cambridge CB3 3QZ

E-mail: roger.downie@glasgow.ac.uk

ABSTRACT

The ever-growing pace of road construction worldwide has become a serious concern for wildlife and natural habitats, resulting in habitat fragmentation and increasing wildlife road fatalities. For amphibians, which are undergoing population declines worldwide, mitigation measures such as road under-passages linked to amphibian-proof fencing may be an effective conservation tool, aiming to reconnect natural habitats and reduce wildlife fatalities. This study assessed the efficacy of road tunnels in a recently developed area by Frankfield Loch, Stepps, North Lanarkshire. Three amphibian tunnels, plus fencing, were put in place during 2010 following the construction of a road in 2006 which separated the loch from a substantial area of marshland, including ponds. In 2015 and 2016, we used custom-made time-lapse cameras within the tunnels to automatically monitor amphibian movements and conducted frog spawn surveys. Numbers of common frogs (*Rana temporaria*), common toads (*Bufo bufo*) and newts (*Lissotriton* spp.) using the tunnels were substantial in both years, though the number of toads decreased significantly between years. We found many frog spawn clumps in the marsh ponds, but rather fewer in the loch. A period of road repair in 2015 was linked with both road mortalities and a change in the pattern of tunnel usage. Additionally, amphibians showed a daily cycle of activity, with nocturnal movements most common. These results indicate important connectivity and usage of both the marsh and the loch, and suggest that this can be effectively sustained through the proper maintenance of tunnels and fencing, which also minimises road mortalities. It remains unknown, however, what proportion of the population crosses the road via the tunnels and how that compares with movements prior to road construction.

INTRODUCTION

Global amphibian declines are increasingly recognised (Nystrom *et al.*, 2007), with over 30% of species listed as threatened including 8.1% critically endangered (IUCN, 2015). These declines have been attributed, in part, to climate change (Kiesecker *et al.*, 2001), pathogens (Pounds *et al.*, 2006), and direct impact of pollution and habitat fragmentation through urbanisation (Foley *et al.*, 2005). The mobile nature of amphibian species, combined with the majority requiring both aquatic and terrestrial habitats (Richter *et al.,* 2001), and associated breeding site fidelity (Santos *et al.*, 2007), make them particularly vulnerable to anthropogenic disruption. This has led to a need to better understand the mechanisms underpinning amphibian declines (Beebee & Griffiths, 2005), and to limit negative impacts through a variety of mitigation measures (Petranka *et al.*, 2007).

The anthropogenic influences that lead to habitat fragmentation, particularly the increasing number of roads constructed to connect developed areas, result in the isolation of wildlife habitats pocketed between such areas (Cushman, 2006). Great Britain had a combined road length of 395.7K km in 2015, an increase of 9.3K km since 1995 (GOV.UK, 2016). Additionally, with over 64 million kilometres of roads recorded worldwide (Van der Ree *et al.,* 2015), it is not surprising that road systems often cut directly across wildlife migration routes and can result in significant wildlife mortality (Roos *et al.*, 2012), with amphibians particularly susceptible (Gryz & Krauze, 2008). In response to these problems, a wide range of mitigation measures has been employed to prevent animal road deaths, such as warning signs, fences and assisted frog crossings (Al-ghamdi & Algadhi, 2004; Van der Ree *et al.*, 2015). Fencing along motorways and other roads can reduce wildlife-vehicle collisions and is often used to keep large mammals off the roads, but is ineffective if not managed properly and can also make population fragmentation worse (Jaeger & Fahrig, 2004). An increasingly utilised solution is the provision of wildlife passages, allowing animals to cross roads without encountering traffic, which have been successfully applied to mammals (Sawaya *et al.*, 2014) and amphibians in the form of road crossing

tunnels combined with amphibian-proof fences to channel animals towards the tunnel openings (Hamer *et al.*, 2015; Matos *et al.*, 2017). However, varying degrees of successful implementation indicate that the specifications of such mitigations often depend on the species and tunnel design and that more data are required in order to assess their short and long-term effects (Beebee, 2013; Smith *et al.,* 2018).

Frankfield Loch lies on the north-east edge of Glasgow and is one of many "kettle ponds" that were formed in Scotland by glacial retreat after the last Ice Age. It is a component of the Seven Lochs Wetland Park being developed on 16 km^2 of terrestrial and freshwater habitat between Glasgow and Coatbridge (www.sevenlochs.org). Woodland and reed beds extend around most of the circumference of the loch, with a large area of marshland and woodland patches to the northeast. This makes the site excellent wildlife habitat, supporting a wide range of species, including four species of amphibian: common frog, common toad, smooth newt (*Lissotriton vulgaris*) and palmate newt (*L. helveticus*). These species and their migration paths were identified in preliminary surveys, carried out in 2002 and 2006 (Heritage Environmental, 2006). A housing development has been constructed to the south east of the site and a road was built (2006) connecting the houses to Cumbernauld Road in the north, separating the loch from the marsh and cutting through the likely migration paths of the four amphibian species.

In response to concerns for the wildlife on the site, four road crossing tunnels, three primarily for amphibians and one for water voles (*Arvicola amphibius*), were built under the road and combined with the installation of barrier fencing, to assist any amphibians and other small animals migrating between the loch and marsh. However, no monitoring of the site or the tunnels had taken place since their installation in 2010. Once housing development has been completed, it is planned that the loch and the undeveloped land will be passed over to North Lanarkshire Council Greenspace Development Services and designated as a Local Nature Reserve.

This study used cameras installed in the amphibian tunnels in 2015 and 2016 to collect data on the animals using the tunnels, with an aim to clarify factors that affect amphibian movements between the marsh and loch sides of the road. The application of modern automatic monitoring techniques allowed for the continuous recording of wildlife activity in the tunnels, without the presence of human beings who could act as a predation risk stimulus (Frid & Dill, 2002). We aimed to assess: 1) the effectiveness of the tunnels and fences in reducing amphibian road mortalities; 2) the overall patterns of usage of the tunnels during the amphibian breeding season; 3) the relative population size of the amphibian species in terms of tunnel usage and locations.

MATERIALS AND METHODS

Site visits

An initial site visit was conducted on 3rd February 2015 with North Lanarkshire Council, University of Glasgow and Froglife all represented, to examine the condition of the site and tunnels (Figs. 1, 2). The tunnels had been installed in 2010 when Loch Road went through its final phase of construction. Amphibian fencing (Herpetosure, Scalford, U.K.) had been installed approximately one year prior to the first visit and was intended to channel the migrating amphibians towards the tunnels and to prevent them crossing the tarmac road. The three amphibian climate tunnels (ACO, Germany) are spaced along 100 m of road, about 50 m apart with the fencing on both sides extending between 56 m and 105 m beyond the tunnels at either end. Each tunnel has a dome-shaped entrance 30 cm high and 50 cm wide, guarded by a metal grid, with fencing arranged to channel animals towards the tunnel entrances. The solid grey, recycled polypropylene, fencing projects about 47 cm vertically above the ground and has a bevelled top section projecting towards the marsh or loch. Each tunnel is about 13.4 m long and has a series of 6 cm x 3 cm ventilation holes running across the road, allowing air, rainwater and light into the tunnels. Tunnel design and lay-out are similar to the European guidelines summarised by Hamer *et al.* (2015).

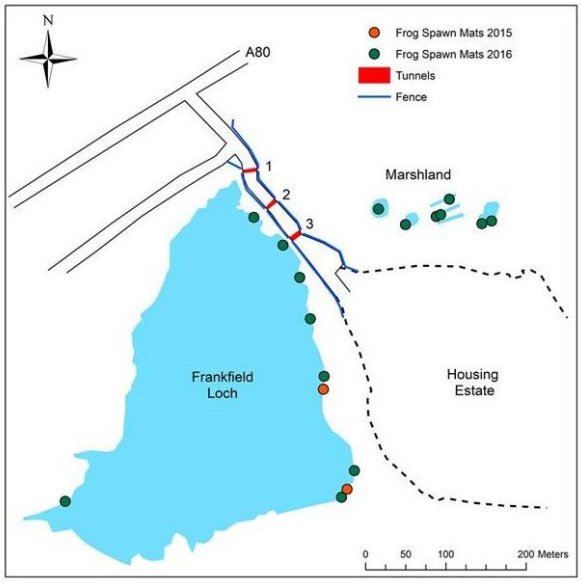


Fig. 1. Frankfield Loch, Stepps, North Lanarkshire and adjacent marshland, showing the positions of the amphibian tunnels (red lines), numbered 1-3. Amphibian fencing is shown as blue lines. Orange dots represent spawn mats identified in 2015, and green dots represent spawn mats identified in 2016, when a more extensive survey of the area was conducted.

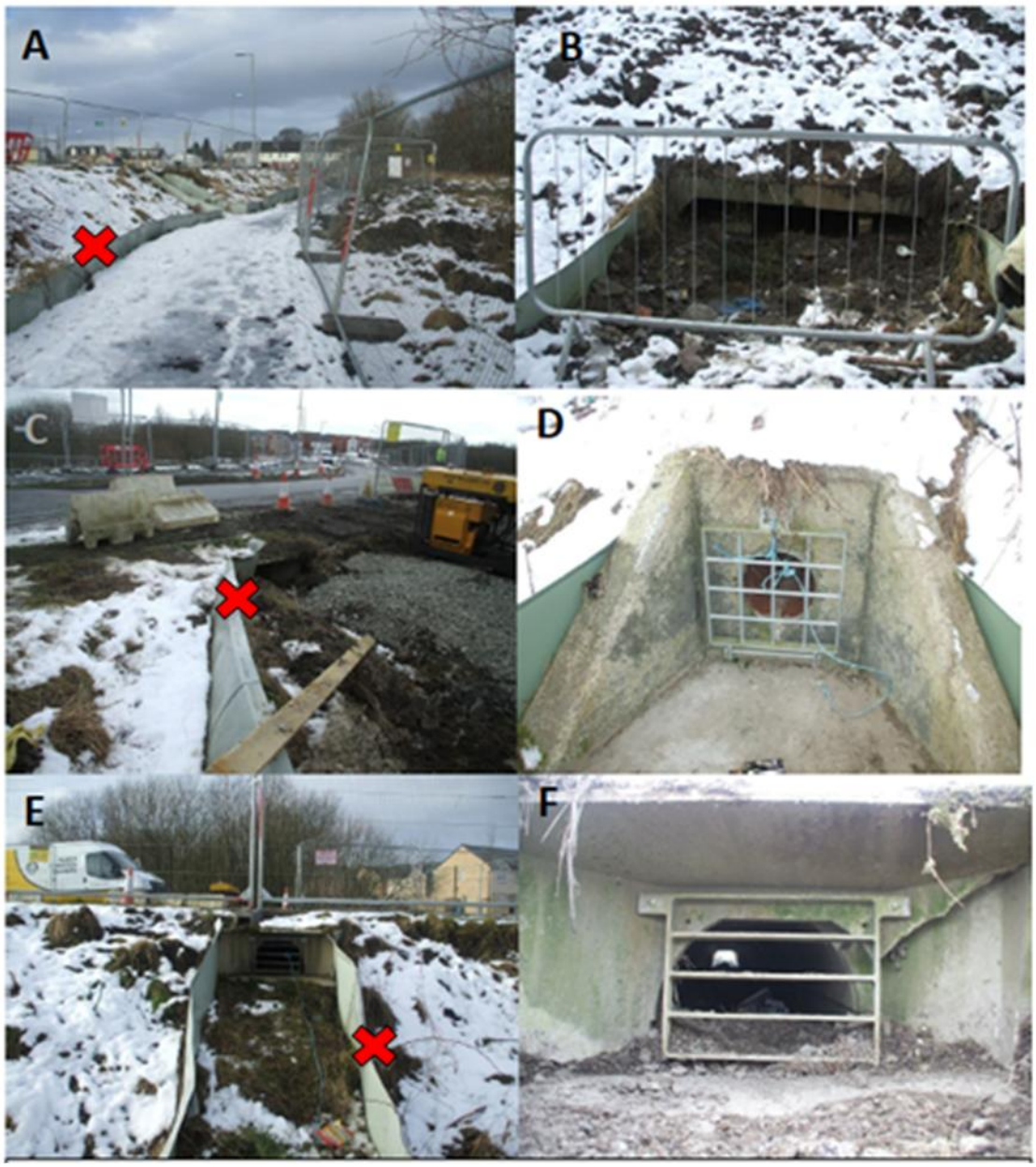


Fig. 2. During a site visit to Frankfield Loch, Stepps, North Lanarkshire on 3rd February 2015 inspection was made of the temporary tarmac footpath (A), the water vole (*Arvicola amphibius*) tunnel (B), the work being carried out at the loch side entrance to tunnel 1 (C), the water culverts (D) and the frog tunnel entrances on the marsh side of the Loch Road (E and F) which are 30 cm high and 50 cm wide. Herpetosure barriers are marked with a red X in (A), (C) and (E). (Photos: I. Hill)

In 2015, road works had recently led to the closing of part of the road and a temporary tarmac path had been laid parallel to Loch Road on the marsh side. This cut across the amphibian fencing, creating a gap to the north at the beginning of Loch Road and to the south, beside tunnel 3 (Fig. 2). The ventilation holes had also been tarmacked over and not reinstated until the summer. Concerns were raised about the site being in this condition during the migration period, but assurances were given that all road works were due to finish by 9th March 2015 as well as the tarmac path removed and amphibian fencing would be put back in place in time for the end of winter hibernation. Despite these intentions, a complication resulted in roadwork completion and fence reinstatement on 2nd April 2015.

There were no road repair issues at the initial site visit in February 2016, but it was noted that burning had damaged a section of the amphibian fencing, and arrangements were made for this to be repaired. On a repeat visit in May 2018, we found that the fencing was in good condition, and that a community group had recently cleared the area of litter.

Camera installation and servicing

Installation of the camera brackets took place on 18th March 2015. The entrance grids were removed in preparation and frames for the cameras were placed onto a converted carjack that was raised into position approximately one metre from the entrance of the tunnels, at the marshland side. To reduce the chance of theft of the cameras, a blacksmith altered

the tunnel entrance grids to allow access to the equipment before the grids were further secured into position by filling the boltholes for them with grip adhesive. This meant that the cameras could only be extracted with the use of an extendable pole that had the appropriate thread size to fit the attachment site at the rear of the camera/battery housing. The cameras were installed on 19th March 2015 and data recording began at 10:33 on that day and continued until 9th May 2015. The cameras pointed downwards, using a fish-eye lens to capture images every 10 s of the tunnel bottom, extending the full width of the tunnel and from the tunnel entrance 1.5 m back. Illumination was provided by low intensity infrared LEDs attached to the camera mounting. At the end of the first recording period, the cameras were removed and stored away until the next year's data collection, when the cameras were reinstalled and recorded from 8th March 2016 until 30th May 2016. Within both data collection periods, memory cards and batteries were replaced every four days.

A custom-made analysis programme was then run that isolated images that indicated animal movements. The software had been blind-tested by different researchers in a previous project, using a large volume of data, and the results indicated near perfect detection, with less than 0.5% differences between fully manual and automated analyses (Helldin *et al*., 2015).

Image analysis

The images selected post motion-detection analyses were analysed frame by frame (Fig. 3) to identify and record each individual as frog, toad, newt, mammal, invertebrate or other. In the rare instances when an amphibian was only partly visible and therefore indistinguishable between frog and toad, it was recorded as "unknown amphibian". The dorsal field of view did not allow us to identify to species level for small newt females and juveniles (only palmate and smooth newts are known to occur at the site, but their identification requires a clear view of underside markings). The direction of travel was recorded as "West" if the individuals were moving from the marshland to the loch (i.e. from the tunnel entrance to the inside of the tunnel) or, "East" if moving from the loch to the marshland (i.e. from the inside of the tunnel towards the entrance on the marshland side). If the individual turned around under the camera, this would be taken as a U-turn and considered as potential tunnel "rejection". During the analysis, different time periods from the three tunnels were mixed between observers to minimise observer bias.

Human disturbance

Data collection in 2015 was interrupted by road works and this period has been used to assess what effect human disturbance had on the use of the tunnels. This consisted of three time periods: "path" was the period in which the temporary tarmac path ran along the marsh side of Loch Road (mid-January to 30th March 2015); "work" was the two days (according to the workmen) when the temporary path was being broken up, the earth that had been cleared was placed back over this and the gaps in the amphibian fence were put back in place (31st March 2015 and 1st April 2015); "clear" was the period after this was completed (2nd April 2015 onwards**).**

Amphibian road mortalities

In both years, amphibian road deaths were investigated and counted during each visit (every four days, coincident with camera servicing). Only fresh carcasses were recorded in order to avoid double counting.

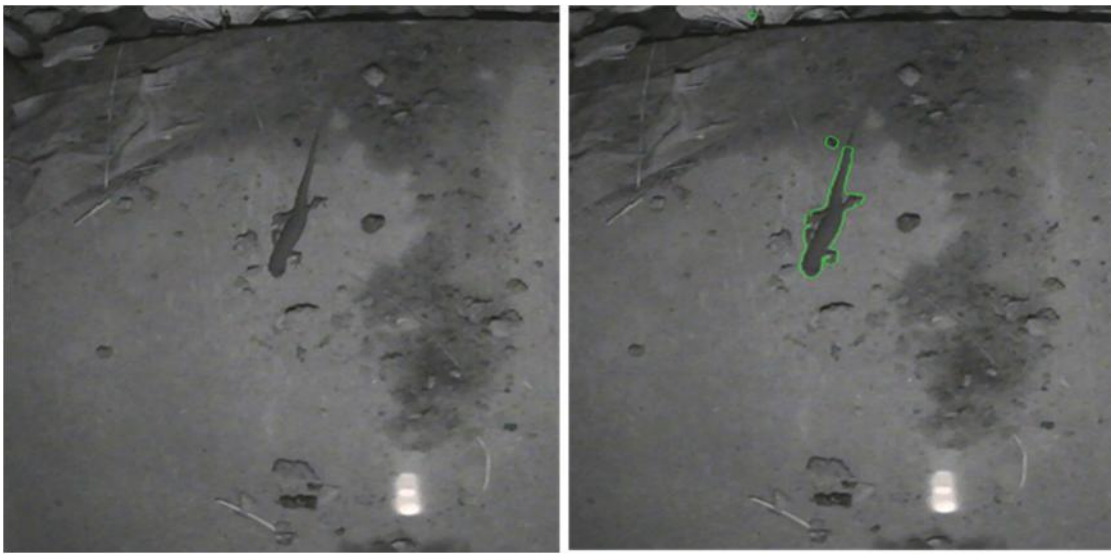


Fig. 3. Image analysis of a newt (*Lissotriton* spp.) passing through a tunnel at Frankfield Loch, Stepps, North Lanarkshire. When the newt moves the image software analysis outlines in green the contour area where pixel change was detected. (Photos: Froglife)

Frog spawning locations
In 2015, four complete circuits around the loch were conducted, searching as far into the reed beds as possible to locate frog spawning sites, on the following dates: 19th, 21st, 25th and 29th March 2015. In 2016, two complete circuits around and loch were conducted locating frog spawn sites. The marshland was also surveyed for frog spawn sites at each pond area (Fig. 1) on 24th March 2016 and 24th April 2016.

Temperature and light periods
Temperature data for Glasgow, and the sunset/sunrise times during the period of data recording were regularly taken from the website: http://www.timeanddate.com/weather/uk/glasgow/historic

Statistical analysis
General linear models (GLMs) were used to examine the differences in "successful" tunnel usage (movement "West" and "East" through tunnels combined) between years for each species group and total amphibian usage numbers. In addition to the standard packages provided in R 3.1.1 (http://www.R-project.org/), the ggplot2 R package was also used.

RESULTS
Species usage
In both years, newts comprised the majority of amphibian observations (Table 1) and frogs and newts had the widest range of dates recorded (Fig. 4), whereas toad activity had a narrower range of dates, indicating a more restricted migration period. "East" movements, particularly in 2015, were greater for frogs and toads than newts (Table 1). Additionally, a greater number of frogs were moving "East" rather than "West" during 2015. Overall, "West" movements (from the marshland towards the loch) were greater than "East" movements in both years. Though "successful" tunnel uses by frogs and newts increased marginally, "successful" uses by toads decreased significantly from 2015 to 2016 (Toad t_1=-4.207, p=<0.0001) (Fig. 5).

The proportions of immediately visible tunnel "rejections" were fairly consistent (8-9% of all amphibian observations) over the two years, though rejections by frogs were less frequent than by toads or newts in both years (Table 1).

Initial migration date cannot be determined from current data, as some activity was recorded on the first recording day during each year. However, numbers recorded in 2015 were very low until day 87 (28th March) and a similar increase in 2016 occurred 15 days earlier. There was some movement throughout the recording period, but numbers declined sharply after day 115 (25th April) in 2015 and day 105 (15th April) in 2016. Toad movements tended to cluster over a fairly narrow time period (days 87-105 in 2015; 82-104 in 2016), whereas movements of newts and frogs extended over a wider period.

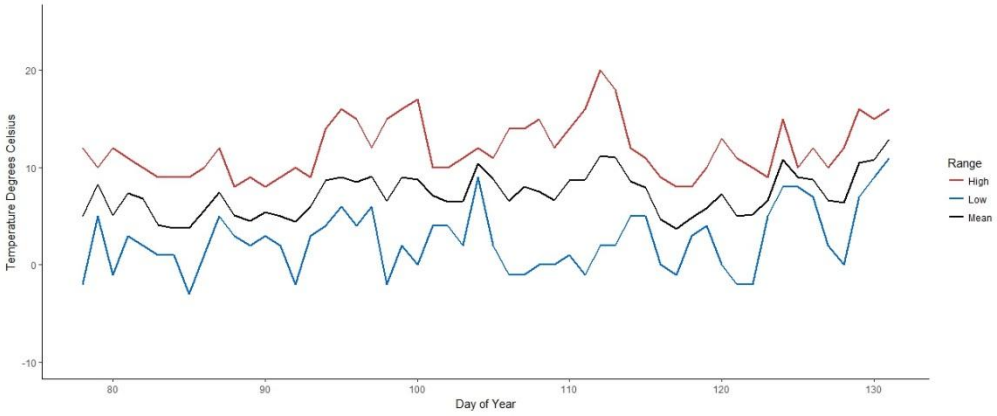
Though not included in analysis, instances of invertebrates, small mammals and birds were also recorded.

Tunnel usage and human disturbance
The patterns of tunnel use were strikingly different over the two years (Table 2; Fig. 6). In 2015, well over half of the "successful" uses occurred through tunnel 3, furthest from the roadworks. In 2016, tunnel use was much more even, with tunnel 2 recording the largest number of uses. Fig. 6 shows that, if we can consider 2016 as the "normal" pattern unaffected by roadworks, there were several migration "peaks" over an extended period, whereas in 2015, there was a single higher peak after the roadworks were completed. In the disturbed period, use of tunnel 1 was particularly low compared with 2016.

Year	**2015**			**2016**		
Species	**West**	**East**	**Rejection (%)**	**West**	**East**	**Rejection (%)**
Toads	371	311	77 (10.1)	219	176	33 (7.7)
Frogs	80	206	16 (5.3)	171	190	15 (4.0)
Newts	730	378	101 (8.4)	756	583	176 (11.6)
Total	1181	895	194	1146	949	224

Table 1. Total numbers of amphibians recorded using the tunnels, moving "West" towards the loch and "East" towards the marsh, or turning within the tunnels ("rejection"), at Frankfield Loch, Stepps, North Lanarkshire in 2015 and 2016. Toads (*Rana temporaria*), frogs (*Bufo bufo*), and "newts" includes palmate newt (*Lissotriton helveticus*) and smooth newt (*L. vulgaris*).

A



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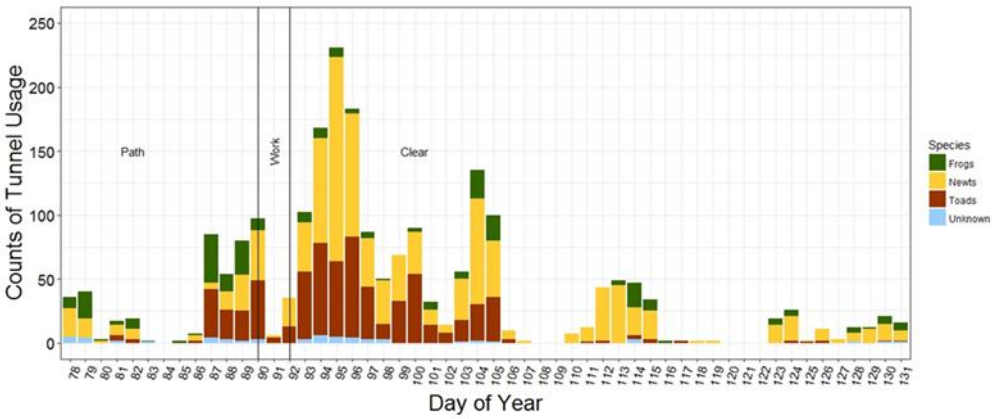

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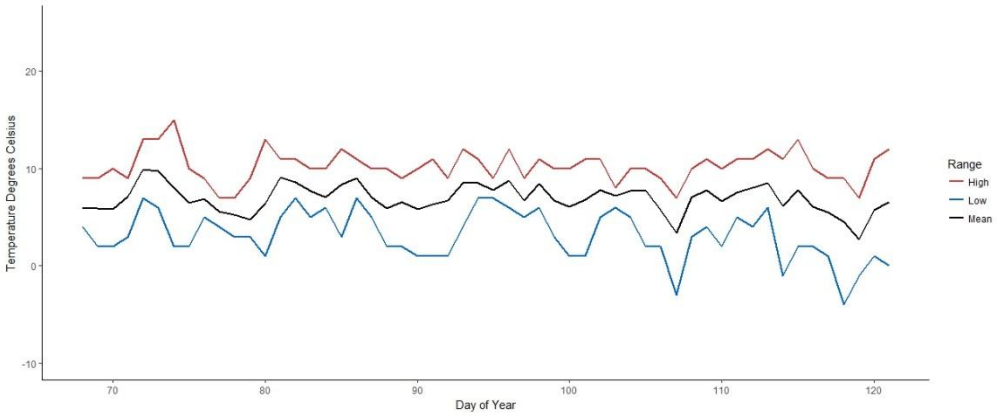

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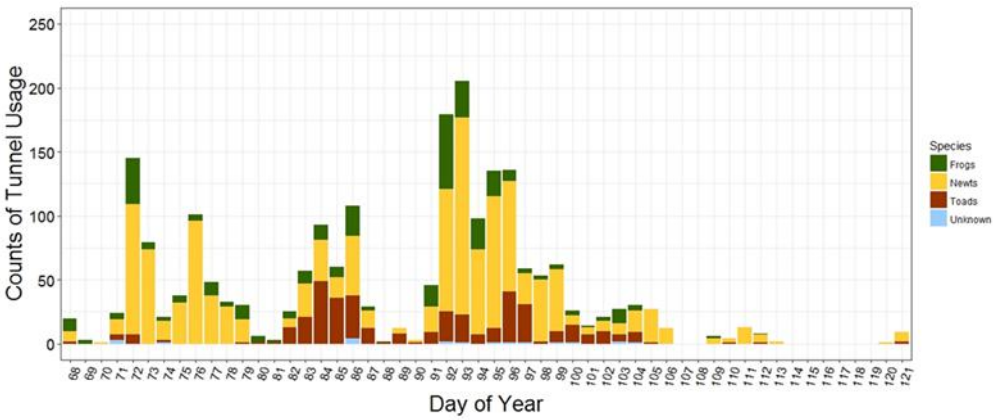


Fig. 4. Line plot of high, low and mean temperatures of the area of Frankfield Loch, Stepps, North Lanarkshire for 2015 (A) and 2016 (C). Successful tunnel uses ("West" and "East" uses summed) recorded for 2015 (B) and 2016 (D). Day of the year is given as a count from 1st January. Frogs (*Rana temporaria*), toads (*Bufo bufo*) and "newts" includes both palmate newt (*Lissotriton helveticus* and smooth newt (*L. vulgaris*). "Unknown" includes indistinguishable frogs and toads. Periods of roadworks during 2015 shown as "Path", "Work" and "Clear".

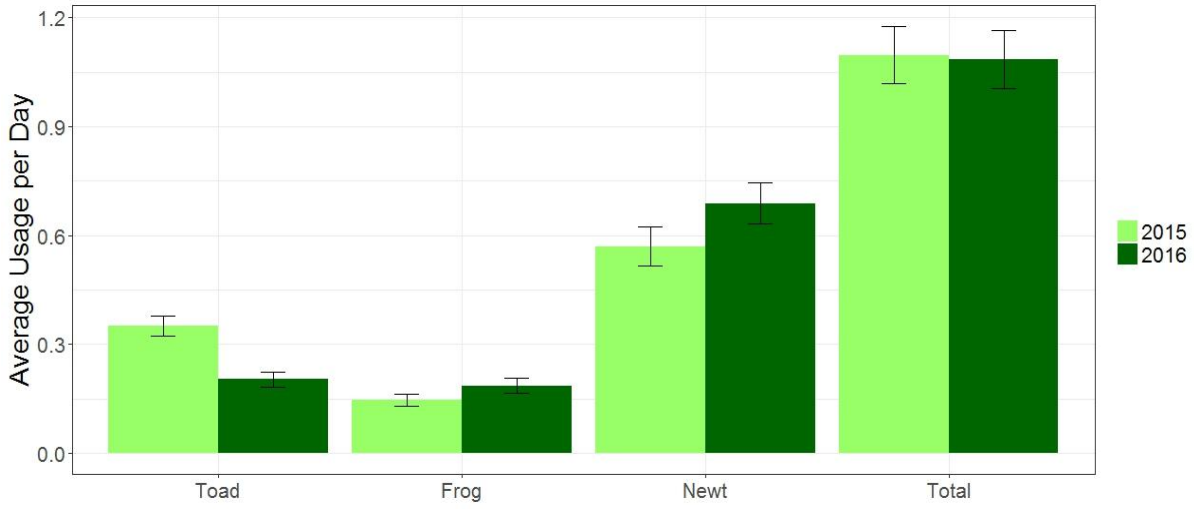
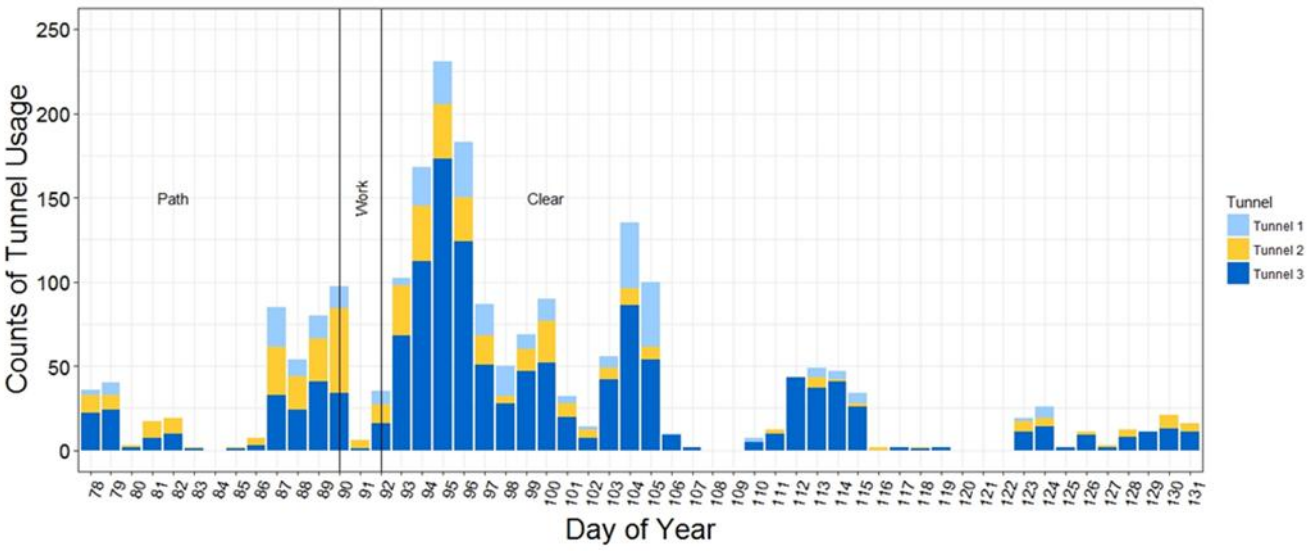


Fig. 5. Average (+/- SE) tunnel usage per day ("West" and "East" uses summed) by amphibians at Frankfield Loch, Stepps, North Lanarkshire from 2015 and 2016.

A



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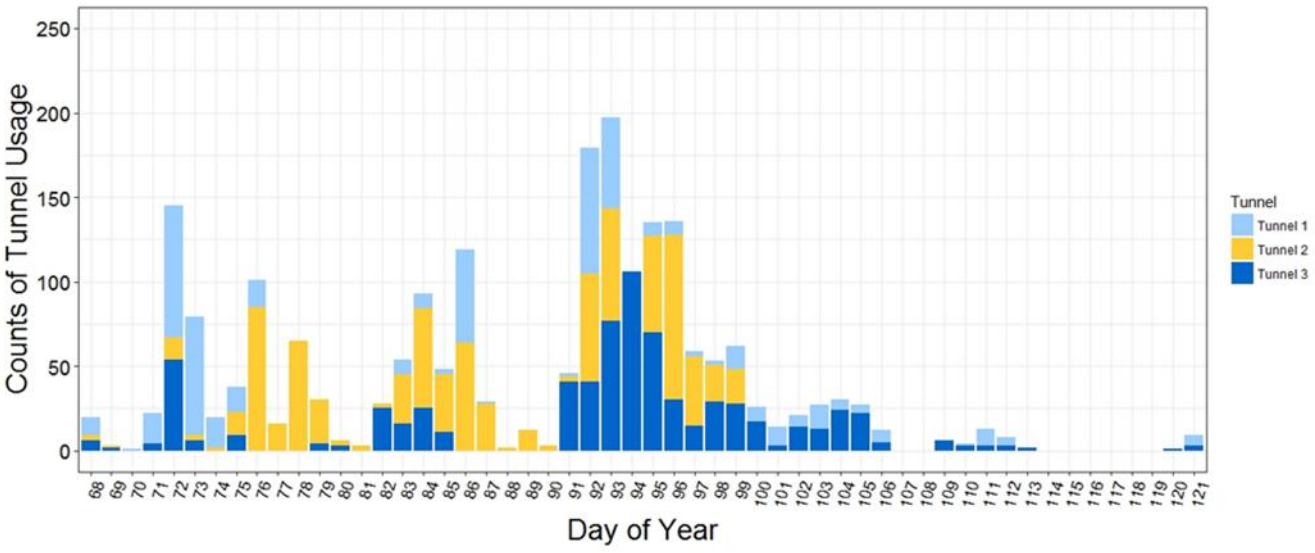

Fig. 6. Successful tunnel usage ("West" and "East" uses summed) by amphibians at Frankfield Loch, Stepps, North Lanarkshire recorded for 2015 (A) and 2016 (B). Legend details same as in Fig. 5.

Tunnel	2015	%	2016	%
1	344	16.13	550	26.07
2	447	20.96	839	39.76
3	1342	62.92	721	34.17
Total	2133	100	2110	100

Table 2. Total successful amphibian uses of tunnels by tunnel ("West" and "East"), year (2015 and 2016) and percentage at Frankfield Loch, Stepps, North Lanarkshire.

Amphibian road mortalities

The high level of frog road deaths recorded in 2015 (Table 3) was associated with the gap in the amphibian fencing during the "path" period and a high number of frog movements around day 88 (Figs. 4, 6). The high incidence of newt deaths over days 96-101 corresponds with a peak in their movement and a gap in the amphibian fencing identified during the initial "clear" period, indicated by high numbers of road mortalities near the gap beside tunnel 3. Although monitoring for road mortality also occurred in 2016, no road deaths were observed.

Date	Toads	Frogs	Newts
21/03: day 80	0	2	0
29/03: day 88	0	18	0
02/04: day 92	0	2	0
06/04: day 96	0	4	57
11/04: day 101	1	4	26
15/04: day 105	0	2	0
24/04: day 114	0	0	1
28/04: day 118	0	0	1
06/05: day 126	0	0	1
Total	1	32	86

Table 3. Dates and counts of observed amphibian road deaths at Frankfield Loch, Stepps, North Lanarkshire in 2015. Toads (*Rana temporaria*), frogs (*Bufo bufo*), and "newts" includes palmate newt (*Lissotriton helveticus*) and smooth newt (*L. vulgaris*).

Frog spawning locations

Common frog breeding sites around the loch were observed in 2015 through the presence of spawn mats. A more thorough investigation carried out in 2016 identified a number of breeding site locations within the marsh (including ponds installed as part of mitigation procedures during housing development) and around the circumference of the loch (Fig. 1).

Temperature and light periods: relationships with tunnel interactions

Mean temperatures between the two years were similar in their range with no consistent rise, though fluctuations were greater in 2015 (Fig. 4A,C). Amphibian activity was highest at night and lowest during daylight hours for all species. A further preference for sunset periods rather than sunrise was indicated for all species in both years (Fig. 7; Table 4).

DISCUSSION

Hamer *et al.* (2015) noted that amphibians are often the vertebrate group most at risk from road mortalities, and that the challenge of any amphibian mitigation measure is "to prevent road-kills and to maintain habitat connectivity". These tunnel monitoring results at Frankfield demonstrate that there is substantial two-way movement of all four local amphibian species between the marsh and the loch areas. The common frog breeding data show that spawning occurs both in the marsh ponds and in the reedy shallows around the loch edge. We have no data on toad or newt spawning, but it is likely that it occurs on both sides of the road, given the suitability of the habitat. Clearly, the loch and the marsh constitute a connected amphibian habitat and the tunnels are allowing animals to move from one side to the other.

We found amphibian road-kills only in 2015, coinciding with the period of disruption to the fencing caused by the roadworks. Hamer *et al.* (2015) noted that road construction "should be timed to avoid periods of high amphibian activity" and that regular inspection and maintenance of both fencing and tunnels are essential aspects of successful road mitigation. Unfortunately, the 2015 roadworks violated these requirements, with the road repair work extending into the migration season. There was no such problem in 2016, but an early season inspection showed that a section of the fence had been damaged, presumably by vandalism; this was repaired in time for the breeding season, and no further road-kills were observed. The roadkill data are likely to be underestimates, given the speed with which scavengers can locate and feed on or remove carcasses (Santos *et al*., 2011). However, the difference between the 2015 and 2016 data indicates an effect of the road repairs. We hope that the need for regular inspection and maintenance has been fully recognised.

The use of automatic tunnel-sited cameras allowed us to assemble a much more comprehensive picture of amphibian movements than is possible with other methods and with a modest expenditure of fieldwork time (this equipment carries a comparable cost to modern trail cameras, but please contact Froglife for further details).

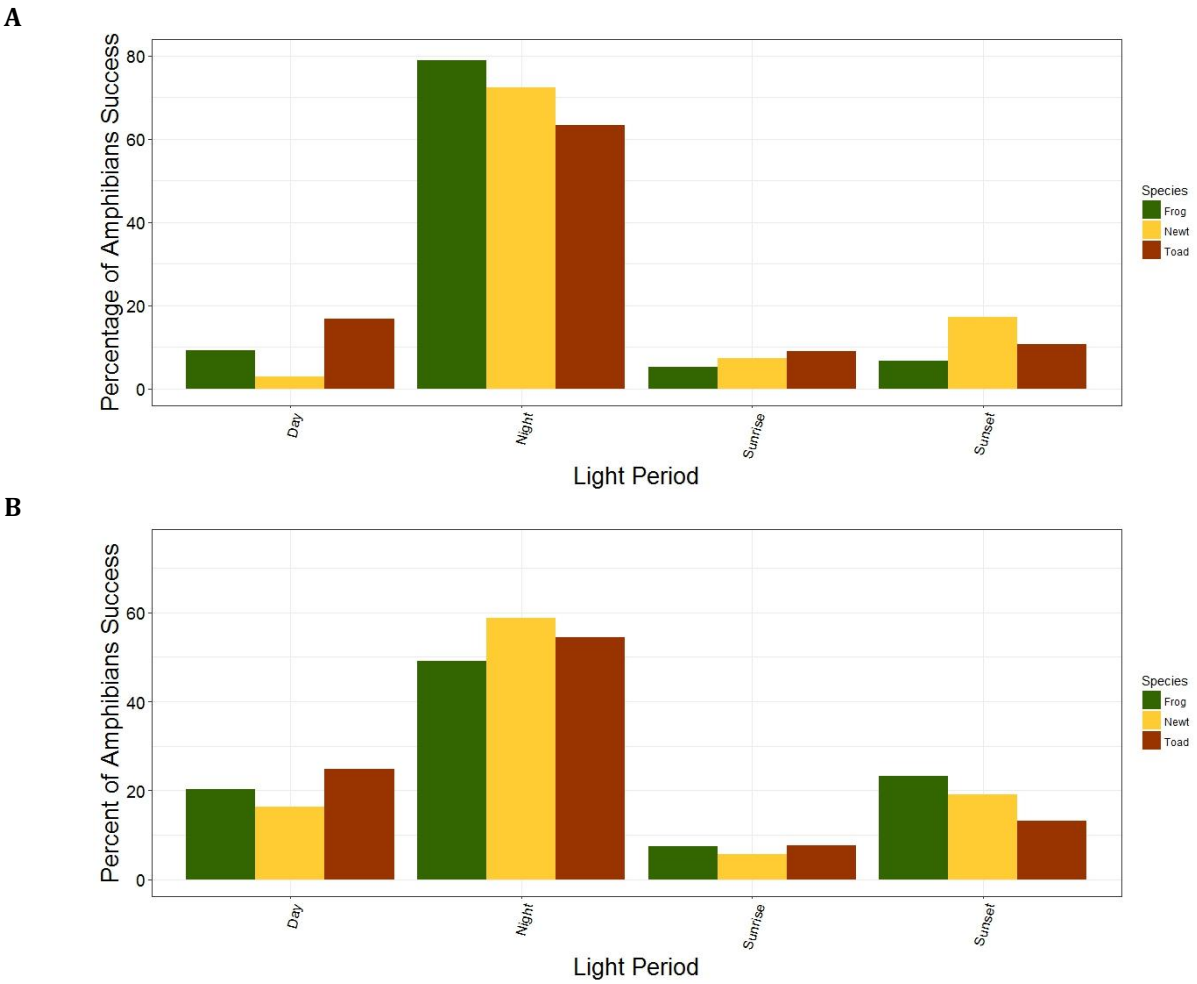

Fig. 7. Percentage of successful uses ("West" and "East" uses summed) by amphibians at Frankfield Loch, Stepps, North Lanarkshire by light period (sunrise and sunset representing the two-hour period around these times), for 2015 (A) and 2016 (B). Frog (*Rana temporaria*), toad (*Bufo bufo*), and "newt" includes both palmate newt (*Lissotriton helveticus* and smooth newt (*L. vulgaris*).

	Toads		**Frogs**		**Newts**		**Total**	
	2015	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
Sunrise	9.09	7.59	5.26	7.48	7.31	5.68	7.61	6.35
Day	16.86	24.81	9.12	20.22	2.98	16.28	8.39	18.57
Sunset	10.71	13.16	6.67	23.27	17.24	19.19	13.64	18.76
Night	63.34	54.43	78.95	49.03	72.47	58.85	70.36	56.32

Table 4. Percentages of total successful tunnel use ("West" and "East") by amphibian species, year and light period, at Frankfield Loch, Stepps, North Lanarkshire in 2015 and 2016. Toads (*Rana temporaria*), frogs (*Bufo bufo*), and "newts" includes palmate newt (*Lissotriton helveticus*) and smooth newt (*L. vulgaris*).

Matos *et al*. (2017) reported on five years of data from road tunnels on Hampton Nature Reserve. Great crested newt numbers entering and leaving the tunnels were assessed from animals caught in pitfall traps. This study provided valuable data, but the method is labour intensive and therefore costly, typically resulting in a highly restricted period of monitoring. Additionally, catching amphibians in pitfall traps biases the data by introducing a significant disturbance factor (i.e. animals trapped at the tunnel entrance cannot complete the crossing until released, but also, since movements are often bi-directional, trapping on one side only potentially underestimates the number of movements). Pagnucco *et al*. (2011) monitored salamander movements into tunnels using motion-triggered cameras, timed-interval images and pitfall traps as a control. Although they concluded that the cameras were effective, their data indicate that over 40% of

the animals failed to trigger the motion detectors due to small size and slow movements.

In this study, frog and newt movements extended throughout the monitoring period in both years from March to May, but with movement numbers low in May. Movements of toads tended to be more tightly clustered around a peak from late March to mid-April. Significant frog and newt movements began later in 2015 than in 2016, most likely related to the cold weather persisting into March.

Although all species showed large numbers of "successful" tunnel uses (assuming that smooth and palmate newts, which we could not distinguish, behaved similarly), there were fairly consistent species differences across the two years in the proportion of individuals which turned around and exited a tunnel after entering it. Such tunnel rejections occurred more commonly among newts and toads than among frogs. We do not have a good explanation for these differences, but they might be related to how microclimate variations within the tunnels are perceived by different amphibian species. Although animals other than amphibians used the tunnels, including occasional instances when we detected opportunistic amphibian predators such as brown rats, hedgehogs and domestic cats, if predator-avoidance was a cause of tunnel rejection, we would expect toads, with their good anti-predator defences, to show the lowest level of rejection. In an experimental set-up, Hamer *et al.* (2014) found that several species of Australian frogs were reluctant to enter tunnels, but they noted that such aversion had not been detected in European studies.

The pattern of tunnel usage differed markedly between the two years, with tunnel 3 the most heavily used in 2015, while usage was more evenly spread in 2016. The most obvious explanation for this difference is the disturbance related to the 2015 roadworks, located closest to tunnel 1 and furthest from tunnel 3.

The continuous automatic monitoring allowed an assessment of the diurnal patterns of amphibian movements. Although night-time was the most common movement period for all species, daytime and sunset periods were not negligible, and the lowest numbers of movements were at sunrise for all species.

These results do not allow us to measure amphibian populations at the site. We have no data on how many individuals hibernate and breed on the same side of the road, without having to move thorough the tunnels. In addition, we do not know whether each tunnel use was by a separate individual, or whether some or all individuals used the tunnels more than once. It is likely that the early movements up to the peaks in late March represent individuals moving to a breeding location, with little reason to return immediately. Later, however, movements are likely to represent dispersals to foraging sites after breeding, and therefore represent individuals recorded moving in the opposite direction to their earlier movement. Numbers were fairly consistent over the two years, except that toads showed a significant decline in 2016. More data are needed to determine whether this finding reflects the overall decline in U.K. toad populations reported by Petrovan & Schmidt (2016) or simply annual variation, which can be high in this species.

One caveat is needed when considering these results. We imply that each amphibian detected is migrating through the tunnel to the other side, unless it turns around within the camera's field of view (an area of about 0.5 m x 0.5 m extending 1.5 m from the tunnel entrance within a 13 m long tunnel), which we class as a tunnel rejection. This method cannot make this certain and it is likely that more amphibians make a U-turn at other points within the tunnels. Verification would require cameras to be installed at both ends and the images of amphibians at the start and end correlated, a difficult and laborious task. However, where this technique was used at two sites over three years, using the same camera equipment, the majority of amphibians, especially newts, spent little time in the tunnels, normally using them only for crossing, although male toads might spend more hours in tunnels as refuge, sometimes spending daytimes there and moving out during the subsequent night (Petrovan, unpublished data). We believe that the assumption that most amphibians entering the tunnels continue through to the other side is justified for the following reasons. Early spring migrations are about breeding, and most of the species we have monitored tend to breed in their natal ponds. They locate these ponds using a combination of physical and chemical cues (Sinsch, 1991; Joly & Miaud, 1993). A narrow tunnel may seem artificial to us, but is likely to provide a rich linear source of breeding-pond data, such as volatile chemicals, to a small amphibian.

At 13 m long, the Frankfield tunnels are shorter than many other tunnels installed in the U.K., which are typically 20 m long, but can be as much as 30-40 m (Matos *et al*., 2017; White *et al*., 2017). Shorter tunnels are likely to have significantly lower rejection rates compared to longer tunnels. In addition, the climate tunnels used at Frankfield have ventilation holes at the top, flush with the road surface, and these greatly facilitate air exchange and minimise differences in temperature and humidity between the tunnels and the external environment. However, ventilation holes allow road pollutants, including heavy metals and salt to enter the tunnels, especially during rainfall, and these can have detrimental effects on amphibians using the tunnels (White *et al*., 2017). A simple solution would be to flush the tunnels with water each spring before the

main amphibian migration, as is commonly done in other European countries. This would also allow the removal of accumulated dead leaves and litter, which can act as barriers to amphibian migration. Although the tunnel entrance grids at Frankfield prevent the entry of larger items of litter, images did show many smaller litter items such as cigarette packet wrappers.

This case study of amphibian mitigation tunnels at Frankfield Loch has demonstrated the efficacy of non-invasive amphibian monitoring using custom-built cameras located in the tunnels. The results show that the tunnels are well used by the local species of amphibians and that the combination of tunnels and amphibian-proof fencing can prevent road mortalities when the system is intact and well maintained. Reference to the literature on road mitigation indicates some desirable management practices such as tunnel flushing to remove pollutants. In detail, the results show interesting interannual and interspecific differences. To build a picture of amphibian dynamics at the site, further research is needed, including more breeding data of the sort reported here from a two-year period.

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Long-term monitoring for adders: an evolving methodology

R.J. Hodges & C. Seabrook

Kent Reptile and Amphibian Group, c/o KMBRC, Brogdale Farm, Brogdale Road, Faversham, Kent ME13 8XZ

E-mail*:* RickHodges123@gmail.com

ABSTRACT

Currently, there is no recommended methodology for long-term population monitoring of European adders (*Vipera berus*). To open a debate on a preferred methodology, we describe an approach based on 10-years' experience of monitoring in a chalk grassland reserve. The main elements are: 1) selection of a site with areas offering contrasting environmental conditions; 2) detection of adders along standard survey paths (transects) combined with paired artificial refuges of corrugated iron and roofing felt that are essential for detecting immature stages; 3) recognition of individual adders based on head-scale and neck patterns; 4) frequent site visits throughout the reptile active season; and 5) adoption of an Encounter Index (E.I.) that combines data from standard paths and refuges and normalises them for variations in survey effort and for shifts between years in the encounter rates along paths and at refuges. E.I. values correlate strongly with the numbers of known adders in the reserve but in some years E.I. values have been disproportionally high. Future objectives of the project are to explain variations in detectability and to estimate adder detectability associated with the current monitoring approach. Effective long-term monitoring is achievable by deploying "sufficient" refuges and, within practical limits, maximising path lengths and site visits. Future analysis of our own results will likely confirm our methodology as a "rule of thumb" for adder monitoring on, at least, chalk grassland.

INTRODUCTION

Significant contributions to the conservation of adders have come from long-term monitoring programmes (≥10 years). In Great Britain, the negative impacts of forestry activities have been highlighted by reports from the Wyre forest between 1978 and 2016 (Sheldon & Bradley, 2016). Details of reproductive biology, sub-populations and the longevity of adders have emerged from studies on the Dorset Heaths from 1986 to 2003 (Phelps, 2004a, 2004b), and in Somerset and Wales from 1989 to 2002 (Phelps, 2007). More recently, a programme in Belgium initiated in 2000 has provided an important understanding of the survival rate of the immature stages and the crucial influence of this on population growth in lowland wet heaths (Bauwens *et al.*, 2016; Bauwens & Claus, 2018), indicating that conservation efforts should address summer habitats where young adders reside and forage.

Long-term monitoring, together with adequate gathering of data on other relevant variables, offers considerable opportunities. It may demonstrate the impacts of land management and future climate change; assist with the interpretation of short-term survey data by clarifying the relationship between detection rates and climatic variables; and may provide information on how life histories may vary based on the records of individual adders from birth to relative old age.

At a national level, long-term monitoring is needed in diverse habitat types and contrasting latitudes. Previous long-term studies have been undertaken using various methodologies although all have relied on purely visual survey, i.e. did not employ artificial refuges. For the future in Britain, a more co-ordinated approach to long-term adder monitoring with broad agreement on methodology is likely to yield greater comparability between studies and a better national effort for adder conservation. As a basis for discussion on preferred methodology, we present the methods adopted by a monitoring programme on chalk grassland in Kent, England that has now passed its tenth year and is planned to continue for many years to come. The programme detects adders using a combination of standardised survey paths (transects) and artificial refuges.

METHODS

Important features of the monitoring site

The site is a chalk grassland wildlife reserve on the North Downs in west Kent, England managed by the Kent Wildlife Trust. The reserve is bordered by open farmland, housing, woodland and, for a short stretch, by a road so that it is relatively isolated with little prospect of exchange with other adder populations. The site is managed by extensive cattle grazing, usually from August to November, and scrub clearance by hand. This arrangement keeps survey paths and refuge positions open so that there is no need to change them between years. The location of the monitoring site is not disclosed in this paper to protect both the adders and their habitat.

The site consists of three areas that slope differently and present contrasting environmental conditions. The sloping areas are separated from each other by tall scrub (Fig. 1), with about 5% to 10% of each area covered in low scrub. Each area has a different aspect (Table 1): Area 1 slopes west into the relatively narrow Darenth valley, Area 2 slopes predominantly to the south-west, and Area 3 slopes predominantly south; the last two areas face into a wide open vale. The differences in topography give potential differences in exposure to climatic variables. These include duration and strength of sunshine exposure and wind action which together may give different desiccation rates. Across years these differences may have varying impacts on the populations of adders and other reptiles. This arrangement gives advantages over a more uniform site since in time informative comparisons may be made between the three areas themselves, and in the future one of the areas could provide better comparisons with sites elsewhere.

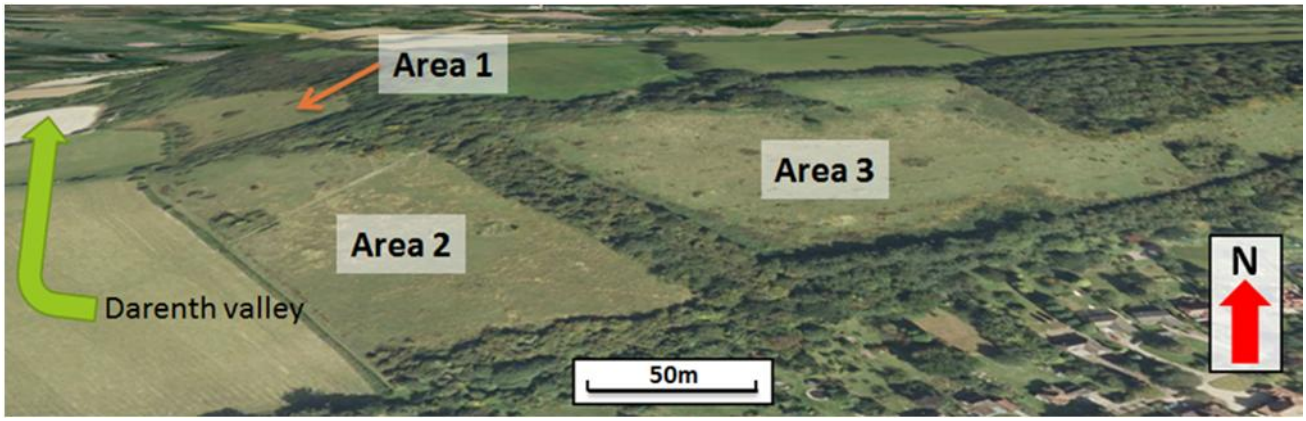

Fig. 1. Chalk grassland long-term monitoring site for adders (*Vipera berus*) on the North Downs in west Kent showing each of three contrasting areas (Areas 1, 2 and 3) separated by tall scrub. Details of each area are given in Table 1. (Photo: Google Earth)

	Main aspect and slope	No. refuge positions	Survey path		Open area		
			Length (km)	Path length/refuge pair (m)	Area (ha)	Refuge pairs /ha	Survey path (m)/ha
Area 1	West, 5-20°	16	1.03	64	3.2	5.0	322
Area 2	South-west 5-20°	14	0.89	64	2.7	5.2	330
Area 3	South 11-20°	20	1.30	65	3.8	5.3	342

Table 1. Details of three survey areas in a chalk grassland reserve on the North Downs in west Kent, managed by the Kent Wildlife Trust. Areas and path lengths measured on Google Earth.

Detecting adders

Adders are observed along a standard survey path (transect) in each area. The paths run along the top, middle and bottom of each slope and vary in length more or less in proportion to the open space in each area at around 330 m path/ha (Table 1). In addition, there are pairs of felt and corrugated iron ("tin") artificial refuges, effectively fixed waypoints, along the survey paths. Refuge tins consist of galvanised corrugated-iron sheets (0.5 mm thick and 0.5 g/cm^2) and felts are prepared from domestic roofing felt (Garage felt, green slate finish, Homebase, #242805, 2 mm thick and 0.3 g/cm^2). They are both cut to the same dimensions (50 cm by 65 cm) and pairs, comprising one of each type, are placed in sunny but inconspicuous locations backed by vegetation cover. To minimise disturbance by humans, tins are camouflaged by spraying their upper surface with brown paint (Espresso, satin finish, Rust-oleum). Refuges usually remain located in same position from year to year although are occasionally moved short distances away from ants nets or other problems. At the start of the study there were 31 refuge pairs but more were added so that at the time of writing there are 50 pairs. Consequently, from the start of monitoring to the time of writing the frequency of refuge positions along paths has risen from one every 104 m to one every 64 m (Table 1). The spacing of refuge positions is only roughly even along the paths, as priority is given to using less disturbed locations backed with good cover and the prospect of sunshine for at least some of the day. In relation to open space, the addition of refuge pairs over time resulted in an increase in density from 2.7-5.3 pairs/ha (Table 1). For general reptile monitoring the recommended density is 5-10 refuges/ha (Froglife, 1999) but much higher densities are recommended for short-term, detailed assessments although in this case the critical factor is the distance of spacing between refuges (Reading, 1997; Schmidt *et al*., 2017).

Experiences with refuge use on the site in the period 2008 to 2015 have already been described in detail (Hodges & Seabrook, 2016a). In summary, adult encounters along paths and at refuges both contribute significantly to records but immature stages were rarely encountered along paths so that records of them largely depend on the use of refuges. All life stages are encountered at tins more frequently than felts. Felts were included as although fewer adders were observed under them than tins, they are more effective for other species, especially slow worms (*Anguis fragilis*). In March and April, refuges are rarely used by adders and study of the thermal ecology of refuge use suggests that at this time of year it is usually more efficient for adders to be exposed to direct sunlight than to obtain warmth beneath a refuge (Hodges & Seabrook, 2016b, 2016c). However, it should be noted that over the years there have been variations in the rates of observations along standard paths and at refuges, with a trend towards more observation along paths (Fig. 2). Consequently, the proportion of encounters at refuges has declined from 80% to 40%. In due course this will need some explanation and may relate to changes in the prevalence of mature and immature adders, and influences of climate.

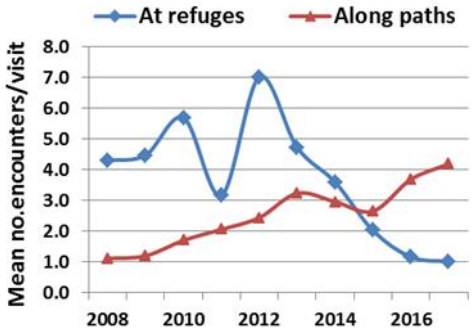

Fig. 2. Mean number of adder (*Vipera berus*) encounters/visit along paths and at refuges from 2008 to 2017 on the Kent long-term monitoring site. These data exclude neonates since these are observed only sporadically.

The survey team make 7 to 12 site visits per month from March through to the end of October, resulting in 56 to 78 annual visits for each of the last 10 years. A survey including all three areas can be completed in about 150 minutes and all areas are surveyed on each visit. Visits are only made on days when there is a reasonable prospect of encountering reptiles, i.e. not on heavily overcast days with dark cloud, significant rainfall, or temperatures below 8°C. If weather allows, most site visits commence around 09.00 h and are initiated in Area 3 (Fig. 1) which receives any early sunshine. The rate of movement by surveyors around the site allows the sun to reach all three areas prior to survey and so optimises the chances of finding adders under refuges or basking in sunshine.

Life stages are defined as follows: neonates are in their year of birth before their first hibernation; juveniles have undergone a single hibernation; sub-adults that have undergone at least two hibernations. Males are adult when they have completed at least 4 hibernations and in the case of females they are adult after at least five hibernations. The life stages are known from a combination of their size, colour pattern and from individual records kept across years, but are likely subject to occasional inaccuracy. The gender of juveniles, sub-adults, and adults is determined by colouration and body proportions (Smith, 1951; Beebee & Griffiths, 2000), although neonates and juveniles are likely more subject to errors.

Individual recognition

Recognition of individual adders is an important component of the study. Individuals are recognized by their head-scale and neck patterns (Benson,

1999). Head-scale instability is thought to be rare; we have observed only a single case (Hodges & Seabrook, 2014) and so this factor is unlikely to have any significant effect on our results. To minimise any disturbance, no adders are handled. Instead photographs are taken of adder heads either in the open at a distance of 2-4 m, using a long focal length lens, or much closer when individuals are under refuges. The scale patterns are coded and then entered into a database of our own design. To date there are records of 461 different individuals across all life stages. Of these adders, 45 (9.7%) have been monitored successfully from their first year through to adulthood. Adders not seen for three years are assumed to be dead.

Individual recognition allows estimation of population size by the exclusion of repetitive counts of the same individual. It also allows the presence of individuals to be inferred when they are not recorded in every year. Furthermore, the individual count data can be used in the future for the analysis of detection rates and the impact on these of other variables, especially those relating to climate.

Detection metrics

Over time several components of the monitoring system have varied. In particular, there have been annual increases in the numbers of refuges deployed, variations in the numbers of site visits made, and as mentioned earlier, variation between the proportion of adders encountered at refuges and along paths (Fig. 2). In addition, the lengths of the survey paths differ between the three areas. These variations prevent simple comparisons in the encounter rate for adders, or other reptiles, between years and between areas. For this reason an Encounter Index (E.I.), a "normalising" metric, has been devised that gives equal weight to encounters at refuges and along paths and corrects for survey effort.

At the heart of the E.I. is the extent of monitoring infrastructure. This is defined for each area by reference to the length of the survey path along which records can be made and the number of refuge pairs along the path. As any changes in the number of refuge pairs or path length are on different scales, monitoring infrastructure is defined as the geometric mean of these two variables:

Monitoring infrastructure = $\sqrt{2}$(No. refuge pairs x path length)

Monitoring infrastructure has increased over the years and a measure of how thoroughly each area has been assessed can be obtained by considering monitoring infrastructure in relation to the number of hectares of open space present in each area (Fig. 3). In recent years (2016/2017), there has been strong convergence between areas in infrastructure per unit area making the three areas more readily comparable (Fig. 3).

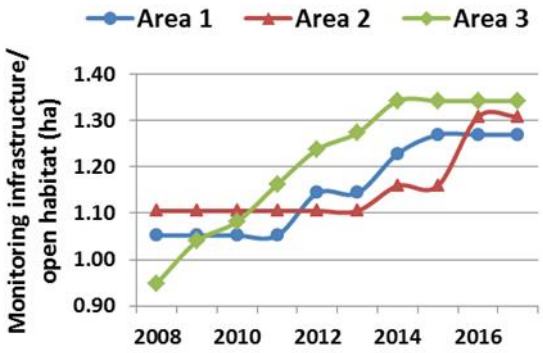

Fig. 3. Annual changes in monitoring infrastructure in Areas 1, 2 and 3 on the Kent long-term monitoring site relative to the amount of open space in each.

Monitoring effort for each year can be estimated by multiplying the monitoring infrastructure by the number of annual visits:

Monitoring effort = monitoring infrastructure x no. annual visits

The E.I. for any particular year can then be estimated as the geometric mean of encounters at refuges ($Encounters_r$) and those along the path ($Encounters_p$), normalised by the annual monitoring effort as follows:

$$\text{Encounter Index} = \frac{\sqrt{2}(Encounters_r \times Encounters_p)}{\text{Monitoring effort}}$$

A plot of E.I. against the total un-normalised number of adder encounters shows the extent of correction made by adopting the E.I. (Fig. 4). As the variables were already kept within fairly close limits the corrections are relatively small.

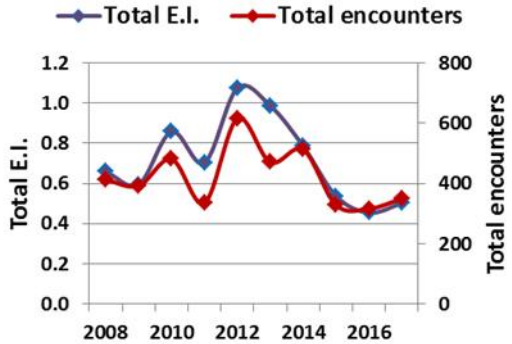

Fig. 4. Total Encounter Index (E.I) on the Kent long-term monitoring site (Areas 1, 2 and 3 combined) plotted with the un-normalised total number of adder (*Vipera berus*) encounters for 2008 to 2017 to show the extent of corrections made by the E.I.

It would be expected that the E.I. correlates strongly with the number of adders present on site, i.e. the more adders there are, the more will be encountered. When E.I. and the number of known adders on site (this excludes neonates as observations of these

tends to be sporadic) are plotted together (Fig. 5), there is a strong positive correlation for the whole period of observation ($r = 0.73$, $df = 8$, $p<0.02$) and even stronger one for the period 2013 to 2017 ($r = 0.92$, $df = 3$, $p<0.05$). If, instead, the adder encounters from refuges and along the path (as shown in Fig. 2) had been simply summed, then the correlation with the number of known adders would have been weaker ($r = 0.626$, $df = 8$, $p<0.1$). It can be seen that adder encounters and the number of known adders have declined since 2012 (Fig. 5). Although this is generally the case, the three areas have behaved differently in this respect and by disaggregating the data by area a more informative picture will emerge during future, detailed analysis.

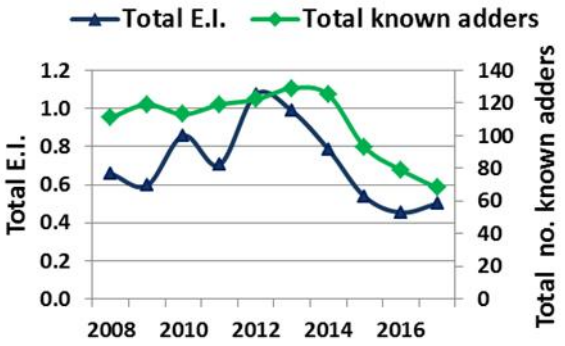

Fig. 5. Total Encounter Index (E.I) for the Kent long-term monitoring site (Areas 1, 2 and 3 combined) plotted with the number individual adders (*Vipera berus*) known to be on site 2008 to 2017, excluding neonates. NB: data for known adders in 2017 are still subject to increase by inference from 2018 and beyond.

An estimate of the extent to which the average adder is encountered each year can be made by dividing the E.I. by the number of known adders for that year. Such a plot (Fig. 6) demonstrates the especially high encounter rates in four out of ten years: 2010, 2012, 2013 and 2017. Preliminary analysis suggests that climatic factors may be responsible for this effect; it is a future task for the project to explain this in detail.

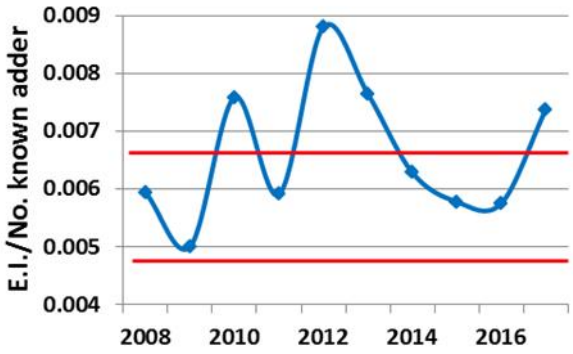

Fig. 6. Total Encounter Index (E.I) of Areas 1, 2 and 3 combined, on the Kent long-term monitoring site, corrected by the number of individual adders (*Vipera berus*) known to present on site each year 2008 to 2017, excluding neonates. Values falling outside red tram lines appear to represent exceptionally high encounter rates.

Other variables being monitored

Data are being gathered on other relevant variables. These include site management by grazing, presence of small mammals, and climatic factors. Live trapping of small mammals in September was undertaken from 2012 to 2015 to indicate food availability for adders at the end of the season. However, manpower constraints made this unsustainable. Climate data are collected both on site and from a private weather station at Tonbridge (Davis Vantage Pro2 Plus hardware, at N51°11'2'', E0°15'34'' and 148 m asl). The weather station is 16 km due south of the reserve. Daily data are provided free of charge and include temperature and humidity (mean, maximum and minimum for both), wind speed, and precipitation. Bright sunshine hours have been provided by a separate Tonbridge weather station but this ceased operation in October 2017 and, for the future, this variable will have to come from Heathrow, which is 48 km to the west. In addition, on site there are temperature loggers (Tinytag) placed in a shaded location. One logger is buried at 5 cm below the soil surface and the other is 30 cm above the soil surface. Since 2018, we have also been making records of soil moisture content to monitor desiccation.

DISCUSSION

For the long-term monitoring of adders on chalk grassland, a methodology employing both standard paths and artificial refuges has to date proved satisfactory, with refuge use essential for collecting data on immature stages. Likewise, a monitoring protocol using a standard path (3 km) along which corrugated iron refuges were placed (one per 136 m of path) was successfully adopted for grass snakes (*Natrix helvetica*) in Norfolk (Sewell *et al*., 2015), although there are several differences from the methodology describe here for adders. Previous advice on refuge deployment has typically expressed the number of refuges required as a proportion of the area of a site (Froglife, 1999). However, it is frequently unclear what sized area is actually being used by adders, i.e. it is unclear where the boundaries are to an adder population. Since the length of the survey path is known, this problem is avoided by expressing refuge deployment as the frequency of refuge positions along the length of the survey path, in this case one pair of felt/tin refuges for each 64 m. This is equivalent to expressing refuge deployment in terms of the spacing in refuge arrays that are used in more intensive short-term monitoring of other reptiles (Reading, 1997; Schmidt *et al*., 2017).

The initial choice of a site with distinct areas of contrasting topography offers the prospect of useful comparisons based on differences in environmental conditions. However, care needs to be taken that lack of uniformity across a site does not accentuate any systematic biases in recording effort. In our case we ensured that the three different areas were given an

equal chance of reptile observations by starting surveys at the area with the earliest sun and working round the site following the sun. Other precautions may be required at different sites.

When presenting monitoring results, these could be given as separate observations from the standard paths and from refuges. However, a metric, the E.I., is proposed that can be used to combine these two and normalise the data for varying effort between years and for shifts between years in the encounter rate along paths and at refuges. When plotted against an alternative (although not entirely independent) measure of adder abundance - the number of adders known to be present on site, there is a strong positive correlation with E.I. (Fig. 5). There are, however, deviations from this relationship (Fig. 6) where encounter rates appear exceptionally high. To date we have some evidence that these relate to responses to particular climatic conditions. This suggests that with more detailed analysis leading to correction of the E.I. for climatic variables, the revised E.I. might be taken as a proxy for the numbers of adders present on site.

An agreement on a preferred methodology for long-term adder monitoring would give useful future opportunities for comparability between studies. In the case of the methodology described here, estimation of the associated adder detection probabilities is still required to confirm its efficacy. There are also other some important considerations. In studies of other habitats (Griffiths *et al*., 2006; Walter & Wolters, 1997; Reading, 1997) it appears that refuges have performed much less well than reported here on chalk grassland; it is not clear why this was the case. Also, undertaking long-term studies (>10 years) is always a significant commitment and the number of annual site visits made in this study (59 - 78) requires a focused team effort, which is rarely available. It is possible that future analysis of our data may give some indication of the extent to which the visit rate could be lowered and still deliver equally useful results. Furthermore, if there are large numbers of individual adders that require recognition then using the freely downloadable Interactive Individual Identification System (I^3S, Version 4) software may make this element of the work quicker and easier. The E.I. needs to be explored further to confirm that it is robust for making comparisons between the results of different monitoring programmes especially in the absence of data on the numbers of known adders detected. At this stage it seems wise only to make comparisons where there monitoring infrastructure ratios are similar, i.e. in the range used in this study of one refuge pair per 64-104 m of path, which appears "sufficient". Less frequent refuge deployment is likely to provide poor data on the presence of immatures. For long-term monitoring, much increased refuge deployment is unlikely to be suitable for use in habitats where human visitors are frequent and aesthetic considerations important. In these situations effective monitoring is achievable by deploying at least "sufficient" refuges, while maximising path lengths and site visits within practical limits. Future analysis of our own results will likely confirm our methodology as a "rule of thumb" for adder monitoring on, at least, chalk grassland.

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Scottish Dragon Finder evaluation

V. Larcombe & J. Stead

Froglife, 1 Loxley, Werrington, Peterborough PE4 5BW

E-mail: james.stead@froglife.org

BACKGROUND

Froglife is a national wildlife charity concerned with the conservation of the U.K.'s amphibian and reptile species and their associated habitats. Froglife's vision is a world in which reptile and amphibian populations are flourishing as part of healthy ecosystems.

Froglife's *Scottish Dragon Finder* project was a 4.5 year project that began in 2014. It was supported by the Heritage Lottery Fund and brought together practical conservation, interactive educational activities and data collection throughout Scotland to help conserve native reptiles and amphibians. *Scottish Dragon Finder* is now finished and here we describe the activities undertaken in the project, and some of its achievements.

SCOTTISH DRAGON FINDER ACTIVITIES

The project had many different approaches to engaging the public, with diverse activities developed to interact with a wide-ranging audience.

Dragon Tails

Dragon Tails were interactive educational sessions aimed at children. They were split into education workshops for primary school pupils and drama workshops for youth groups. School sessions, linked to the Curriculum for Excellence, taught primary school children skills in Science, Maths and English whilst introducing native amphibian and reptile species (Fig. 1). Drama workshops worked with youth groups across Scotland, where children were taught an amphibian and reptile-themed play, which they performed to their families and friends after spending time practising and learning about how native species look and move. The *Dragon Tails* team has engaged over 2,600 people through its activities, visiting 51 schools and 20 youth groups.

Dragons on the Move

The *Dragons on the Move* activity travelled across Scotland bringing free wildlife experiences to a diverse range of people and enthusing them about our native species. The activity offered educational amphibian crafts, walks to spot amphibian and reptile species and Froglife stalls to answer questions and provide advice. Over 29,000 people attended *Dragons on the Move* activities across Scotland at 72 events.

Fig. 1. *Dragon Tails* at Sciennes Primary School, City of Edinburgh. *Dragon Tails* are interactive educational sessions for primary school pupils, teaching skills in science, maths and English whilst introducing native amphibian and reptile species.

Dragons in your Garden

The *Scottish Dragon Finder* team visited therapeutic gardens all over Scotland (Fig. 2). It delivered workshops teaching gardeners with mental health issues or physical or learning disabilities about native amphibians and reptiles, and showed them how to garden in a wildlife-friendly way. This was followed by a model wildlife garden activity, where workshop attendees created their own table-top wildlife garden from a blank slate to give them ideas for the future. Finally we headed out to the garden with the group creating wildlife friendly features including hibernacula, bog gardens, ponds or raised ponds. In addition, *Scottish Dragon Finder* also included a number of "train the trainer" workshops to share skills, and teach groups of garden leaders how to run wildlife gardening sessions with their own teams. The team engaged over 530 garden users and visited 36 therapeutic and community gardens.

Dragons on the Hills

Not enough is known about the distribution of amphibians and reptiles in remote areas of Scotland, as these areas can be hard to access on a regular basis or unfeasible for staff to reach for survey and monitoring purposes. However, hill-walking and other outdoor pursuits are popular pastimes in

Scotland, with many people heading to the outdoors in their free time. *Dragons on the Hills* aimed to enthuse and educate people who regularly visit Scotland's wild areas, teaching them how to spot and identify native amphibians and reptiles and submit their sighting using Froglife's free *Dragon Finder* app. Over 200 outdoor enthusiasts joined the *Dragons on the Hills* sessions on the ten walks we held, and all participants were introduced to the *Dragon Finder* app.

Fig. 2. *Dragons in your Garden* at Hansel Alliance Group, South Ayrshire. These workshops teach gardeners with mental health issues, or physical or learning disabilities about native amphibians and reptiles, and show them how to garden in a wildlife-friendly way.

Dragon Finder app

The *Dragon Finder* smartphone app allows anyone with an Android/iPhone device to learn more about the U.K.'s amphibian and reptile species: their ecology, identification features, calls and more. The app can answer questions to aid their identification and by submitting sightings they are building a clearer picture of the distribution of native and non-native amphibian and reptile species throughout Scotland and the rest of the U.K. Photos of sightings can be submitted which can be extremely useful if these are of diseased animals. The app also includes a species database offering pictures, calls and information on the basic ecology of native and non-native species.

Habitat creation/restoration

Scottish Dragon Finder aimed to create and restore reptile and amphibian habitats across Scotland, which included creating new ponds, restoring old ponds that had developed problems and creating reptile habitats suitable for basking. The project created 52 new ponds and restored 16 ponds across 25 locations, alongside terrestrial improvements.

Evanton Wood near Dingwall, Highlands was one of the early *Scottish Dragon Finder* project sites. It is owned by the Evanton Wood Community Company (http://www.evantonwood.com/index.asp) who were motivated to establish ponds within their community woodland site. The clay soil on site allowed for ponds to be created with artificial liners and, with the help of hired contractors, three ponds were created with different shapes, sizes and depths – the largest measuring 26 m x 19 m. A fourth pond was created with local volunteers which resulted in a network of ponds offering varied habitats for amphibians and associated pond wildlife.

Strathnairn Community Woodland near Inverness, Highland, also locally owned and managed, was completed at the end of 2016 (http://www.strathnairn.org.uk/strathnairncommunitywoodlands.asp). A network of ponds was created with a timber boardwalk to allow access for visitors (Fig. 3). Volunteers helped to create hibernacula nearby for overwintering reptiles and amphibians. The success of the ponds allowed the *Scottish Dragon Finder* team to run an outdoor school session with Farr Primary School, followed on another date by an evening amphibian survey training event, which attracted many local people.

Fig. 3. Three ponds created at Strathnairn Community Woodlands near Inverness, Highland, for amphibians and pond wildlife as part of the *Scottish Dragon Finder* project.

Tay View Community Garden (https://en-gb.facebook.com/tayviewcommunitygarden/) was one of the final sites of *Scottish Dragon Finder*. Set in the heart of Dundee on the site of a nursing home, Froglife worked with Dundee City Council to create a new lined pond with a pond-dipping platform for educational sessions. The new pond is a welcome feature within the developing garden site for local users and for the many people passing through the gardens, which are bordered by residential properties, a medical centre, and a school.

Training and volunteering

To ensure the longevity of the habitat projects, a programme of training and volunteer days was set-up. These allowed local people to learn more about the habitats around them and a chance to get outdoors and be active. They also learned skills in

amphibian and reptile surveying and identification, and skills in pond creation and management. Forty training and volunteer days were held throughout Scotland, with over 440 local people attending and learning new skills.

Traineeships

Two salaried 18-month traineeships were offered as part of the project to give those looking to start their career in nature conservation the chance to learn skills in public engagement and practical habitat work. We are delighted that both trainees have found employment since completing their traineeships; one has gone on to do a Ph.D. in conservation and the other now works for Buglife.

And finally...

Scottish Dragon Finder has worked across 31 of the 32 council areas within Scotland, with Shetland being the only council area we were not able to visit. During this time we have had some great feedback from those involved!

"I learned all about what a newt is and all about amphibians and reptiles that live in Scotland." - Louise age 10 – *Dragon Tails*

"This was a good experience as it's something I never thought to do."

- Highlands – Amphibian training session attendee

"Gained knowledge about frogs, toads, slow-worms and snakes. The garden designing exercise was excellent - fun, creative and thought provoking."

- Falkirk – *Dragons in your Garden* attendee

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Linking water quality with amphibian breeding and development: a case study comparing natural ponds and Sustainable Drainage Systems (SuDS) in East Kilbride, Scotland

R.J. Bird, E. Paterson, J.R. Downie & B.K. Mable

Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ

E-mail: roger.downie@glasgow.ac.uk

ABSTRACT

Amphibians have declined due to habitat loss and alteration. Sustainable Drainage Systems (SuDS) provide potential habitat for amphibians in urban landscapes. However, the contaminants they accumulate may cause increased pollutant exposure, and limited research has addressed whether differences in water quality between SuDS and natural ponds might restrict their use by amphibians. This study aimed to explore the effects of water quality on amphibian breeding and development in SuDS and natural ponds in East Kilbride, Scotland. A generalised linear modelling approach was employed to determine sources of variation in common frog (*Rana temporaria*) breeding and development in relation to pond size, pH, electrical conductivity (EC), chlorophyll and heavy metal concentrations. Key findings included that EC indicative of salt pollution was higher in SuDS than natural ponds, amphibians bred in both site types, but frog spawn clump densities were lower in SuDS, and tadpole development rates were higher in SuDS sites but decreased when aluminium concentrations exceeded concentrations those of surface water standards. However, pond desiccation was a significant element in the 2018 study year. It was concluded that natural ponds and SuDS varied in water quality but were important in supporting amphibian populations. However, water quality might influence amphibian development more than breeding attempts; implications and management recommendations are highlighted based on these observations.

INTRODUCTION

The unprecedented rate at which amphibians are declining globally is attributed to numerous human induced pressures, of which the loss and alteration of habitat is especially detrimental (Stuart *et al.,* 2005; Sodhi *et al.,* 2008). The necessity of aquatic habitats for breeding in most amphibian species can render anthropogenic landscapes inhospitable, as water bodies can be sparse, inaccessible, and polluted. Furthermore, amphibians face a major ontogenetic shift following metamorphosis, which results in different habitat requirements during different life stages (Werner & Gilliam, 1984). Continued alteration and loss of habitat presents novel challenges for species such as amphibians, which are a group that generally reacts negatively to urbanisation (Scheffers & Paszkowski, 2012). Yet, some artificial landscapes can serve as viable habitat for wildlife species, and contemporary studies have documented amphibian populations utilising certain drainage systems such as stormwater ponds, detention/retention basins, and artificial wetlands (Birx-Raybuck *et al.,* 2010; Brand & Snodgrass, 2010; Hamer *et al.,* 2012; Holzer, 2014; O'Brien, 2015). Additionally, Brand & Snodgrass (2010) found that amphibians were predominantly found in artificial ponds and other human-made wetlands while surveying both suburban and forested watersheds, highlighting the importance of some urban water systems for amphibians; but also that specific habitat characteristics are strongly associated with successful amphibian breeding and occupancy.

A caveat to wildlife species using urban habitats is the potential for pollutant exposure. This is especially the case for amphibians due to their permeable skin (across which a high proportion of respiratory exchange occurs) and the physiological and habitat shifts they undergo through different life stages. The transition between aquatic and terrestrial habitats exposes most amphibian species to a variety of substances and environmental conditions, resulting in their characterisation as effective ecological indicator species (Carignan & Villard, 2002). Thus, variation in water quality parameters is likely to influence their breeding and survival at different life stages. For example, salts applied to road systems can flow into adjacent waterways, leading to increased salinity levels and posing a risk for aquatic species such as pond breeding amphibians that dwell in these water bodies (Collins & Russell, 2009; Karraker *et al.,* 2016). Salt exposure can lead to increased mortality, increased severity and frequency of developmental deformities, heightened stress levels, reduced activity, and can deter pond occupancy in amphibians (Sanzo & Hecnar, 2006; Collins & Russell, 2009; Chambers, 2011; Hopkins *et al.,* 2013;

Hua & Pierce, 2013). These negative impacts from salts can occur at different amphibian life stages and have long-lasting effects (Wu *et al*., 2012).

Nitrogen and phosphorus are common and essential for biological organisms, but can become pollutants when in high concentrations (Zhang *et al.,* 2015; Garnache *et al.,* 2016). Nitrogen and especially phosphorus in aquatic systems can lead to excess algal growth and eutrophication, which can create hypoxic conditions and adversely affect aquatic organisms and trophic interactions (Perkins & Underwood, 2002; Guo, 2007). Furthermore, anthropogenic eutrophication can promote shifts in species composition and increase the prevalence of intermediate parasite hosts such as snails (Johnson & Chase, 2004). Direct exposure to these chemicals can also harm amphibians, and nitrogen-based fertilisers such as ammonium nitrate have been shown to decrease the survival of wood frog tadpoles (Burgett & Wright, 2007), and impair anti-predator behaviour by decreasing predator cue recognition in western spadefoot toad tadpoles (*Pelobates cultripes*) (Polo-Cavia *et al.,* 2016). Eutrophication via nitrogen and phosphorus results from increased plant activity usually in the form of an algal bloom, and one way to indirectly measure nitrogen and phosphorus is through the presence of chlorophyll *a*. Heightened chlorophyll *a* concentrations can be indicative of negative effects on aquatic biota, and one study documented the prevalence of three amphibian species being inversely related to chlorophyll *a* concentrations (Jarosiewicz *et al.,* 2014). Given the effects of nitrogen and phosphorus on amphibians and their prevalence in human dominated landscapes, additional research focusing on their impact at different magnitudes can inform conservation efforts to improve water quality in both urban and natural landscapes.

Metals are another category of naturally occurring substances that can become pollutants at unnatural concentrations. For example, larval amphibians exposed to chronic copper concentrations exhibited various negative effects, including hindering percent of animals reaching and time to metamorphosis, reduced survival, increased tail resorption time, and slower swimming speed (Chen *et al.,* 2007). Exposure of Zhenhai brown frog (*Rana zhenhaiensis*) tadpoles to low copper concentrations induced a decrease in body mass, having negative implications for later life stages (Wei *et al.,* 2015). Zhenhai brown frog tadpoles exposed to zinc had decreased mass and length as well as increased frequencies of abnormal erythrocytic nuclei. In addition to impairing growth and development, elevated zinc levels can compromise the ability of larval amphibians to escape predators by decreasing fright response (Lefcort *et al.,* 1998). For gilled aquatic species such as newt larvae and early stage tadpoles, aluminium toxicity in water systems can hinder respiration through the upset of ion exchange and can also cause blockage through excess secretion of mucus (Gensemer & Playle, 1999). Sensitivity to aluminium likely varies between amphibian species; for example, Pacific chorus frog (*Pseudacris regilla*) and long-toed salamander (*Ambystoma macrodactylum*) hatchlings and larvae exposed to aluminium treatments showed only sub-lethal effects (body size reduction, early hatching), but no reduction in survival (Bradford *et al.,* 1994). The extent to which amphibians can tolerate metal pollution is still not fully understood and further research is needed.

With the increase of human population size and continued urbanisation, it has been realised that managing and improving urban wildlife areas foster many benefits. "Sustainable Drainage Systems" (SuDS) is a term used to describe systems of managing urban surface water with a "multi-benefit" approach of improving four main categories: water quality, water quantity, amenity (related to humans and aesthetics), and biodiversity (Woods Ballard *et al.,* 2015). SuDS can take many forms, but examples that can directly benefit amphibians are artificial ponds, wetlands, swales (linear depressions, typically long and narrow), and retention basins (basins for holding water), all of which can be found in urban areas. SuDS are a relatively new scheme, which are increasingly being implemented in urban and suburban areas and their water management approach distinguishes them from previous drainage systems that focused solely on redirecting and caching water. How amphibians fare in SuDS is relatively unknown as few studies have addressed this topic. SuDS may harbour pollutants due to their proximity to developed areas and exposure to urban runoff, and a recent study found that fine sediments in SuDS networks were resuspended in the water column subsequent to multiple rainfall events (Allen *et al.,* 2017a). These fine sediments can contain salts, metals, and other pollutants that are potentially harmful to aquatic wildlife and can hinder benefits to biodiversity. However, the contamination of sediments can vary greatly between SuDS types as well as specific sites; methods for removing sediment pollution accumulation have been suggested as the best way to ensure water quality and biodiversity benefits are maintained, while still allowing for large water quantities to be managed (Allen *et al.,* 2017b). SuDS are still in their pilot stages and their benefits are not fully understood, but attempts to monetarily quantify their value have shown that in many cases the perceived benefits of SuDS (aesthetics, providing habitat, sustainable drainage solution) outweigh the capital and maintenance costs (Jarvie *et al.,* 2017). Perhaps what is paramount from a biodiversity standpoint is to understand the specific characteristics that make SuDS viable habitats for species such as amphibians, and the chemical and physical barriers that inhibit species from colonising and persisting in them. The continued incorporation of SuDS into developing

areas can enhance sustainability and improve connections with nature among urban dwellers. However, further evidence to support the merits of SuDS is crucial to facilitating the transition to improved urban water systems.

Scotland has a high rainfall and successful water management strategies are important in urban areas. Glasgow is a post-industrial city with a history of coal mining and heavy industrial manufacturing, and deposits of heavy metals and other pollutants are prevalent. East Kilbride is a region of the greater Glasgow area where amphibian populations have been documented using both natural ponds and SuDS. Paterson (2016) reported on a six-year study of annual variation in amphibian populations in both SuDS and natural ponds, but SuDS only represented six of 33 sites, and no comparisons were made between SuDS and natural ponds regarding amphibian spawning and tadpole development.

This study aimed to test the effects of water quality parameters and urban pollutants on amphibian breeding and development patterns in SuDS and natural ponds in East Kilbride, Scotland. Specifically, we aimed to determine whether: (1) amphibians were present in SuDS sites and if so, which species; (2) there was a difference in water quality between SuDS and natural ponds; (3) water quality or site type influenced common frog (*Rana temporaria*) breeding activity; and (4) water quality or site type influenced common frog development. The results contribute to the growing body of knowledge relating to how wildlife species use novel water systems in developed areas, which will inform future management decisions.

METHODS

Study site

An area encompassing water bodies on the outskirts of East Kilbride, South Lanarkshire was chosen for the study site. Nine natural ponds and ten SuDS were selected based on the combination of information obtained from South Lanarkshire Council and knowledge about local amphibian activity in the area (Fig. 1). The Google Earth area calculator tool was employed to determine the area of each site in m^2 and was used in conjunction with field observations for sites with tree cover. Each of the 19 sites was visited during the day four times over a period of four months during 2018 (April through July, one visit per month), with two additional night visits in May. Each of the day visits included on-site water quality testing, as well as water sample collection for laboratory analysis. Common frog spawn clump surveys were conducted on the first day visit in April, with tadpole development monitored on subsequent monthly visits. Torchlight surveys to determine frog, toad, and newt presence were conducted on two additional night visits in May. General observations took place at each site during each visit, which included documenting fish presence.

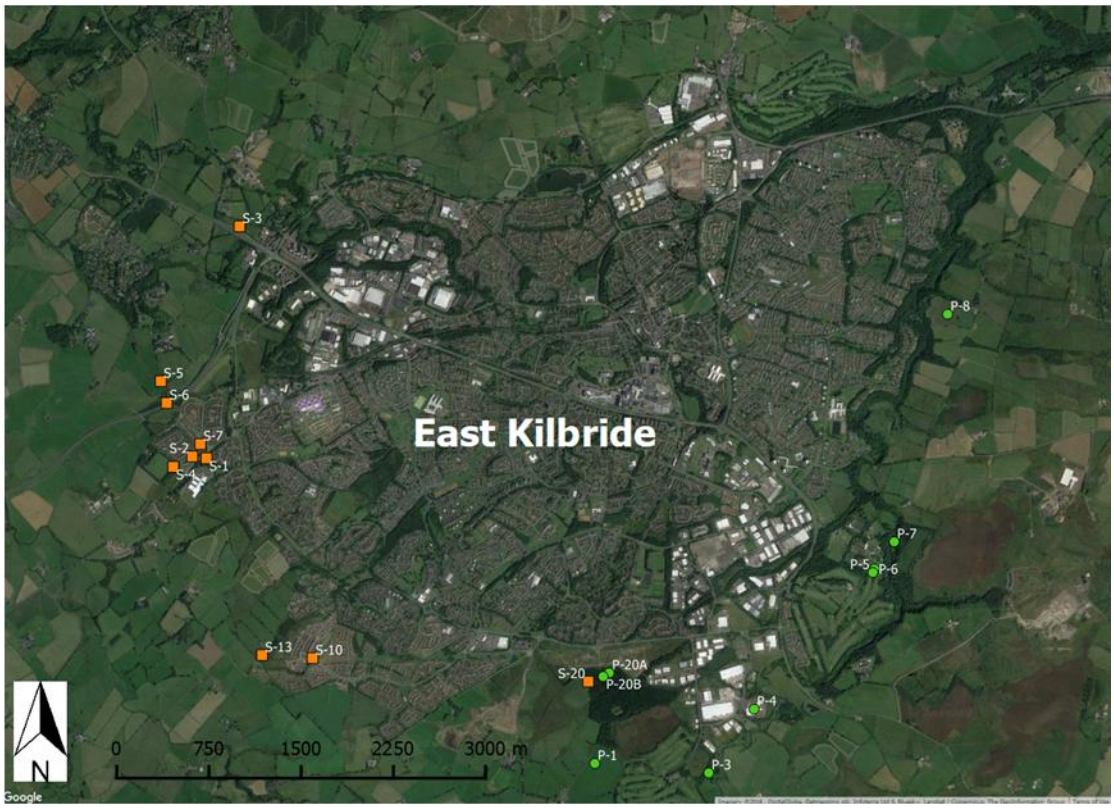

Fig. 1. The East Kilbride, Scotland study site, showing locations of SuDS (orange) and natural ponds (green) surveyed during 2018.

Water quality analyses

During each day visit, water quality was tested using a calibrated Hanna HI-98129 combo meter to measure pH and electrical conductivity (EC). EC is measured in microsiemens per centimetre (µS/cm) and is a measure of a solution's ability to pass an electrical current, so is directly related to the number of ions in a solution. Multiple processes can alter the EC of freshwater, one of which being the application of salt as a road de-icer, which can elevate EC levels of freshwater and result in salt pollution (Sanzo & Hecnar, 2006; Chambers, 2011; Karraker *et al.,* 2016). Three readings of water quality parameters were taken at each visit and the mean of these values constituted the measurement for each site. Readings were taken at a depth of approximately 10 cm at the water's edge, and distant from any visible source flowing into the water body.

Water samples were collected for chlorophyll and metal analyses. On each day visit, 500 ml of water was collected from each site for chlorophyll analysis. Samples were stored to inhibit light exposure and ensure no additional photosynthetic activity occurred. Each sample was then filtered within 8 h through GF/C microfibre filters. Methods by Strickland & Parsons (1972) were followed, using a Gallenkamp VISI-SPEC spectrophotometer to determine chlorophyll *a* concentration in µg l^{-1}. An additional 10 ml of water was collected at each site in May, June, and July for analysis of dissolved metal concentrations. Samples were filtered through a Minisart RC 0.45 µm filter into 0.1 ml of Aristar nitric acid to form a 1% nitric acid solution (EPA, 1994). Samples were delivered to the Scottish Universities Environmental Research Centre (SUERC) for inductively coupled plasma atomic emission spectroscopy (ICP-OES) analysis to determine dissolved concentrations of aluminium (Al), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in µg l^{-1}. Of the five metals, Cu, Fe, Mn, and Zn are "specific pollutants" that are discharged in significant quantities in the U.K. and have potential to negatively impact biological entities (SEPA, 2018). Harmful effects can occur at levels exceeding 1 µg l^{-1} for Cu, 1 mg l^{-1} for Fe, 123 µg l^{-1} for Mn, and 10.9 µg l^{-1} for Zn. Aluminium is a "non-statutory" substance as the standards set are not in legislation, but the current standard is 15 µg l^{-1} (SEPA, 2018).

Amphibian surveying

During the first visit in April common frog spawn mats were identified. Numbers of common frog spawn clumps were determined using published methods (Griffiths & Raper 1994), which involved measuring the area of the spawn mat and using the equation (2.27 + (73 x (spawn mat area in m^2)) + individual clump count) to determine total number of spawn clumps per site. In the subsequent three visits, tadpole development at each site was monitored using Gosner staging (Gosner, 1960). Dip netting was conducted at each site for a duration of 10 min or until 20 tadpoles were caught. Due to the lack of newt and toad tadpoles at most sites surveyed, Gosner staging focused on common frog tadpoles. Torchlight surveys in May consisted of visiting each site following methods described by Griffiths *et al.* (1996) using a 1,000,000-candlepower Clulite Clubman CB3 LED torch. Torchlight survey data were combined with site observations to generate a measure of presence/absence of common frogs, common toads, and newts (not identified to species) at each site. Fish presence/absence was also recorded.

Statistical analyses

A generalised linear modelling approach was implemented in R version 3.4.1 to determine which variables explained the most variation in biological and physical parameters (R Core Development Team, 2017). The first set of models aimed to test whether water quality parameters differed significantly by pond type or over time. EC, pH, chlorophyll *a*, and concentrations of each of the heavy metals were used as response variables. Pond type (SuDS or natural pond), date, and type x date interaction were included as potential variables and likelihood ratio tests were used to test their significance based on stepwise elimination.

The next series of models aimed to explain variation in spawn clump density of common frogs, and presence of spawn clumps in SuDS sites using data collected in April. Two sets of models were created: for the first set, spawn clump counts for each site were divided by the area of the water body in square metres to calculate spawn clump density per site, which was used as the response variable; for the second set, egg presence in SuDS sites was used as a response variable. EC, pH, and chlorophyll *a* concentrations were treated as covariates in these models, while water body type (SuDS or natural pond), fish presence, newt presence, and toad presence were treated as factors. The EC x type interaction was also considered for the spawn clump density models to determine if differences in EC between water body type could explain variation in spawn clump density. This interaction along with the covariates and factors were included as potential explanatory variables and likelihood ratio tests were used to test their significance based on stepwise elimination.

The final series of models aimed to explain variation in Gosner development stage. The mean Gosner stage characterises the general trend of development for each month and has been used in previous studies pertaining to amphibian development (Walsh *et al.,* 2008, 2016). Gosner stage progression is not a strictly linear phenomenon, but slope can be informative regarding general rate of development as well as mean stage reached when approaching the latter parts of the development season in July (Walsh *et al.,* 2008).

Thus, two sets of analyses were performed: one using the slope of the three mean Gosner values for sites for which development data were collected in May, June, and July, and one using the final stage of development reached at each site where frog eggs were observed. For these response variables, site type was considered as a factor and EC, pH, heavy metal concentrations, and area of the water body were considered as covariates. Due to the large number of variables considered, relative to the sample size, the final model was determined by successively adding each variable to a simple model and eliminating variables not significantly contributing to the variance, using likelihood ratio tests. For heavy metal concentrations (Al, Cu, Fe, Mn and Zn), maximum dissolved concentrations across the three sampling periods were determined and percent of the U.K. specific Environmental Quality Standards and Standards for Discharges to Surface Waters (SEPA, 2018) were calculated and used as explanatory variables in the model. In the results section, average rates are shown as mean ± standard deviation.

For each final model, model fit was tested by calculating percent explained deviance (or pseudo R^2 value) using the equation:

$$\left(\frac{\text{null deviance} - \text{residual deviance}}{\text{null deviance}}\right) \text{x } 100$$

RESULTS

Site characteristics

There was substantial variation in size of both the SuDS (575 ± 432.21 m^2) and natural ponds (215 ± 241.57 m^2) (Table 1). Amphibians were present in all SuDS and natural ponds. Newts were present in eight of the ten SuDS and six of the nine natural ponds, while toads were present in six of the ten SuDS and two of the nine natural ponds (Table 1). Only three SuDS and two natural ponds included fish, which did not appear to be related to size of the sites (Table 1). Two of the 19 sites had no common frog eggs (SuDS #4 and SuDS #7), but toads were present in SuDS #4 and newts were present in SuDS #7 (Table 1). Since these sites did not contain any frog eggs they were not sampled for tadpoles after April. During July, tadpoles could only be sampled at nine sites due to excessively hot, dry conditions, thus these nine sites were the only ones with Gosner data for all three months (Pond #3 dried up in July but emerging froglets were seen near moist ground and vegetation and were staged) (Table 1). Therefore, comparisons of water quality parameters in relation to site type and date were based on all 19 sites, assessments of common frog spawn clump densities and maximum amphibian development stage reached were based on 17 sites, and assessment of amphibian development slope was based on nine sites.

Water quality in relation to site type

On-site water quality testing yielded differences in EC between SuDS and natural ponds, with SuDS generally showing higher levels but more variation in relation to time at some sites (Fig. 2). The EC generalized linear model (GLM) determined that neither the date x site interaction (χ^2 = 4.11, DF = 3, P-value = 0.25) nor the date (χ^2 = 1.25, DF = 3, P-value = 0.74) were significant in the model, leaving type as the only significant variable and estimating an increase of 236.1 µS/cm for SuDS sites (χ^2 = 17.41, DF = 1, P-value = <0.0001) with a pseudo R^2 value of 29.49. Chlorophyll *a* concentrations varied at individual sites (Fig. 6, supplementary information), but no significant differences were found in relation to site type or date. The pH GLM determined that the date x site interaction was significant (χ^2 = 15.34, DF = 3, P-value = 0.0015), and estimated a pH decrease in SuDS of 0.28 and 0.43 on 6th April and 7th May respectively, but a pH increase in SuDS of 0.19 on 7th July with a pseudo R^2 value of 55.06 (Fig. 7, supplementary information).

There was considerable variation between site and date and between the five metals measured (Figs. 8-12, supplementary information). The date x type interaction was not significant in any of the GLMs for metal concentrations, and none of the tested terms were significant in explaining aluminium concentration variation. However, type was significant in explaining iron variation with an estimated decrease of 762.2 µg l^{-1} for SuDS, and date was significant in explaining variation in copper (-4.79 µg l^{-1} on 7th May and -4.95 µg l^{-1} on 7th July), manganese (-1066.0 µg l^{-1} on 7th May and -715.6 µg l^{-1} on 7th July), and zinc (-6.95 µg l^{-1} on 7th May and -9.46 µg l^{-1} on 7th July) (Figs. 8-12, supplementary information).

Variation was also apparent regarding the percent at which the five metals exceeded standards (Fig. 3). For example, most of the sites exceeded standards for aluminium, manganese, and copper, with SuDS #2 at over 3000% of the set aluminium standard and SuDS #10 at over 5000% of the set manganese standard. Aluminium and manganese concentrations were measured at highly variable magnitudes relative to standards, whereas the highest levels above standards for zinc and iron were less variable at approximately 400-800%. Most sites were below the zinc and iron set standards, with only two SuDS and two natural ponds exceeding zinc standards, and two SuDS and four natural ponds exceeding iron standards (Fig. 3).

Amphibian breeding in relation to site type and water quality

Spawn density was on average higher in natural ponds (0.70 ± 0.60 clumps) compared to SuDS (0.16 ± 0.24 clumps) (Fig. 4).

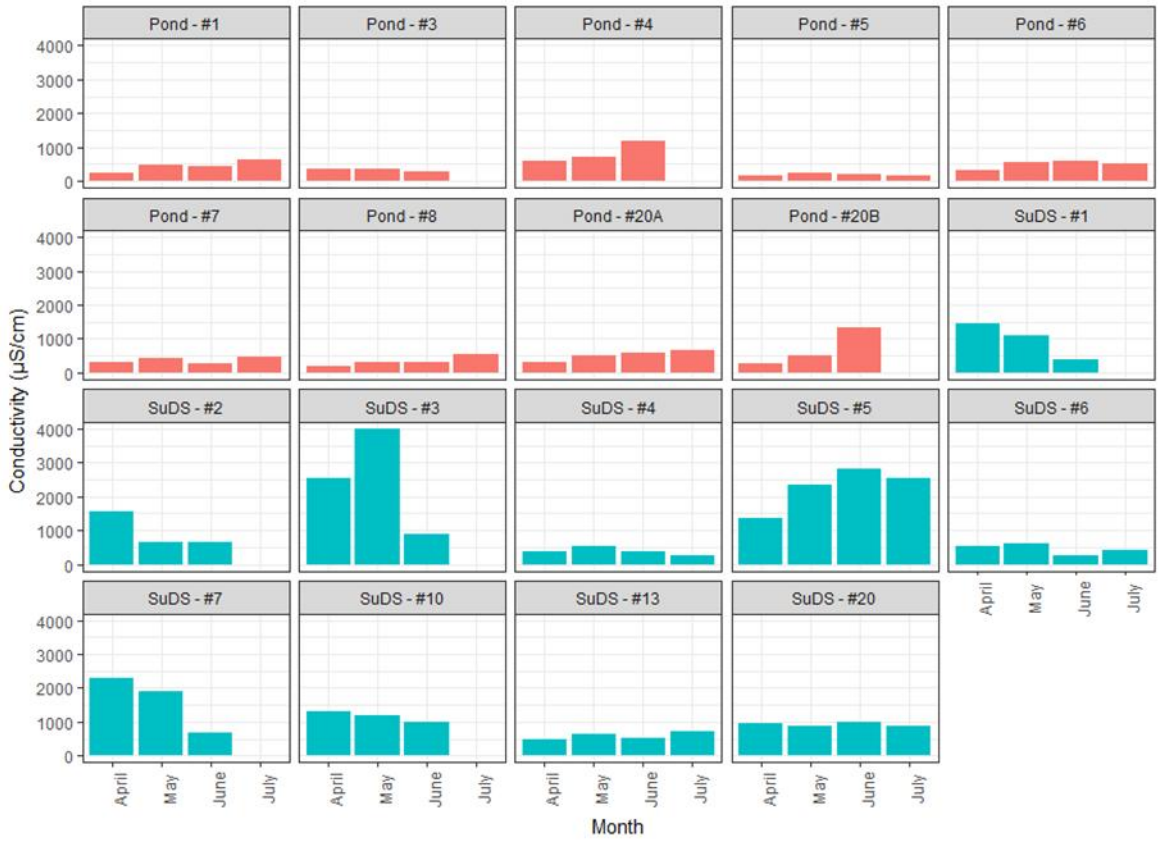

Fig. 2. Electrical conductivity (µS/cm) measured in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland on 6th April, 7th May, 3rd June, and 7th July 2018.

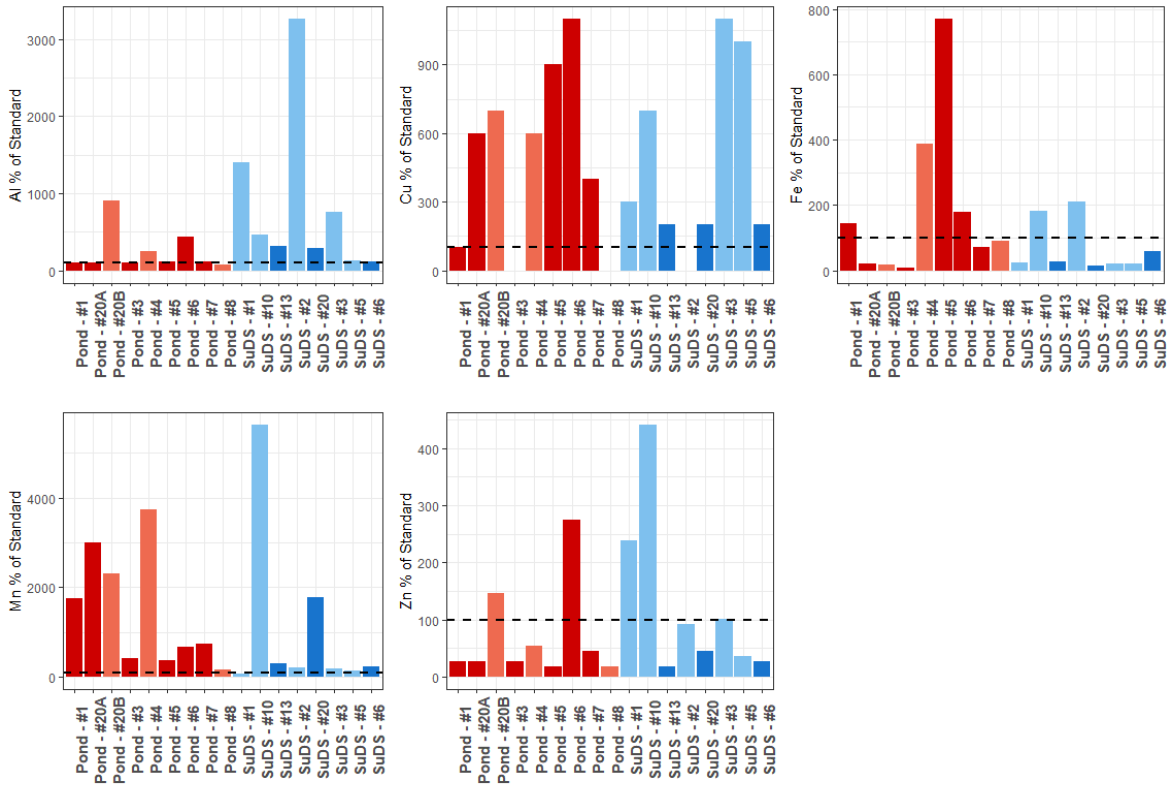

Fig. 3. Concentration percent exceeding the maximum U.K. surface water standards for five heavy metals (aluminium, copper, iron, manganese and zinc) at natural ponds and SuDS at East Kilbride, Scotland surveyed during 2018. Six natural ponds sampled over three months (dark red bars) and three natural ponds not sampled over all three months (pale red bars). Three SuDS sampled over all three months (dark blue bars), and five SuDS not sampled over all three months (light blue bars). Black dashed line denotes the U.K. surface water standard for each metal at 100%.

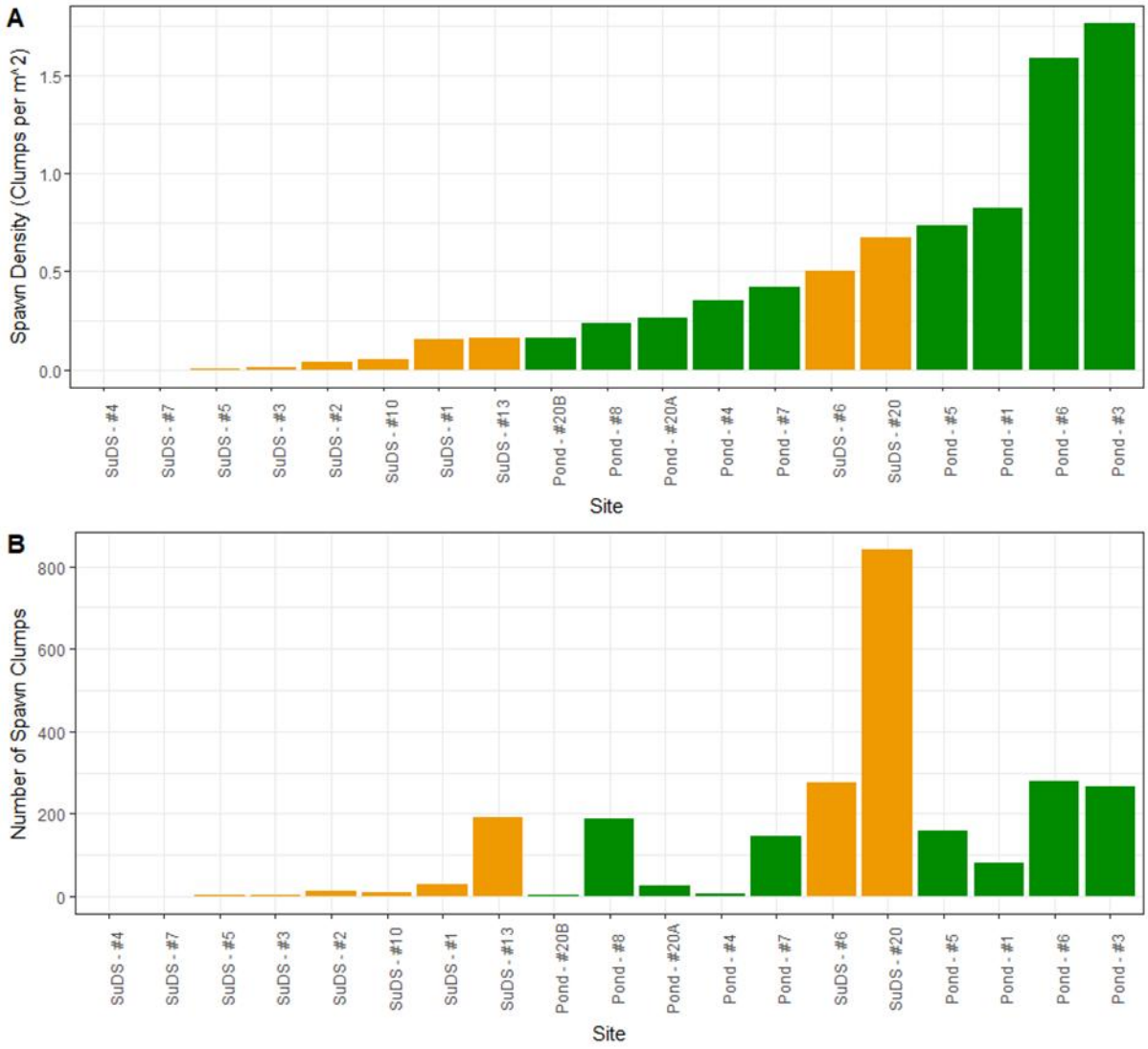

Fig. 4. Common frog (*Rana temporaria*) spawn densities (clumps per m^2) (**A**) and number of spawn clumps (**B**) at SuDS (yellow bars) and natural ponds (green bars) at East Kilbride, Scotland surveyed during 2018. Spawn densities are arranged by ascending order of density, and spawn clumps are arranged in the same order as density.

Site type was the only significant variable explaining variation in spawn density (χ^2 = 6.09, DF = 1, P-value = 0.014), with a predicted decrease in spawn density of 0.54 clumps per square metre in SuDS. The pseudo R^2 value for the model was calculated at 29.49, indicating a small proportion of variation was explained. Pond #3 had the highest spawn density with over 1.75 clumps per m^2, while the lowest eight spawn densities belonged to SuDS sites (Fig. 4). Although spawn densities were on average greater in natural ponds compared to SuDS, the mean spawn counts were slightly higher in SuDS (136.7 ± 265.24) than natural ponds (128.33 ± 105.27) due to SuDS #20 which had excess of 800 spawn clumps (Fig. 4). For SuDS site egg presence, none of the water quality parameters, newt presence, or toad presence were significant in explaining variation; however, fish presence was significant (χ^2 =6.19, DF = 1, P-value =0.013), with an estimated 21.26 lower probability of egg presence when fish were present (Table 1).

Amphibian development in relation to site type and water quality

Based on the nine sites that could be sampled for tadpoles over the three months, tadpoles in SuDS had higher development rates than those in natural ponds (Table 1). The maximum Gosner value for July was 46 which represented froglets observed around the perimeter of the site that had completed metamorphosis with tails fully resorbed, and all but two of the nine sites had individuals at this stage by the July sampling period (Table 1). Pond #5 showed a maximum of 45 (froglets with tail not fully resorbed), but tadpoles at Pond #6 (which showed some of the highest signs of pollution based on the heavy metal analyses; Fig. 3) only reached stage 38 (hindlimb toe differentiation, forelimbs not emerged) by July. The froglets observed at pond #3 and SuDS #6 were the most developed, with all individuals reaching stage 46 by the July sampling period.

Table 1. Common frog (*Rana temporaria*) development data and site characteristics for the 19 study sites at SuDS and natural ponds surveyed at East Kilbride, Scotland (sites numbered based on order of initial visits). The upper nine rows show sites tadpole development data collected for May, June, and July 2018. The lower ten rows are sites that either dried up during data collection so development data were not collected for all three months, or no frog eggs were observed during April.

	Site ID	Frog egg Presence	Site Dried in July	Mean May Gosner Stage	Mean June Gosner Stage	Mean July Gosner Stage	Min./Max. Gosner Stage observed in July	Max. Gosner Stage reached	Gosner Slope	Area (m^2)	Fish Presence	Newt Presence	Toad Presence
Sites with three sets of Gosner data	Pond #1	Yes	No	25.4	35.2	44	39/46	46	9.3	100	No	Yes	No
	Pond #3	Yes	Yes	26.9	36.2	46	46/46	46	9.55	150	No	Yes	No
	Pond #5	Yes	No	25.2	32.4	38.3	36/45	45	6.55	215	No	Yes	No
	Pond #6	Yes	No	25.2	29.5	33.2	28/38	38	4.00	175	Yes	No	No
	Pond #7	Yes	No	25.75	37.33	42.8	39/46	46	8.53	350	Yes	No	No
	Pond #20A	Yes	No	26.95	39.9	42.3	37/46	46	7.68	100	No	Yes	Yes
	SuDS #6	Yes	No	25.2	40	46	46/46	46	10.40	550	No	Yes	Yes
	SuDS #13	Yes	No	27	38.2	45.8	45/46	46	9.40	1200	No	Yes	Yes
	SuDS #20	Yes	No	25.8	35.5	45.7	45/46	46	9.95	1250	Yes	Yes	Yes
Sites without three sets of Gosner data	Pond #4	Yes	Yes	25.1	--	--	--	26	--	20	No	No	No
	Pond #8	Yes	Yes	26	29	--	--	29	--	800	No	Yes	No
	Pond #20B	Yes	Yes	26.2	--	--	--	27	--	25	No	Yes	Yes
	SuDS #1	Yes	Yes	25	41	--	--	41	--	200	No	Yes	No
	SuDS #2	Yes	Yes	--	--	--	--	--	--	300	No	Yes	No
	SuDS #3	Yes	Yes	--	--	--	--	--	--	150	No	Yes	Yes
	SuDS #4	No	No	--	--	--	--	--	--	900	Yes	No	Yes
	SuDS #5	Yes	Yes	--	--	--	--	--	--	800	No	Yes	Yes
	SuDS #7	No	No	--	--	--	--	--	--	200	Yes	Yes	No
	SuDS #10	Yes	Yes	26.5	--	--	--	27	--	200	No	No	No

The minimum Gosner value for July varied more substantially between sites, with the least developed individual seen at stage 28 (hindlimbs at budding stage, no toe differentiation), which was also observed at pond #6. All except one of the natural ponds (#3) showed slower developing tadpoles than the three SuDS sites based on slope (Table 1). Pond #6 also showed a substantially lower Gosner slope (4.0) across the three sampling points than the other sites (maximum of 10.4 in SuDS #6) and natural ponds on average showed a lower slope (7.60 ± 2.08) than the SuDS sites (9.92 ± 0.50). Therefore, differences in the slowest developing tadpoles influenced the mean Gosner stage in July, with the lowest values at 33.2 (pond #6) and 38.3 (pond #5), and the highest value at 46 (pond #3 and SuDS #6).

Of the ten remaining sites that did not have complete Gosner data for the three months, two had no frog eggs (SuDS #4 and SuDS #7), tadpoles were not detected at three (SuDS#2, SuDS #3, and SuDS #5), tadpoles were only detected in May for three (pond #4, pond #20B, and SuDS #10), and tadpoles were detected in May and June in two (pond #8 and SuDS #1) (Table 1). The highest maximum Gosner stage of these ten sites was observed in June at stage 41 (SuDS #1), while the lowest was observed in May at stage 26 (pond #4). The three sites where eggs were observed but no tadpoles were detected were SuDS (#2, #3, and #5). SuDS #2 showed extremely high levels of aluminium (excess of 3000% of the set standard) and high levels of Fe; SuDS #3 showed high EC levels (higher than the maximum detectable by the water quality meter at over 3999 µS/cm in May), high aluminium and copper levels; and SuDS #5 showed high EC levels and high copper levels (Figs. 2, 3).

Based on the GLM model selection, site type, area of the water body, and percent of U.K. standard for maximum aluminium, copper, and zinc concentration were significantly associated with variation in the slope of Gosner stages (as a proxy for developmental rate) for the nine sites that were sampled over all three months. The GLM estimated a Gosner slope increase of 0.86 in SuDS, 0.0015 with area, and 0.0049 with percent of U.K. standard for maximum zinc concentration, but a decrease of 0.0034 with percent of U.K. standard for maximum copper concentration and 0.0095 with percent of U.K. standard for maximum aluminium concentration. The pseudo R^2 value for this model was 99.94, indicating a very strong fit and explaining most of the variation. Based on the maximum Gosner GLM model selection, site type, average EC levels for the four sampling dates, and percent of U.K. standard for maximum aluminium concentration were significantly associated with variation in the maximum Gosner stage reached for the 17 sites that had frog eggs. The GLM estimated a maximum Gosner stage increase of 9.022 in SuDS, and a decrease of 0.022 with average EC level and 0.013 with percent of U.K. standard for maximum aluminium concentration. The pseudo R^2 value for this model was 85.64, indicating a strong fit and explaining most of the variation.

DISCUSSION

The results presented here indicate that SuDS were similar to natural ponds in terms of amphibian presence, with all sites having at least one amphibian species detected, and most sites (79%) having multiple amphibian species (Table 1). Variation in water quality parameters was apparent, but the only significant differences in pollutants based on site type were EC and iron levels, while most others varied based on date of collection (Figs. 6-12, supplementary information). Common frog breeding was not associated with water quality parameters, but rather with site type, as natural ponds facilitated significantly higher breeding densities than SuDS. Moreover, frog egg presence was negatively associated with fish presence within SuDS sites. Amphibian development was significantly associated with variation in water quality parameters; sites with hindered or aborted development tended to have high indicators of pollutant levels (Table 1; Figs. 3, 4), which suggests an increased susceptibility of tadpoles to multiple pollutants. Overall, multiple sites in the sampling area had low levels of pollutants, as well as productive breeding and development rates regardless of site type, demonstrating that both natural ponds and SuDS are important habitat for amphibians in East Kilbride.

Water quality differences in SuDS and natural ponds

A major difference between SuDS and natural ponds relates to proximity to roads and developed areas. A primary goal of the SuDS design is to manage surface water and incorporate sustainable designs around urban and suburban areas (Woods Ballard *et al.,* 2015). The natural ponds in this study were more isolated from major roads than were SuDS, and there was also a difference in geographic location based on site type (Fig. 1). Natural ponds were located to the southeast of the study area while SuDS were to the southwest, which represents a potential confounding effect. However, differences between SuDS #20, and ponds #20A and #20B were apparent even though they were situated in very close proximity, suggesting that differences between SuDS and natural ponds were based on characteristics of the ponds and not just geographic location. The protracted winter weather and unusual snowfall during the early months of 2018 may have resulted in administering road salts at an intensified rate throughout the greater Glasgow area. Salinity and conductivity levels of waterways adjacent to roads and developed areas probably increased initially, and heightened levels may have persisted if salt deposits formed and dissolved repeatedly during precipitation events. Gallagher *et al.* (2014) reported

urban pond chloride levels exceeding toxicity guidelines, and the highest EC value from this study was recorded in SuDS #7 during May, at over 3999 µS/cm. Increased mortality, increased severity and frequency of developmental deformities, heightened stress levels, and reduced activity are among the harmful effects salts can have on amphibians. Thus solutions to abate de-icers or partition roads from nearby aquatic habitats can assist in maintaining ecological integrity and prevent salt pollution (Sanzo & Hecnar, 2006; Collins & Russell, 2009; Chambers, 2011; Hopkins *et al.,* 2013; Hua & Pierce, 2013). Isolating aquatic habitats from roads and developed areas may also prevent metal pollution, as heavy metals in urban waterways often originate from transportation areas and vehicles (Allen *et al.,* 2017a; White *et al.,* 2017). Another possible source of elevated metal concentrations may be the submerged refuse observed at sites, including cans, pipes, trollies, bicycles, engine parts, which could leach metals into these environments.

Factors affecting amphibian breeding

Common frog spawn clump densities were higher in natural ponds than in SuDS, which suggests that in general the natural ponds offer conditions that were more favourable than SuDS. Natural ponds may be more attractive to amphibians because SuDS are artificially developed for drainage and tend to be nearer to homogenous, impervious landscapes (Birx-Raybuck *et al.,* 2010; Woods Ballard *et al.,* 2015; Guderyahn *et al.,* 2016). However, more evidence is necessary as the pseudo R^2 value for the spawn density model was 29.49, indicating that some of the variation was explained but other unknown factors besides site type are likely influencing spawn clump density. Holzer (2014) also found that whether the site was natural or constructed only had a weak association with native amphibian breeding, whereas forest cover and prevalence of aquatic vegetation had a stronger association. Habitat characteristics such as vegetation, impervious surfaces, and shading could be important regarding amphibian breeding and perhaps could have explained some of the variation in breeding between SuDS and natural ponds.

Overall, there was no association of egg absence with water quality parameters, so other environmental characteristics associated with SuDS may deter amphibians from breeding in these sites. All the SuDS sites were at least ten years old (South Lanarkshire Council), indicating that age of the SuDS is probably not a contributing factor to reduced use by amphibians. Although the sample size is small, the presence of fish was associated with the absence of frog eggs in SuDS and may have deterred breeding at these sites. Avoidance of amphibians breeding in water bodies with fish is documented and has been associated with decreased anuran diversity (Drake *et al.,* 2015; Manenti & Pennati, 2016; Pollard *et al.,* 2017). However, both natural pond sites with fish had frog spawn, indicating fish may have a more inhibiting effect on breeding in SuDS than in natural ponds, although a larger sample size is necessary to confirm this.

Factors affecting amphibian development

Gosner slope as well as maximum Gosner stage both significantly increased at SuDS sites, suggesting that SuDS had developmentally favourable characteristics. However, the results suggest that the presence of particular or combinations of heavy metals might negatively affect amphibian development. The amount by which aluminium exceeded standards was associated with both decreased Gosner slope and decreased maximum Gosner stage, indicating a negative effect on development. Aluminium toxicity to wood frog tadpoles (*Rana sylvatica*) significantly decreased body mass as well as protracted development (Peles, 2013). A similar toxic effect may have hindered development of common frog tadpoles in this study. Aluminium was the only metal negatively associated with both Gosner slope and maximum Gosner staged reached. The results suggest that other pollutants that hinder development may work in tandem when in high concentrations.

Of the nine sites having Gosner stage data for May, June, and July, pond #6 had the shallowest Gosner slope and the lowest mean Gosner stage for July at 33.2, the next highest mean being 38.3 (pond #5) and all other sites above 42 (Table 1). Pond #6 had the highest levels of aluminium, copper, and zinc, as well as being above the U.K. standards for all five of the heavy metals tested (Fig. 3) while pond #5 had the highest concentration of Fe, at 770% of the U.K. standard, but also had elevated copper and manganese levels. This could suggest that elevated levels of multiple heavy metals were hindering tadpole development and growth at these sites. Furthermore, fish were present in pond #6, which may have induced higher stress levels in tadpoles, as a consistent field observation was tail damage to tadpoles where fish were present. Combinations of pollutants at high levels may also explain the lack of tadpoles where eggs were present in SuDS #2, #3, and #5 (Figs. 2, 3), and may be indicative of aborted development. Chen *et al.* (2007) found that exposure to elevated copper levels reduced northern leopard frog (*Rana pipiens*) tadpole growth as well as increasing time to metamorphosis. It has also been demonstrated that American bullfrog tadpoles (*Lithobates catesbeianus*) exposed to iron and manganese resulted in delayed time to metamorphosis (Veronez *et al.,* 2016). The negative effect of heavy metals on tadpole development that we found is consistent with current research, and multiple heavy metals may have a compounding hindering effect.

Atypical Scottish weather patterns of 2018
Abnormal weather patterns were observed in Scotland during spring and summer of 2018. Glasgow received 40.6 cm of snow between 28th February and 1st March and temperatures were below 0°C on 10 days (AccuWeather.com). During April, temperatures did not exceed 11°C until the 14th. Temperatures began to rise during late May, where the average high temperature for the last eight days of the month was 22°C. June received continued warm weather and exceeded 30°C for two days during the last week. The warm weather continued in July, with 22 of the 31 days receiving a maximum temperature of at least 20°C. Rainfall also diminished, as recorded levels in 2018 were less than average in April (by 13 mm), May (36 mm), June (20 mm), and July (47 mm) (AccuWeather.com).

The effect of this warm temperature and low rainfall was seen at many of the survey sites. Sites with large numbers of tadpoles during May (Pond #4, pond #20B, and SuDS #10) were devoid of tadpoles in June. Hydroperiod is especially important for most breeding amphibians, as standing water is needed for initial spawning and aquatic larvae development. Longer hydroperiods are thus advantageous for amphibians because they support larger breeding populations and higher species richness (Baldwin *et al.,* 2006; Holtmann *et al.,* 2017).

The high temperatures and lack of rainfall during 2018 resulted in substantial site drying, as eight of the 19 sites (two natural ponds, six SuDS) had desiccated enough to eliminate all standing water, or rendering the site inaccessible in July (Table 1). The high temperature could have accelerated development in tadpoles, as laboratory experiments revealed that increased temperatures resulted in increased development in common frogs compared to lower temperatures (Walsh *et al.,* 2016). Although we did not have comparable data to compare with other years, the variation in Gosner slope documented in this study suggests that other factors also changed developmental rates.

Management implications
During times of drought or unusually warm and dry weather, a potential management solution to pond drying is to extend hydroperiod by manually adding water. Seigel *et al.* (2006) documented a considerable decrease in tadpole mortality and increase in metamorphosis in a pond supplemented by well water to increase hydroperiod. Very limited research has addressed this topic and supplementing surface water bodies with ground water or other water sources may alter their composition with unknown consequences. The drying of multiple SuDS sites due to warm weather and lack of rain has design implications, and it is recommended that SuDS being developed in the future consider a minimum area to avoid drying and potential harm to wildlife. All SuDS 300 m^2 or less dried completely or to the point of water inaccessibility (Fig. 5); thus, it is recommended that future SuDS designs be built to a size of at least 400 m^2. Smaller SuDS designs risk desiccation during unusual bouts of heat such as those seen during this study, and hydroperiod and the ability of artificial ponds to retain water can have major effects on amphibian communities (Hamer & McDonnell, 2008). Although amphibian adults and newly metamorphosed individuals may be able to survive in moist and vegetated areas after pond drying, a major risk is posed for tadpoles in these conditions, as they require adequate standing water to develop.

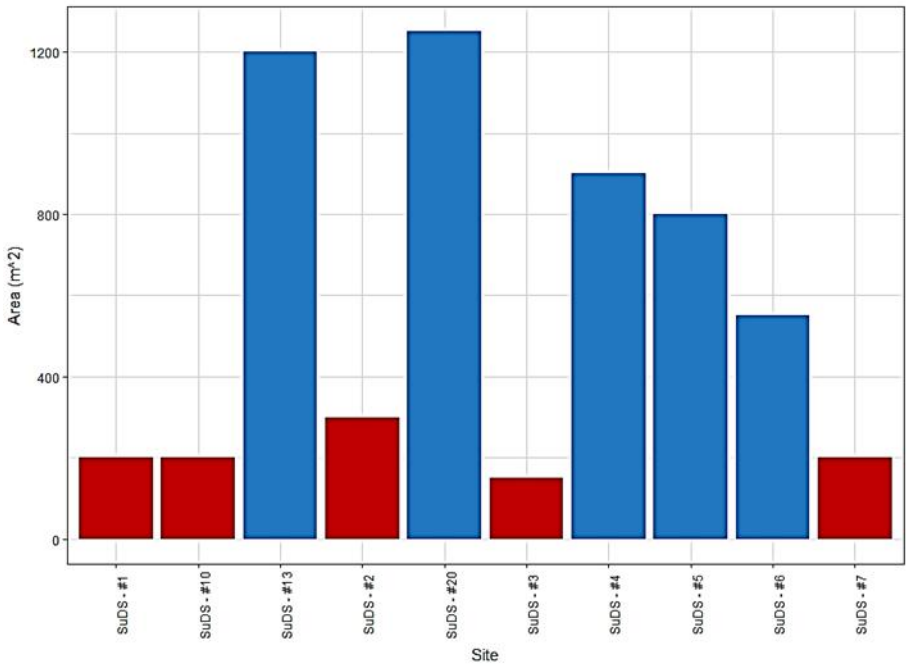

Fig. 5. Surveyed SuDS based on area (m^2) at East Kilbride, Scotland during 2018. SuDS that dried out during July (red bars), and SuDS which did not dry out (blue bars).

The amphibian assemblages present in all ten SuDS from this study exemplify their viability as amphibian habitat and importance in a continually urbanising environment. Common frogs were present and bred in eight of the ten SuDS, which is comparable to a study in Inverness, Scotland where common frogs were present and bred in eight of 12 SuDS in 2011 and 2012 (O'Brien, 2015). However, in our study three of these eight SuDS with breeding activity showed no signs of tadpoles, and one site had tadpoles only in May, suggesting that water quality or other aspects of these sites might not have been as suitable as perceived during breeding. The function of SuDS and other urban drainage systems to sequester pollutants increases their potential to be ecological traps by advertising false cues of suitable habitat (Battin, 2004; Robertson & Hutto, 2006). Ecological traps are a major risk for wildlife in urban areas, but frequent management of SuDS and systematic monitoring and removal of harmful pollutants are one method to ensure these habitats stay suitable and not acquire toxic pollutant levels. Additionally, the installation of vegetation buffer zones around urban drainages can be advantageous for amphibians by creating habitat and potentially sequestering heavy metals through phytoremediation (Birx-Raybuck *et al.,* 2010; Moreira *et al.,* 2011; Puglis & Boone, 2012; Holzer, 2014). Low levels of contaminants and productive breeding activity was seen in some East Kilbride SuDS, which is encouraging. However, more research is required to identify the conditions that make artificial systems habitable, and long-term studies are necessary to illuminate annual and seasonal variations in pollutants and how these conditions influence successful amphibian breeding and development. Research such as this will add to the growing body of knowledge pertaining to the importance of artificial water bodies for wildlife.

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SUPPLEMENTARY INFORMATION

Fig. 6. Chlorophyll *a* concentrations in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland over the four sampling periods during 2018. Data from eight sites are not shown for July, due to the water body drying out.

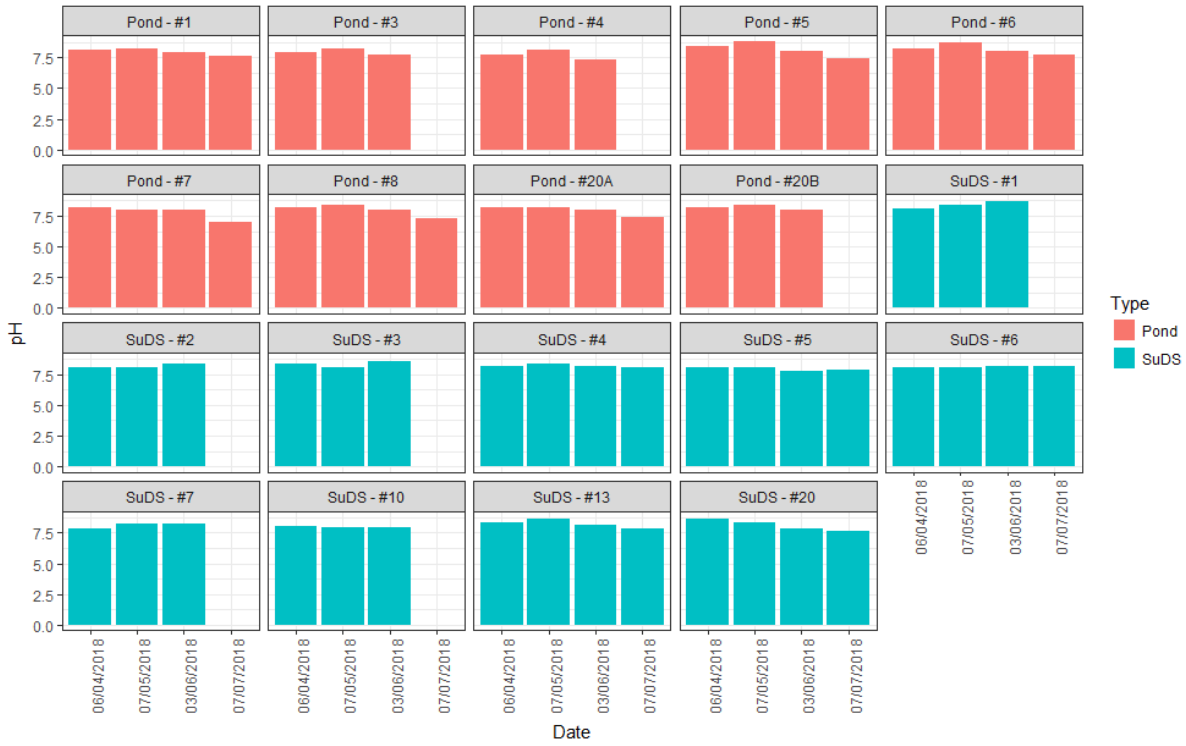

Fig. 7. pH values in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland over the four sampling periods during 2018. Data from eight sites are not shown for July, due to the water body drying out.

Fig. 8. Aluminium concentrations in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland over the three sampling periods during 2018.

Fig. 9. Copper concentrations in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland over the three sampling periods during 2018.

Fig. 10. Iron concentrations in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland over the three sampling periods during 2018.

Fig. 11. Manganese concentrations in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland over the three sampling periods during 2018.

Fig. 12. Zinc concentrations in natural ponds (red bars) and SuDS (blue bars) surveyed at East Kilbride, Scotland over the three sampling periods during 2018.

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The study and conservation of adders in Scotland

C.J. McInerny

University of Glasgow, President Glasgow Natural History Society (GNHS), Chairman Biological Recording in Scotland (BRISC), and Clyde Amphibian and Reptile Group (CARG)
School of Life Sciences, University of Glasgow, Glasgow G12 8QQ

E-mail: Chris.McInerny@glasgow.ac.uk

ABSTRACT
This paper describes two projects that explored the study and conservation of European adders (*Vipera berus*) and other reptiles in Scotland. One involved the implementation of an environmental mitigation plan during the construction of a hydroelectric scheme through an area of high reptile densities. The other involved the monitoring of a population of adders in a highly managed environment on a golf course. In both cases the adder numbers persisted suggesting that the populations were not affected, and so they illustrate situations where this reptile species can co-exist with humans. Finally, more general observations about reptile habitat requirements and conservation in Scotland are inferred from these studies.

INTRODUCTION
Reptile species are declining in numbers and range throughout the UK, which has prompted research to understand the reasons for these trends (Beebee & Griffiths, 2000). Conclusions from this research have resulted in recommendations for conservation, including legal protection of animals and habitat, and the implementation of habitat management practices to benefit these animals (Edgar *et al*., 2010; Gleed-Owen & Langham, 2012).

Scotland has in some areas healthy populations of reptiles, including the adder (McInerny & Minting, 2016). One area in Scotland where adders have been studied is Loch Lomond, 40 km north of Glasgow, which has revealed much information about their biology, distribution and habitat preferences (McInerny, 2014a, 2014b, 2016a, 2016b, 2017).

Here we summarise two projects which addressed the conservation of adders at Loch Lomond. One involved the creation of an environmental mitigation plan to ameliorate the effects of the installation of a hydroelectric scheme (McInerny, 2016a), and the other the study of a population on a golf course in highly managed habitat (McInerny, 2016b). In both cases monitoring of the populations revealed stable numbers of adders, suggesting that these reptiles can, when appropriate consideration and action are put in place, co-exist with humans.

HYDROELECTRIC SCHEME ENVIRONMENTAL MITIGATION PLAN

Study site and survey work
The study site is a replanted mixed native forest on south and west facing hill slopes flanking the east shore of Loch Lomond (McInerny, 2014a, 2017). This contains areas of bracken (*Pteridium* spp.), bramble (*Rubus fruticosus* agg.), gorse (*Ulex* spp.) and heather (*Calluna vulgaris*), amongst scattered small trees and bushes of various species.

Artificial cover objects, made from 50 cm x 50 cm roofing felt, were distributed in March 2012 (McInerny, 2017). These, and areas around the felts, were examined visually about once a week for reptiles from early February until November. The site was monitored from 2012 to 2015, with 36-58 visits each year. The location, number, maturity and gender of reptiles were recorded on each visit; these were recorded as day counts, and are plotted graphically in Fig. 1. Underground reptile hibernation sites were also mapped. Highest reptile densities were found in an area approximately 0.6 hectares in size at the lower parts of the study site.

Environmental mitigation plan
A hydroelectric scheme was proposed for construction in 2014 which involved the diversion of water through a 2 km underground pipe from a burn 300 m down a hillside to a turbine powerhouse, before re-entering the burn (McInerny, 2016a). The proposed site of the turbine powerhouse coincided with the area that contained the highest reptile densities revealed by the 2012 survey work.

As a prelude to the environmental mitigation plan, the entire length of the proposed pipeline and turbine house was surveyed for reptiles. Three visits were made during March and early April 2013, the optimum time of the year to locate these animals in Scotland, when vegetation has not grown, and the reptiles bask for long periods in the cool air and weak early spring sun. The surveys confirmed the distribution of adders revealed by the 2012 survey and formed the basis of the mitigation plan, which had three parts:

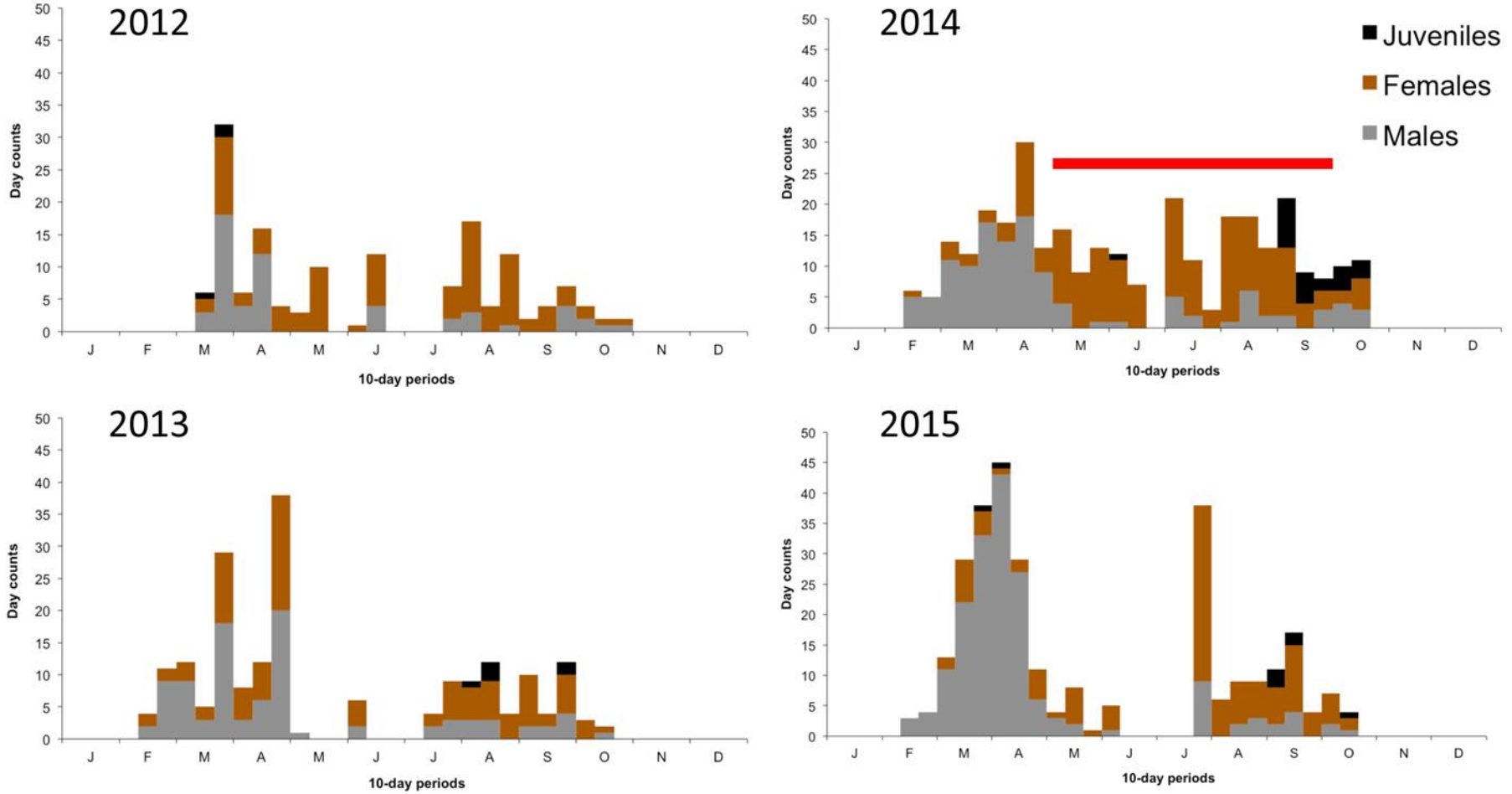

Fig. 1. Numbers of adders (*Vipera berus*) before, during and after a hydroelectric development at a Loch Lomond site, from 2012 to 2015 (McInerny, 2016a, 2017). Total day counts for 10-day periods over the four-year period for adult males (grey), adult females (brown) and juveniles (black) are plotted. The hydroelectric development was completed during 2014. Red bar indicates the construction period from May to September, with the reptile-proof fencing present from January to November.

1. A revision of the route of the underground water pipe to avoid reptile hibernation sites.
2. The erection of a reptile-proof fence around the development corridor at the lower part of the site where highest reptile densities were found. The fence was ~50 cm high plastic with ~10 cm below the ground surface (Fig. 2A,B). The fencing was installed during January 2014 to minimise impact on mapped reptile hibernation sites, when reptiles were hibernating underground.
3. The removal of all reptiles within the developmental corridor to outside the fence, and the maintenance of the fence and the adjacent habitat during construction.

The hydroelectric development lasted from May to September 2014, with the fence removed during November 2014 when reptiles had returned to underground hibernation sites (Fig. 2C).

Results and conclusions

Surveys of adders from 2012 to 2015 in areas adjacent to the development corridor revealed over 200 individuals at the site, with 60-80 different snakes observed each year (Fig. 1; McInerny, 2016a). Importantly, similar numbers of animals were counted each year. Numbers did not decline over the survey period, and instead were stable, indicating that the hydroelectric development had not affected the population, at least in the short term. Furthermore, adders were observed mating and carrying developing young close to the development, showing that they were not disturbed by the noise and activity of the construction site.

GOLF COURSE POPULATIONS

Study site and survey work

The study site is an 18-hole golf course in the vicinity of Loch Lomond, about 50 hectares in size on a south facing slope, with managed areas of cut grass on tees, fairways and greens, interspersed with areas of bracken, gorse and brambles (Fig. 3; McInerny, 2016b).

Artificial cover objects were not used due to public access at the site and the possibility of interfering with rounds of golf, with reptiles identified by visual surveys about once a week from early February until November. The site was monitored from 2012 to 2016, with 10-20 visits each year. The location, number, maturity and gender of reptiles were recorded on each visit; these were recorded as day counts and are shown in Table 1, and plotted graphically in Fig. 4. Underground reptile hibernation sites were also mapped.

Fig. 2. Loch Lomond hydroelectric development at a site containing high densities of adders (*Vipera berus*) and other reptiles (McInerny, 2016a). (A) Arial view of the lower section of the construction site, August 2014. This shows the fenced area encompassing the construction site containing the partially built turbine powerhouse, the cleared areas where the underground water pipes had been laid, a movable heavy sheet "gate" placed across the access road, and the intact habitat surrounding the site. (B) Reptile-proof fencing that surrounded the construction site, February 2014; this was ~50 cm high with ~10 cm below the ground. (C) The completed turbine powerhouse, with fencing removed, November 2014. (Photos: C. McInerny)

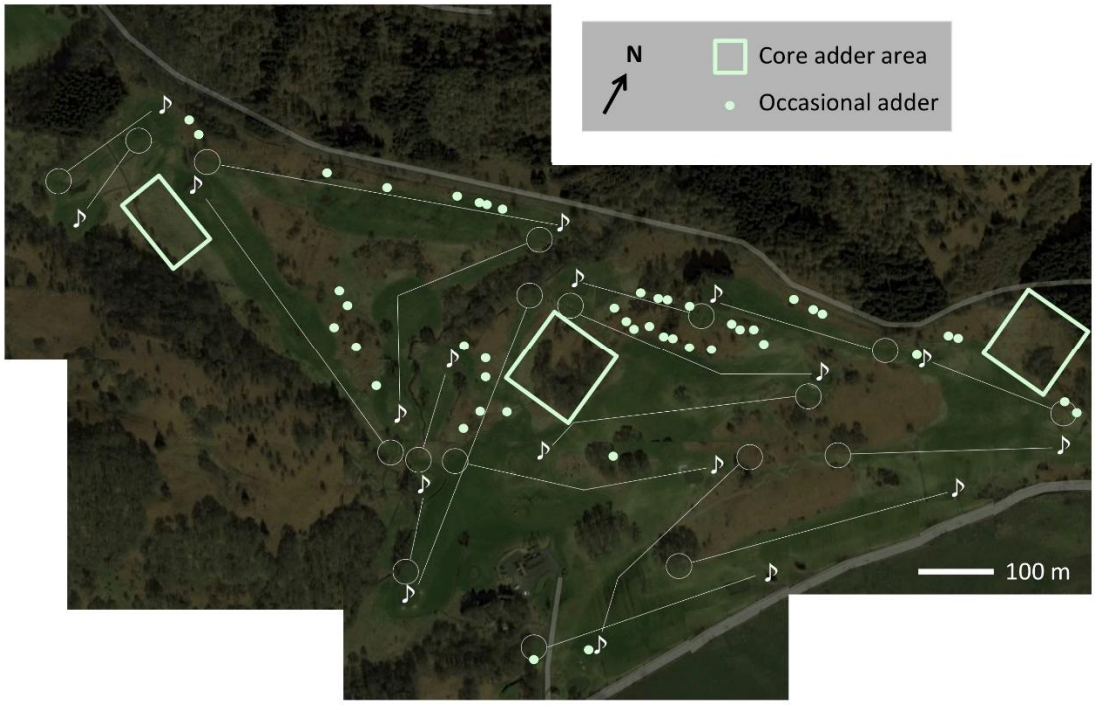

Fig. 3. Distribution of adders (*Vipera berus*) on a Scottish golf course, 2012 to 2016 (McInerny, 2016b). Core areas where snakes hibernated and were seen regularly are indicated by boxes; locations of occasional sightings are shown by dots. The layout of the golf course is mapped, with the 18 holes marked: open circles, tees; lines, fairways; ♪, greens and holes.

Visits		Day Counts	Individuals			
		Total	Total	Male	Females	Juvenile
2012	**20**	**36**	**19**	**6**	**11**	**2**
2013	**17**	**37**	**26**	**14**	**10**	**2**
2014	**16**	**77**	**39**	**13**	**22**	**4**
2015	**10**	**21**	**10**	**6**	**4**	**-**
2016	**14**	**36**	**23**	**13**	**10**	**-**

Table 1. Numbers of adders (*Vipera berus*) on a Scottish golf course, 2012 to 2016 (McInerny, 2016b). For each year, the number of visits, total of day counts, and the minimum numbers of individual adult males, adult females and juveniles are shown. Individuals were identified through their head-scale patterns. During the five-year period a minimum of 54 different snakes were recognised.

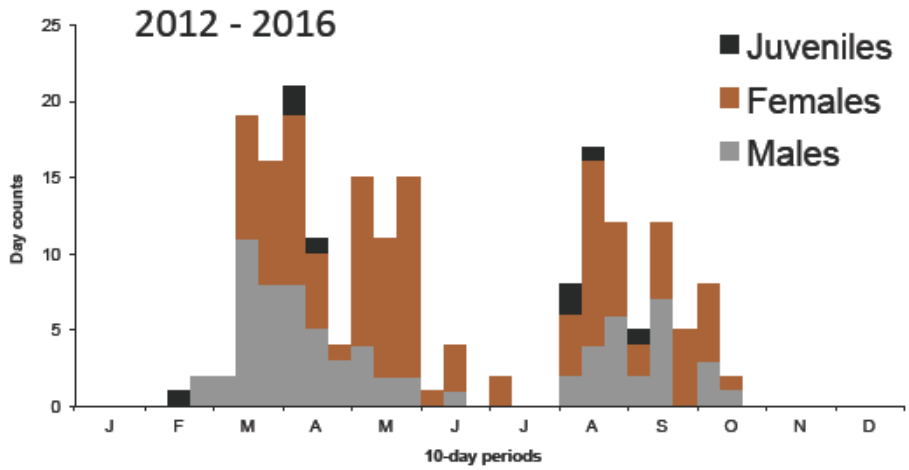

Fig. 4. Total numbers of adders (*Vipera berus*) on a Scottish golf course, 2012 to 2016 (McInerny, 2016b). Total day counts for 10-day periods over the five-year period for adult males (grey), adult females (brown) and juveniles (black) are plotted.

Results and conclusions

Surveys of adders from 2012 to 2016 revealed over 54 individual at the site, with 10-39 different snakes observed each year (Table 1; Fig. 4; McInerny, 2016b). Animals were noted throughout the golf course, though with concentrations in some areas where snakes were seen more regularly, usually near to hibernation sites (Fig. 3). Numbers were constant each year of the survey period, with young observed, suggesting that the life cycles of the adders were unaffected by the management practices of the golf course and that the population was being maintained at the site.

DISCUSSION

Reptiles in managed habitats

This paper describes two different projects, which considered populations of adders in habitats that have been influenced or modified by humans. In both cases the adder numbers were not affected and the populations persisted. These studies suggest that where appropriate actions are put in place or where habitat management is sympathetic, adders and humans can co-exist. In a world increasingly changed by mankind, these studies offer examples where humans and reptiles can live together, to the benefit of both.

Reptile habitats in Scotland

The studies described here identified habitats in Scotland which supported high densities of adders and other reptiles, including common lizards (*Zootoca vivipara*) and slow-worms (*Anguis fragilis*). These were open areas of bracken, gorse and brambles on south and west facing slopes, with small trees and bushes, often with burns and wet areas nearby. Many areas containing this habitat are present in the Loch Lomond National Park where the author has discovered a number of populations of adders, common lizards and slow-worms (McInerny, 2014b). Indeed, adders and other reptiles are found throughout mainland Scotland and some west coast islands (McInerny & Minting, 2016), often present in comparable habitat, which is widespread and familiar. In this context such habitat should be valued as a refuge for reptiles, and so protected and preserved.

ACKNOWLEDGEMENTS

I thank the landowners, wardens, the Club Secretary, golfers and groundsmen for permission and understanding to monitor reptiles at the two study sites. The survey work in 2014-2015 was supported by travel grants awarded by the Glasgow Natural History Society from the Professor Blodwen Lloyd Binns bequest, and Clyde Ecology.

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A study of the reptile populations in the Shielhill Glen, Clyde Muirshiel Regional Park, Scotland

K. McMillan

Greenock Cut Visitor Centre, Clyde Muirshiel Regional Park PA16 9LX

E-mail: Kathleenmcm07@yahoo.co.uk

INTRODUCTION

Scotland is home to three of Britain's native reptiles that have evolved to live in cooler climates: the common lizard (*Zootoca vivipara*), the slow-worm (*Anguis fragilis*) and the European adder (*Vipera berus*) (McInerny & Minting, 2016). Clyde Muirshiel Regional Park covers *ca.* 280 km^2 in the central lowlands on the west coast of Scotland, with habitats that appear suitable for reptiles. However, the species present and their numbers have not been established. Survey work, described here, showed that habitat Shielhill Glen, near the Greenock Cut Visitor Centre, encompassing both moorland and improved grassland, supports a population of common lizards, but slow-worms and adders were not detected.

METHODS

The study took place in Shielhill Glen, near the Greenock Cut Visitor Centre. A nature trail with a 45-minute circular walking path runs through the glen. The trail's boardwalk is a key feature of the study as it provides sites for basking reptiles.

The habitat in the glen around the boardwalk consists of a mosaic of different vegetation types: heather (*Calluna vulgaris*) and grazed open grassland, interspersed with areas of gorse (*Ulex europaeus*) and bracken (*Pteridium* spp.). On the north side of the glen is a Site of Special Scientific Interest (SSSI) of natural regenerating woodland.

Another key feature of the study area in the glen is a stone wall, which acts as a barrier for the sheep farmed on the moorland to prevent them from entering the SSSI. Because of their grazing patterns, the sheep control the growth of new plants and the height of existing plants.

The Shielhill Glen study area, comprising 15.1 ha, has five habitats that have the potential to support reptile populations. All are interconnected, some sharing ecotones or the stone wall. These habitats are: Habitat 1 - open grassland; Habitat 2 - heather (*Calluna vulgaris*) and moorland grasses; Habitat 3 - gorse (*Ulex* spp.); Habitat 4 - bracken (*Pteridium* spp.); and Habitat 5 - stone wall and SSSI.

Fifty artificial refugia, made from black roofing felts 50 cm x 50 cm in size, were placed in suitable locations, with ten in each of the five habitats areas. The felts were numbered and their locations recorded using global positioning system (GPS). Four corrugated iron "tins" were also placed in Habitat 1. Both materials were used to target different reptiles; adders prefer corrugated iron, whereas roofing felts are preferred by lizards (Langham, 2011). Published reptile survey methods were followed (Froglife, 1999).

The felts were inspected when weather conditions were suitable for locating reptiles: an air temperature 8 - 20°C, dry, with open cloud cover to allow the heat from the sun to reach the ground. Surveys times were influenced by weather conditions from 10 a.m. to 7 p.m. Air temperature was taken from the weather station at the Greenock Cut Visitor Centre at the start of each survey. The duration of each survey was recorded. At each felt the number of reptiles above and below was recorded, along with those seen in the surrounding vegetation, or on the adjacent walls or boardwalk.

RESULTS

Over four months, from June to September 2013, 46 survey days were completed. Only one native reptile species was recorded, with 150 sightings of the common lizard. No slow-worms or adders were found.

Lizard numbers

Lizard numbers were recorded as follows: 1) total number seen (all locations: felts, boardwalk, undergrowth); 2) number seen using the felts, both on top and under the felts; 3) number seen on top of felts; 4) number seen under felts; and 5) reptiles seen near the felt using the boardwalk, stone wall or undergrowth.

In total, 150 lizards were recorded. Of these 109 were found using felts, and 41 in other locations, including the stone wall, boardwalk and undergrowth. Out of the 109 reptiles using felts, only 66 were seen on felts with 43 under felts.

Eight surveys were carried out during June, with a total of ten reptiles being seen through the month (average of 1.25 lizards per survey). In July the number of surveys increased to 14 due to improved weather. As a result, the total number of reptiles seen rose to 20 (1.43 lizards per survey). During August 16 surveys took place with a total of 96 reptiles recorded (6.00 lizards per survey). This was due to weather conditions; also August is when young lizards are born. In September the number of reptiles seen was 24, over seven surveys (3.43 lizards per survey).

Lizard habitats

Habitat 1, open grassland

No reptiles were found. This area was grazed by sheep with the vegetation controlled, and no new growth.

Habitat 2, heather moorland

Thirty-two lizards were observed. Within this habitat, lizards were only found using felts, even though alternative basking sites were present.

Habitat 3, gorse

Twenty-three lizards were observed, in all five locations; 22 used felts, with only one lizard seen in another location.

Habitat 4, bracken

Fourteen lizards were observed, in all five locations. Of these, nine lizards used felts. The lower number of lizards within this habitat could be a result of the bracken covering basking sites.

Habitat 5, stone wall and SSSI

Eighty-one lizards were observed, the highest recorded, with 46 of these using felts.

DISCUSSION

Certain landscape features are important for reptiles and are referred to as "key features" (Bray *et al.,* 1997). These key features should be taken into consideration in management plans in areas where reptiles are present. The Shielhill Glen has many key landscape features for reptiles. However, only one species was found, the common lizard. A number of different habitats at the site were identified as supporting lizard populations: heather moorland, gorse and bracken, with the main population at a stone wall and SSSI.

It is unclear why adders or slow-worms were not found within the area. Studying other locations around the Greenock Cut Visitor Centre might reveal these two species. However, it is possible that none was found because they are absent. This could be due to the habitat being unsuitable, or the extinction of populations. Alternatively, the surveys may not have located the species, being at the incorrect time of the day and/or year. The surveys described here were conducted from 10 a.m. to 7 p.m. during June to September. In contrast, surveys for adders and slow-worms elsewhere in west Scotland have revealed highest numbers of both reptiles, monitoring from 8 - 10 a.m. during February to April (McInerny, 2017).

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Developing a strategy for the terrestrial amphibians and reptiles of Scotland

R. Raynor

Scottish Natural Heritage, Great Glen House, Leachkin Road, Inverness IV3 8NW

E-mail: Robert.raynor@nature.scot

INTRODUCTION

Scotland's amphibians and reptiles face a variety of threats, and in order to safeguard them for future generations we need to take a coordinated and planned approach. To facilitate this Scottish Natural Heritage (SNH) has been developing a more strategic approach for their future conservation. An outline "proto" strategy is presented, which describes the current situation, the main threats and opportunities, and the reasons why action is needed. The final version of the strategy will be an SNH document, but the intention is for it to form a framework for future herpetofauna conservation action in Scotland. Once finalised, an implementation plan will be produced to support delivery. With or without a strategy, amphibians and reptiles have intrinsic value in their own right as part of our natural heritage, playing an important role in ecosystems, so we have a moral responsibility to conserve them.

STRATEGY

The overall aim of the strategy is to support the delivery of healthy, self-sustaining populations of all native amphibians and reptiles, and to reverse previous declines where applicable. To achieve this, the strategy has three objectives:

1. To improve people's understanding and appreciation of amphibians and reptiles, reduce persecution and build public support for conserving them.
2. To address the range of ecological threats and pressures affecting Scottish amphibians and reptiles.
3. To improve the quality of information we hold on the distribution and status of amphibians and reptiles through appropriate surveillance and monitoring.

The strategy is intended to help guide the work of public bodies, business and voluntary organisations, recognising that a partnership approach between the relevant organisations is required if we are to achieve our objectives. At its core lies the *2020 Challenge for Scotland's Biodiversity* (Scottish Government, 2013) and the associated *Route Map to 2020*, from which many of the habitat-related actions are derived and the importance of ecological connectivity is highlighted. Projects designed to improve habitat connectivity for reptiles and amphibians contribute to this while benefiting other wildlife generally, and *vice versa*. Associated benefits to people also ensue where such projects enable improved access to the countryside and urban green space, thereby providing opportunities to connect people with nature. More specialist actions are also required, and these will be detailed in the implementation plan.

A key requirement is a better understanding of the current population status and trends of Scottish reptiles and amphibians, as well as the threats and pressures affecting them. The population status and trends of most species are incompletely understood and, although general long-term declines have been identified in the British herpetofauna since the early-mid 20th century (Beebee *et al.*, 2009), trends for most species in Scotland are less clear. So, while there is evidence of pond losses in the last century and land-use change, the lack of long-term systematic monitoring for most species means that the empirical evidence base for population declines and our understanding of the effects of perceived threats and pressures is often inadequate. Examples of species that are known to be in decline in Scotland include natterjack toad (*Bufo/Epidalea calamita*) (McInerny & Minting, 2016) and the European adder (*Vipera berus*) (Reading *et al.*, 1994; Wilkinson & Arnell, 2011). Where such knowledge gaps exist there is a need to implement appropriate measures to strengthen existing populations and promote recovery where declines are believed to have taken place.

The strategy recognises that amphibians and reptiles continue to face a variety of threats but highlights the following in particular:

- Habitat loss and fragmentation
- Pollution, eutrophication and pesticides
- Persecution and exploitation
- Non-native species
- Disease
- Climate change

Habitat loss and fragmentation are considered by the International Union for Conservation of Nature (IUCN) to be the most pervasive threats to amphibians globally. Reversing this through measures designed to improve connectivity and thereby encourage dispersal is regarded as central to achieving greater resilience amongst amphibian and reptile populations. In Scotland, the effects of habitat fragmentation may be most significant in lowland areas, where patches of semi-natural habitat exist within an intensively-farmed or industrial landscape, often with few or no links to other such patches, and the pressure on land for agriculture and urban development is at its greatest. By reinstating the links between these patches, increased gene-flow can take place, thereby countering the effects of isolation. For some species with more southerly distributions, improved habitat connectivity may also prove to be important in facilitating range expansion northward in response to climate change. Such expansion is predicted by climate envelope modelling for species such as the grass snake (*Natrix helvetica*) (Dunford & Berry, 2012).

To improve habitat connectivity the strategy refers to potential wildlife corridors, such as former dismantled railway lines, particularly in the Central Lowlands of Scotland, where they exist at high density in some areas. Some have already been re-developed as long-distance footpaths and/or cycle-ways (e.g. see Scottish Natural Heritage, Sustrans & Scottish Canals, 2014), but these uses need not conflict with their potential value for wildlife, provided sufficient habitat along the margins is retained and managed appropriately. The strategy points to the potential benefits of working with organisations responsible for the development and management of paths and cycle-ways constructed around the former railway network.

Other forms of collaboration are identified that have the potential to benefit amphibians and reptiles. There may be different objectives between collaborating organisations, but where common goals exist, benefits can be delivered for several target species. An example of this is *Roots of Rockingham,* a *Back from the Brink* project in Northamptonshire, led by Butterfly Conservation (Nature Back From The Brink, 2018). This aims to restore and manage a network of woodland sites, creating more habitat for vulnerable species – specifically the chequered skipper (*Carterocephalus palaemon*), but the habitat management work will also be of benefit to adders. Other opportunities like this need to be explored.

Underpinning all of this is the need to involve people, educate and raise awareness, and to generally improve our knowledge and understanding of the distribution and status of Scottish amphibians and reptiles.

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Consultation on SNH draft amphibian and reptile strategy

D.C. McNeill

Kelvin Building, University of Glasgow, Glasgow, G12 8QQ

E-mail: Deborah.Mcneill@glasgow.ac.uk

INTRODUCTION

The conference *Amphibians and Reptiles of Scotland: current research and future challenges* took place on Saturday 9th June 2018 as part of the Glasgow Science Festival. On behalf of Scottish Natural Heritage (SNH), Rob Raynor presented a talk on the SNH draft amphibian and reptile strategy for Scotland. The threats, opportunities and challenges for Scottish herpetological conservation were explored, with a recognition of the importance of effective partnership with relevant organisations to meet conservation objectives. This was followed by three facilitated workshops to enable discussion and comment on the draft strategy.

An audience of over 80 participants took part with representation from key herpetological and conservation organisations, research staff and students, consultancies and interested public. Fifty responses were collated by facilitators/scribes.

THE WORKSHOPS

Participants attended one of three workshops of their choosing, each on one of the draft strategy's priorities:

1. To improve people's understanding and appreciation of amphibians and reptiles, reduce persecution and build public support for conserving them.
2. To address the range of ecological threats and pressures affecting Scottish amphibians and reptiles.
3. To improve the quality of information we hold on the distribution and status of amphibians and reptiles through appropriate surveillance and monitoring.

The following questions were addressed:

1. Are these the right priorities?
2. Can we rank them?
3. Are there any key omissions? (These need to be justified.)
4. How deliverable are they and who would be responsible for their delivery?

WORKSHOP OUTCOMES

Each of the three workshops featured a list of priorities, which are shared below, ranked in order from *most important* to *of least concern* by all participants. A summary of comments pertaining to these priorities was collated.

Workshop 1

Participants ranked the following first three points as equally important. It was also deemed that there was overlap between the questions and as such, they were commented on together.

1a) Increase the number of people actively involved in conservation projects of benefit to amphibians and reptiles.

1b) Increase public understanding and appreciation of amphibians and reptiles.

1c) Promote practical conservation measures involving volunteers and members of the local community to benefit amphibians and reptiles on land owned or managed by Non-Governmental Organisations (NGOs), local authorities and statutory undertakers and on private estates.

Discussion: Participants raised resource issues: for example, the shortage of local authority biodiversity officers needed to develop Local Biodiversity Action Plans (LBAPs), and the comparative shortage of funds for conservation work (in Scotland compared with England). Points on public understanding stressed the need to choose audiences carefully, to use evidence-based approaches, and to be subtle in approaches aimed at gaining public support for amphibians and reptiles.

2a) Promote collaboration amongst conservation organisations to identify synergies between unrelated species-focused projects and strategies of potential benefit to amphibians and reptiles, as illustrated by the *Roots of Rockingham Project* (Nature Back From The Brink, 2018) and the SNH *Pollinator Strategy 2017-2027* (SNH, 2017).

Discussion: Comments supported the idea that strategic priorities could provide a clearer focus for NGO activities. However, there was concern over inconsistency and frequent changes in plans and reporting requirements, especially for LBAPs.

2b) Promote more widespread adoption of amphibian-friendly measures to reduce mortality associated with roads and road drains.

Discussion: This aim was widely agreed, with comments emphasising the need for improved legal protection of amphibians and incorporation of amphibian mitigation into the planning process. Common toad (*Bufo bufo*) patrols provide effective public engagement as well as protection.

3) Reduce persecution of European adders (*Vipera berus*) and slow-worms (*Anguis fragilis*).

No comments were recorded.

Additional comments: Participants emphasised the political battle needed to improve amphibian and reptile conservation in Scotland; the lack of attendance by any Member of the Scottish Parliament (MSP) Species Champions or their staff was telling.

Workshop 2

1) Review how best to optimise habitat connectivity in both urban and rural areas in relation to important amphibian and reptile sites, and work with key stakeholders to integrate their needs into infrastructure schemes and initiatives.

2) Promote the inclusion of more focused habitat management options designed to benefit amphibians and reptiles within future forestry and agri-environment funding schemes.

Discussion: It was felt that current government priorities for forestry and agricultural development are a potential threat to amphibians and reptiles unless their needs are carefully considered. Agri-environment schemes rarely focus on amphibians and reptiles, but this could change in well-chosen areas.

3) Ensure that key best practice guidance is up-to-date and, if necessary, revised with an appropriate Scottish emphasis.

Discussion: Participants would welcome an easily accessible source of comprehensive and up-to-date best practice guidelines hosted on e.g. the SNH website. The question was asked as to whether there could be better connectivity with biodiversity guidelines for Sustainable Drainage Systems (SuDS) and urban development, produced by SNH, the Scottish Environment Protection Agency (SEPA) and Scottish Water.

4) Improve our understanding of how upland land management activities such as spring muirburn may impact on reptiles and amphibians and develop appropriate mitigation guidance as appropriate.

5) Assess benefits of green infrastructure to reptiles and amphibians and how predicted effects of climate change can be mitigated through these and other measures to improve habitat connectivity in general.

Discussion: One example explored was a targeted approach to communicating potential biodiversity benefits of SuDS to developers.

6) Promote awareness of disease risks and remain vigilant, ensuring an appropriate and rapid response in accordance with the Invasive Non-Native Species (INNS) code of practice (Scottish Government, 2012) to suspected outbreaks of harmful infectious diseases.

7) Build on previous studies to better understand how climate change might affect Scotland's amphibians and reptiles.

8) Promote a responsible attitude towards species translocations generally, particularly in relation to the assisted movement of fish between water bodies.

9) Remain vigilant and ensure an appropriate and rapid response in accordance with the INNS code of practice to reports of any new non-native species of amphibian or reptile in Scotland. This includes on-going monitoring of existing non-native populations and the identification of potential new threats.

10) Promote the guidance on biosecurity provided by Amphibian and Reptile Groups of U.K. (ARG UK, 2017).

Additional comments: Participants discussed the need to identify and address current knowledge gaps. A better understanding of the effectiveness of herpetological sites that are currently afforded protection will offer insights into e.g. where resources might be best utilised for improvement. Utilising tools such as GIS mapping of key populations can feed into predictive mapping to identify potential new sites, underpinned by a programme of baseline population surveys. One suggestion was to link the strategy to appropriate regulatory bodies so SNH is not solely responsible for guidance and regulation.

Workshop 3

1) Increase collaboration between the key organisations involved in amphibian and reptile conservation and build partnerships with other organisations that can contribute to recording and monitoring schemes.

Discussion: There was consensus that the partnership landscape should extend to include other wildlife and conservation organisations. There is a need to map out this landscape to identify who these stakeholders could be. This should include community partners, e.g. churches, schools.

2) Maintain and, where possible, expand the existing surveillance and monitoring schemes and improve coverage.

3) Review the distribution of active volunteers involved in amphibian and reptile recording with a view to targeting areas with poor such representation for future training in identification and monitoring.

Discussion: With reference to priorities 2 and 3, participants discussed shared experiences around the difficulties in recruiting and maintaining a large enough cohort of volunteers across the required geographical range. This can lead to "volunteer fatigue", where a minority of volunteers may be undertaking significant amounts of monitoring, often across a range of projects and priorities. Potential new sources of volunteers were explored, including targeting clubs and groups of people who regularly access the underserved geographical locations, e.g. hill walking clubs, rambling groups. This would require training and/or tools to make monitoring and recording easy and accessible, e.g. Froglife's *Dragons on the Hills* app. This raised the question as to who would be responsible for the delivery of the monitoring schemes and a reflection on whether schemes could be expanded to include monitoring for disease and genetic bottlenecks.

4) Ensure that all data collected through monitoring schemes are accessible via Scotland's Environment Web (SEWeb).

Discussion: It was noted that there are ongoing discussions around the creation of a central biological recording system which will be widely accessible. Concern was voiced around information on sensitive species being available publicly.

5) In light of concerns about adder populations in Scotland, clarify the status of the species and assess any changes since 1994.

6) Clarify the status of grass snakes (*Natrix natrix*) in Scotland and their likely provenance.

7) Expand the use of eDNA sampling in great crested newt (*Triturus cristatus*) surveillance to provide reliable trend information for Scotland, and trial the use of meta-barcoding eDNA analysis to enable the inclusion of additional species in the surveillance.

Discussion: The method is expensive (£100-£200 per sample) and requires a standardisation of techniques. To support this priority, funding may be available from ScotRail for biodiversity schemes near railway lines. Reference sequences are available on public databases, e.g. Genbank.

Additional comments: There is an untapped resource from surveys carried out by industry and private companies, which is currently not fed into the biological records centres. These, however, are too often regarded as commercially sensitive and not made available.

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Infectious disease threats to amphibian conservation

A.A. Cunningham

Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY

E-mail: A.Cunningham@ioz.ac.uk

ABSTRACT

The unexplained decline of amphibian populations across the world was first recognised in the late 20th century. When investigated, most of these "enigmatic" declines have been shown to be due to one of two types of infectious disease: ranavirosis caused by infection with FV3-like ranavirus or with common midwife toad virus, or chytridiomycosis caused by infection with *Batrachochytrium dendrobatidis* or *B. salamandrivorans*. In all cases examined, infection has been via the human-mediated introduction of the pathogen to a species or population in which it has not naturally co-evolved. While ranaviruses and *B. salamandrivorans* have caused regionally localised amphibian population declines in Europe, the chytrid fungus, *B. dendrobatidis*, has caused catastrophic multi-species amphibian population declines and species extinctions globally. These diseases have already caused the loss of amphibian biodiversity, and over 40% of known amphibian species are threatened with extinction. If this biodiversity loss is to be halted, it is imperative that regulations are put in place – and enforced – to prevent the spread of known and yet-to-be discovered amphibian pathogens. Also, it is incumbent on those who keep or study amphibians to take measures to minimise the risk of disease spread, including from captive animals to those in the wild.

INTRODUCTION

When the first *World Congress of Herpetology* was held in Canterbury, Kent in 1989, no papers were presented about the amphibian conservation crisis. It was only during social interactions at coffee and tea breaks and in the evenings that many herpetologists from around the world realised that it was not only their own study species that appeared to be undergoing declines or even local disappearances, but also those being studied by many of their colleagues. Whilst "localised" declines were initially thought to be due to natural population cycles or local, short-term factors such as extreme weather events, it soon became clear that there was a larger issue affecting amphibians and a call for action was made.

In response, the International Union for the Conservation of Nature (IUCN) set up the Declining Amphibian Populations Task Force (DAPTF) to investigate if the reported declines of amphibians was a true phenomenon and, if so, what was, or were, the cause(s) of it. The DAPTF brought together experts from across the world and from across disciplines to promote research into amphibian declines and to collate and evaluate evidence that showed amphibians were undergoing unprecedented declines around the world including in protected areas and in pristine habitats. Indeed, it is now known that 41% of known amphibian species are threatened with extinction, which is a much higher percentage than for mammals (25%) and over three times the percentage for birds (13%) (IUCN, 2018). Perhaps just as worrying is that over 61% of known amphibian species are either "Not Evaluated" or have out-of-date assessments; this compares to just 0.2% of mammals and 0% of birds (Tapley *et al*., 2018).

Initially, habitat destruction, overexploitation, excessive UV-B irradiation, pesticide use, acid rain and other pollutants were all put forward as likely causes of the amphibian decline phenomenon. Through the work of the DAPTF's Diseases and Pathology Working Group, however, infectious disease - and one in particular, chytridiomycosis due to infection with the non-hyphal, zoosporic fungus, *B. dendrobatidis* - was identified as elevating amphibian mortality rates and driving amphibian population declines across multiple continents (Cunningham, 1998). This fungus was discovered contemporaneously as the cause of multi-species mortality of wild amphibians where frog populations were declining catastrophically in the rain forests of Panama and Australia (Berger *et al.,* 1998) and was named following its isolation from a captive blue poison dart frog (*Dendrobates azureus*) (Longcore *et al*., 1999).

While previously dismissed as being irrelevant to species demographics, in recent decades infectious disease has been increasingly identified as a driver of species declines and extinctions (Daszak *et al.,* 2000; Cunningham *et al*., 2017). For amphibians, in particular, there is now substantial and irrefutable evidence of the role of infectious disease in multiple species declines and in some species extinctions (e.g. Schloegel *et al*., 2006; Skerratt *et al*., 2007). The

presence of disease, however, should not always be seen as a negative thing. Disease-causing agents, such as pathogens, are important components of ecosystems, often crucial for the regulation of species abundances and for ecosystem function; for example, the experimental removal of fungal pathogens from rain forest plants has been shown to adversely affect biodiversity through the reduction of species richness (Bagchi *et al*., 2014). When infectious disease threatens species conservation it is inevitably a consequence of human-mediated factors that have decreased the resilience of a species to a pre-existing pathogen, or that have introduced a "novel" pathogen to a naïve species (Cunningham *et al*., 2003).

When pathogens are in the "wrong" place (i.e. in species or locations in which they did not co-evolve), their presence can lead to adverse effects on biodiversity and ecosystem function (Cunningham 1996; Daszak *et al.,* 2000; Cunningham *et al.,* 2017). This usually is consequent to human-mediated introduction: a process which has been termed "pathogen pollution" (Cunningham *et al.,* 2003). The introduction of pathogens can have long-term, profound and unpredictable impacts on nature. One example of this is the introduction of myxomatosis to the rabbit (*Oryctolagus cuniculus*) population in the United Kingdom (U.K). Who could have predicted that, through complex interactions between rabbits, grasses, wild thyme (*Thymus polytrichus*), and a red ant (*Myrmica sabuleti*), that the introduction of a rabbit virus would, over a course of 30 years, lead to the extinction of the large blue butterfly (*Maculinea arion*) in the U.K. (Sumption & Flowerdew, 1985)?

Until recently, amphibians were very much the "Cinderella" of the wildlife disease world, with very little attention being given to identifying, understanding or mitigating the causes of amphibian morbidity or mortality. Many advances in this field have been made in the past 30 years or so. However, although many infectious diseases of amphibians are now recognised – and there are undoubtedly many more waiting to be discovered – only two have been shown to cause amphibian population declines in wild populations: ranavirosis and chytridiomycosis. The key points about these diseases, with particular reference to the situation in the U.K., will now be reviewed.

AMPHIBIAN RANAVIROSIS

Amphibian ranavirosis is caused by infection with any one of a large number of different types of virus in the genus *Ranavirus* (known as ranaviruses) that can infect amphibians. First identified as a cause of amphibian mortality in the 1960s when the mortality of North American bullfrog (*Lithobates catesbeianus* – formerly *Rana catesbeiana*) tadpoles was investigated (Wolf *et al.,* 1968), over recent years there has been a large increase in the number of ranaviruses, and ranavirus-related mortality incidents, described globally (Duffus *et al.,* 2015). Of these, only two have been shown to cause long-term amphibian population declines: a frog virus 3-like ranavirus in the U.K (Teacher *et al.,* 2010) and common midwife toad virus (CMTV) in Spain (Price *et al.,* 2014). Ranavirus-associated amphibian mortality was first detected in the U.K. in the early 1990s, although it had probably been occurring for some years before this (Drury *et al.,* 1995; Cunningham *et al.,* 1996). Large disease outbreaks involving the deaths of tens or hundreds of animals, usually the common frog (*Rana temporaria*), but also the common toad (*Bufo bufo*), were reported (Cunningham *et al.,* 1996; Cunningham *et al.,* 2007). In most cases, outbreaks affected adult animals, occurred in the summer months and were peracute (i.e. very short period from first signs of illness to death), with a large number of dead animals being found in the vicinity of a breeding pond over a short period of time (hours or days) (Fig. 1). In the U.K, two types of disease (or syndrome) have been described due to ranavirus infection: haemorrhagic syndrome and skin-ulceration syndrome (Cunningham *et al.,* 1996; Cunningham, 2001). With haemorrhagic syndrome, affected animals – although dead - usually appear normal from the outside, although bleeding might be seen from the mouth or vent and the skin can appear reddened, especially on the underside of the hind legs and body. On post mortem examination, animals are often in good body condition but with evidence of bleeding throughout most of the body systems, including into the gastro-intestinal tract, reproductive tract, skeletal musculature, and even into the fat bodies (stores). These animals are killed very quickly by the virus; usually within a matter of hours after the first onset of illness. Animals which develop skin-ulceration syndrome, however, can be ill for weeks or even months before they die. These animals develop skin ulceration, which can be extensive and which can occur over any part of the body but is most frequently seen over the ventral hind legs (Fig. 2). Affected animals can also undergo necrosis (death) of the limbs. While, if present, this usually affects the hind and fore feet, it can extend higher up the limbs to reach the body. During the period of illness, animals stop feeding and become listless and lethargic. By the time they die they are usually in very poor body condition and sometimes are emaciated. There is, however, some evidence that a small proportion of common frogs affected with skin-ulceration syndrome might recover as apparently-healthy animals have been found a year after an outbreak with scarring indicative of healed skin ulcers (Cunningham, 2001).

Molecular characterisation of the FV3-like virus causing mortality in British frogs showed the virus to be indistinguishable from FV3-like ranaviruses in the U.S.A., and to be distinct from those infecting amphibians or fish elsewhere, including in continental Europe (Hyatt *et al.,* 2000). It has, therefore, been suggested that the virus is a recent

Fig. 1. Mass mortality of common frogs (*Rana temporaria*) in the U.K. due to ranavirus infection. Affected animals in such mass mortality events exhibit systemic haemorrhagic disease (see text). (Photo: Frog Mortality Project)

Fig. 2. A dead common frog (*Rana temporaria*) with lesions typical of the skin ulceration form of ranavirosis. Note the long, thin (linear) ulcer on the animal's left ventral thigh and the loss of digits on the right forefoot along with part of that foot. (Photo: Zoological Society of London)

incursion into the U.K., possibly along with North American bullfrogs, which were imported into the U.K. by the pet trade in the 1980s: this species is known to be a carrier of FV3-like ranaviruses (Cunningham, 2001).

Teacher *et al.* (2010) compared frog populations at 18 ranavirus-positive sites with recurring frog mortality with those at 16 ranavirus-negative sites where no unusual mortality events had been reported. Over the period 1996 to 2008, there was no overall change in population size at the ranavirus-negative sites, but at the ranavirus-positive sites there was an overall decline in the frog populations of 83%. It is clear, therefore, that in addition to causing one-off mass-mortality events, ranavirus can lead to long-term population declines of the common frog in the U.K.

The ranavirus, CMTV, was first described as a cause of systemic haemorrhagic disease causing high mortality in tadpoles of the common midwife toad (*Alytes obstetricans*) in the Picos de Europa National Park in northern Spain (Balseiro *et al.,* 2009). Subsequent to the initial disease outbreak, CMTV, which is in a sister clade to FV3-like ranaviruses, caused recurring multi-year disease outbreaks in multi-species amphibian assemblages in the National Park, leading to long-term, severe population declines with no evidence of recovery over at least a five-year period (Price *et al.,* 2014).

AMPHIBIAN CHYTRIDIOMYCOSIS

Amphibian chytridiomycosis is the name given to disease in an amphibian caused by infection with a chytrid fungus. To date only two such fungal pathogens of amphibians are known: *Batrachochytrium dendrobatidis* and *B. salamandrivorans*. *B. dendrobatidis* specifically infects keratinised cells; these cells form the outer surface of the skin of metamorphosed amphibians and the mouthparts of some anuran larvae. While infection of the latter can cause loss of mouthparts and possible delayed development of larvae, generally it is infection of metamorphosed animals that causes mortality. Ironically, infected larvae can survive to metamorphose, only to then develop a skin infection and die, usually within 2 - 3 weeks of metamorphosis. As *B. dendrobatidis* is an intracellular pathogen, meaning that it invades, lives and grows within amphibian cells, the infection fails to elicit a noticeable inflammatory cell response yet interferes with normal skin function, in particular with osmoregulation (the skin's ability to regulate electrolytes within the body) (Voyles *et al.,* 2010). This leads to ion imbalances, one of the most dangerous of which is a decrease in the concentration of potassium ions in the blood. Reduced potassium levels lead to abnormal electrical activity in the heart, with eventual cardiac arrest (Voyles *et al.,* 2009). *B. salamandrivorans* also infects amphibian skin, often causing skin ulceration, but how it kills its host is currently unknown (Martel *et al.,* 2013).

B. dendrobatidis was discovered in the 1990s consequent to the DAPTF Diseases and Pathology Working Group disseminating the latest results and then bringing researchers and their findings together from across the world (Berger *et al.,* 1998; Cunningham, 1998; Longcore *et al.,* 1999). This was the first time a cause of unexplained amphibian declines had been described and, although at the time many herpetologists were sceptical that an infectious disease could be the cause, the weight of subsequent research has dismissed any such doubt. For example, a close association has been shown between the passage of a north-to-south wave of amphibian declines through Central America and a (retrospectively determined) contemporaneous wave of first identification of *B. dendrobatidis* in amphibians collected across the region (Lips *et al.,* 2006). The introduction of the pathogen to the Caribbean islands of Dominica (in 2002) and Montserrat (in 2009) led to the rapid and almost complete extirpation of the mountain chicken frog (*Leptodactylus fallax*) due to chytridiomycosis (Hudson *et al.,* 2016) (Fig. 3). In Montserrat, the absence of *B. dendrobatidis* had been established in the early 2000s (Garcia *et al.,* 2007), so its emergence on the island must have been a result of pathogen introduction, and this was almost certainly the case also in Dominica (Hudson *et al.,* 2016). There is compelling evidence that *B. dendrobatidis* infection is

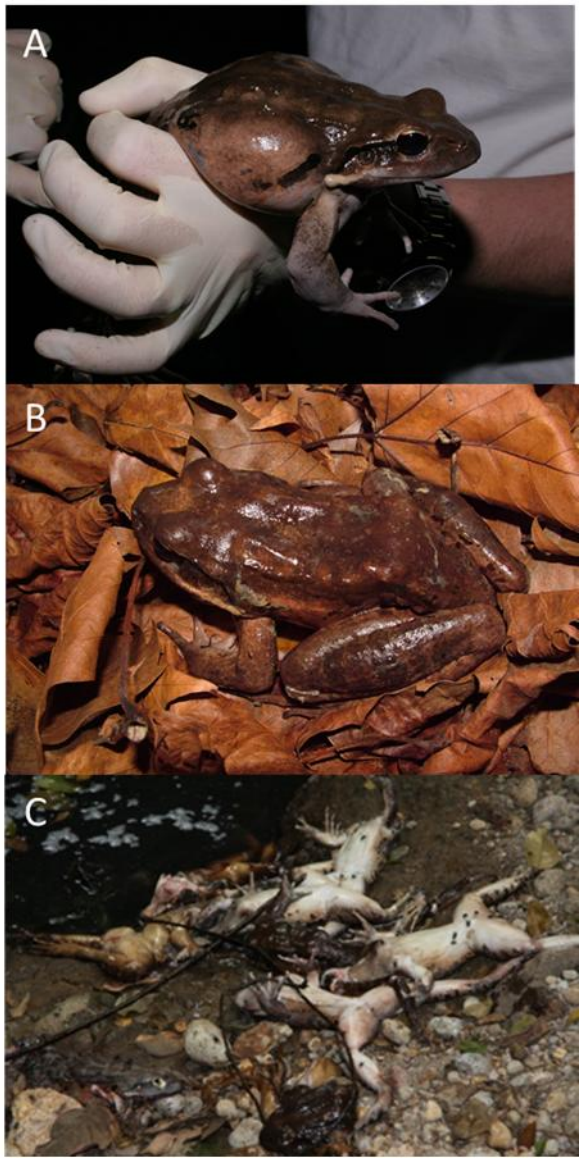

Fig. 3. Mountain chicken frog (*Leptodactylus fallax*). (A) A healthy individual in Dominica. (B) An individual with chytridiomycosis due to *Batrachochytrium dendrobatidis* infection. (C) Dead individuals in Dominica killed by chytridiomycosis. (Photos: Zoological Society of London and A. James)

a driver of southern Darwin's frog (*Rhinoderma darwinii*) declines in Chile, South America (Soto-Azat *et al.,* 2013; Valenzuela-Sánchez *et al.,* 2017), where it is also an introduced pathogen (Valenzuela-Sánchez *et al.,* 2018; O'Hanlon *et al.,* 2018). Indeed, infection with *B. dendrobatidis* has now been identified as a cause of amphibian mortality and population decline on every continent where amphibians exist. In the U.K., the pathogen has been commonly found in captive amphibians in which it sometimes has been found to be a cause of mortality, whilst nationwide surveys of *B. dendrobatidis* have shown wild amphibians to be infected with the pathogen at multiple sites across mainland Great Britain, but without obvious signs of disease (author's unpublished observations). Further studies involving long-term population monitoring

and infection surveillance are required to determine if native British amphibians are unaffected by *B. dendrobatidis* infection, or if the pathogen is causing the long-term decline of any species in the same, cryptic, way that it is doing so with the southern Darwin's frog in Chile.

As research into *B. dendrobatidis* has progressed, it has become apparent that there are multiple lineages of the fungus, of which infection with one – termed the global pandemic lineage (BdGPL) – is responsible for almost all known cases of amphibian population decline due to chytridiomycosis resulting from infection with *B. dendrobatidis*. The natural area of endemicity of *B. dendrobatidis*, appears to be South East Asia; more specifically, the Korean peninsula (O'Hanlon *et al.,* 2018). Movement of the pathogen from this geographical location probably occurred inadvertently with the growth in the international trade of amphibians. It has been hypothesised that this led to different lineages of *B. dendrobatidis* coming into contact with each other, probably through the co-infection of amphibians, and hybridising to form BdGPL (Farrer *et al.,* 2011). Indeed, Farrer *et al.* (2011) used molecular clock analyses to date the emergence of BdGPL to around the time of the emergence of the global amphibian trade. Using data from a much larger number of isolates of *B. dendrobatidis* collected from around the world, O'Hanlon *et al.* (2018) have since verified these results, concluding that BdGPL emerged during the early 20th century, when the long-distance international trade in amphibians was becoming established. It is likely that, subsequent to the emergence of BdGPL, it was spread globally via the international amphibian trade, which is now huge in both monetary and volume terms, and which is largely unregulated and unrecorded (e.g. Schloegel *et al.,* 2010; Peel *et al.,* 2012; Wombwell *et al.,* 2016). While thought to be a hybrid itself, there is recent evidence that BdGPL is hybridising with other *B. dendrobatidis* lineages (e.g. in Brazil and in South Africa) and, where it is doing so, the hybrid lineage is even more virulent to amphibians than BdGPL (Greenspan *et al.,* 2018; O'Hanlon *et al.,* 2018). Thus, even if a country or geographical region is known to be positive for *B. dendrobatidis*, it is important to prevent the movement and mixing of lineages as this could facilitate even more catastrophic amphibian population declines through the creation and spread of hybrids.

B. salamandrivorans was discovered in 2013, when it was identified as the cause of fire salamander (*Salamandra salamandra*) mortality, with subsequent population extirpation, in the Bunderbos forest on the Netherlands-Belgium border (Martel *et al.,* 2013). It has since spread to Belgium and western Germany, where it is also causing localised extinctions of the fire salamander and possibly also other species of urodele (Spitzen-van der Sluijs *et al.,* 2016). Unlike *B. dendrobatidis*, which has been shown to infect and kill species in all three orders of the Amphibia (Anura, Urodela and Gymnophiona) (Doherty-Bone *et al.,* 2013; Gower *et al.,* 2013), to date *B. salamandrivorans* is known only to kill urodeles (newts and salamanders) – although it is known to be able to infect at least some members of the Anura, hence they might also be able to act as vectors of the pathogen (Martel *et al.,* 2014; Stegen *et al.,* 2017). As with *B. dendrobatidis*, in the locations where it is causing amphibian mortality and declines, *B. salamandrivorans* is an introduced pathogen; its area of natural endemicity is south east Asia (Laking *et al.,* 2017; Martel *et al.,* 2014) and it is most likely to have been introduced into Europe via the amphibian pet trade (Martel *et al.,* 2014; Nguyen *et al.,* 2017; Yuan *et al.,* 2018).

Despite extensive surveillance, *B. salamandrivorans* infection has not yet been found in any wild amphibian in the U.K. (author's unpublished observations), but the infection has been found to be widespread in captive collections in Great Britain and elsewhere in Western Europe (Fitzpatrick *et al.,* 2018; Sabino-Pinto *et al.,* 2018) (Fig. 4). At least one native British amphibian, the great crested newt (*Triturus cristatus*), is known to be highly susceptible to lethal infection with *B. salamandrivorans* (Martel *et al.,* 2014), therefore measures should be taken to prevent the spread of this pathogen into wild amphibian populations.

Fig. 4. A captive Bosca's newt (*Lissotriton boscai*) in the U.K. with chytridiomycosis due to *Batrachochytrium salamandrivorans* infection. Note the emaciated body condition and lack of grossly visible skin lesions. (Photo: L. Fitzpatrick)

CONCLUSIONS AND RECOMMENDATIONS

Since the mid-1990s, there has been a growing body of evidence that infectious diseases caused by chytrid fungi and ranaviruses have been causing unprecedented rates of mortality and population declines of amphibians. While ranaviruses and *B. salamandrivorans* have caused regionally localised amphibian population declines in Europe, *B. dendrobatidis* has caused catastrophic multi-species amphibian population declines and species

extinctions globally (e.g. Skerratt *et al.,* 2007). The identification of different lineages of *B. dendrobatidis*, including the emergence of multiple hyper-virulent hybrids (including BdGPL, which is thought to have emerged in the early 20th century) resulting from the human-mediated movement of these fungi around the world, is of particular concern. Thus, even if populations of wild amphibians appear to be stable while infected with *B. dendrobatidis*, an incursion of a different lineage (or hybrid) could result in declines due to chytridiomycosis. Also, the occurrence of a stable host-pathogen system – as appears to be the case in the U.K. - could be deceptive, as has been shown for the southern Darwin's frog in Chile (Valenzuela-Sanchez *et al.,* 2017).

In order to protect amphibian populations and diversity, therefore, it is important that steps are taken to prevent the national or international spread of amphibian pathogens, and particularly their incursion into the wild. Both *B. dendrobatidis* and ranaviruses were listed by the World Organisation for Animal Health (OIE), meaning that, under World Trade Organisation agreements, governments are allowed to put measures in place to prevent the intentional or unintentional import of these pathogens into their countries (Schloegel *et al.,* 2010). This was the first time that any pathogen had been listed on the grounds of biodiversity protection; previously all pathogens were listed to protect livestock or public health. Despite this listing, no government has actually enacted or enforced protection measures against these amphibian pathogens.

More recently, the European Commission has implemented controls to prevent the spread of *B. salamandrivorans* within the European Union (EU). Measures include the requirement for amphibians to be quarantined and to test negative (using a pathogen-specific qPCR; Blooi *et al.,* 2013) for *B. salamandrivorans* infection before entry to the EU or before being traded across national borders within the EU (European Commission, 2018). At the time of writing, however, in the U.K. (and most probably elsewhere in the EU), there are no official quarantine facilities, testing laboratories or methods of certification that animals are free of infection. Also, there are no measures in place for the enforcement of these controls.

Public pressure is required in order to encourage politicians and other policy makers to take infectious disease threats to amphibian conservation seriously and to bring in, and enforce, regulations to minimise the spread and impact of these diseases. There are, however, a number of steps that herpetologists, ecology consultants and others can take to minimise their role in the introduction and spread of diseases to new locations or amphibian populations. These include taking simple biosecurity measures to prevent infections from spreading from captive amphibians to those in the wild and measures to minimise the chances of spreading diseases from one wild population to another.

Steps to prevent the spread of infectious diseases from captive to wild animals are summarised in Table 1. These are detailed in a leaflet which has been distributed by the pet trade, herpetological and conservation non-governmental organisations, and the government which is available from https://www.ornamentalfish.org/wp-content/uploads/2015/07/Amphibian-disease-alert_June-20151.pdf

Steps to prevent the spread of infectious diseases between different wild amphibian populations are detailed in the Amphibian and Reptile Groups' Advice Note 4: Amphibian Disease Precautions: A Guide for UK Fieldworkers, which is available at www.arguk.org/ Additional information about biosecurity procedures when visiting amphibian habitats is available on the "BsalEurope" web site http://bsaleurope.com/hygiene-protocols/

It is up to us all – whether we work with amphibians on a professional basis, keep them as pets or as a hobby, or just appreciate their existence – to do whatever we can to halt the rising tide of amphibian extinctions, so long as those actions are based on evidence and sound science. Actions, such as disinfecting equipment between enclosures or disinfecting footwear between amphibian habitats in the wild, might appear small but they all play important and significant roles in reducing disease spread and pressure on amphibian populations.

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Table 1. Biosecurity measures that should be taken to minimise the chances of infectious disease spread from captive to wild amphibians.

Source of Disease Threat	Biosecurity Measures
Captive amphibian trade	· Ideally, only obtain amphibians that have tested negative for chytrid fungi. · Even if the infection status of the new animals is known, always quarantine new arrivals and screen for chytrid infections on arrival. · Any positive animals should be treated under veterinary supervision and test negative before being added to your collection.
Keeping captive amphibians	· Do not assume that a healthy looking animal is free of infection; some animals can act as carriers without exhibiting signs of disease. · Adopt the precautionary principle and manage all amphibians as if they are infected. · Know the health status of your collection. Get your animals tested for chytrid fungi and ensure any dead amphibians are submitted for post mortem examination, including testing for chytrid fungi and ranaviruses.
Maintenance procedures for amphibians in captivity	· Do not clean tanks or vivaria outside where there is a possibility of contaminating areas used by wild animals. · To avoid spreading disease within a collection, disinfect equipment between enclosures or have dedicated equipment for each enclosure. · Equipment and furnishings should be regularly cleaned and disinfected. · Disinfect all waste water from amphibian enclosures. Bleach, Virkon, F10 and Anigene are some disinfectants that will kill the majority of amphibian pathogens provided the manufacturers' guidelines are followed. · All waste water, once disinfected, should be discharged down a drain connected to a sewer. · Substrates (soil, sand, gravel, etc.) can harbour infections and should be discarded carefully. Ideally these should be sent for incineration by a registered company that can dispose of clinical waste (e.g. those used by veterinary practices). If this is not possible, disinfect and then dispose with the household refuse for collection by your local authority.
Enclosures	· Avoid keeping amphibians in outdoor enclosures as they may come into direct or indirect contact with native wild amphibians and infect them with disease agents (even if the captive animals appear healthy).
Contact with wild amphibians	· Never share equipment, such as nets, between amphibians in captivity and those in the wild. · Never release any (native or exotic) amphibians from captivity into the wild. (This includes releasing into a garden pond, as this will also enable any pathogens present to get into the wild.)

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