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Outcomes and lessons from a quarter of a century of Sand lizard *Lacerta agilis* reintroductions in southern England

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Despite occurring widely across Europe and Asia, the Sand lizard *Lacerta agilis* is threatened in the north-western part of its range and had disappeared from much of its former habitat in England and Wales prior to concerted conservation action. A breeding population established at Marwell Zoo, UK, contributed to the re-establishment of 26 populations of Sand lizards at heathland and coastal dune sites across southern England as part of a wider multi-stakeholder response to reverse the decline of the species. Knowledge about the biology of Sand lizards was accrued during the process, which helped to refine the management of the breeding population that was maintained in a naturalistic setting within the indigenous range of the species. These successes were underpinned by coordinated collaborative actions and long-term institutional commitments against a backdrop of considerable change in the statutory framework governing Sand lizard conservation. The management of this project was not without cost or risk, including protection of valuable founder stock, incomplete knowledge about the health and disease status of Sand lizards, intrinsic constraints of limited founder representation, and the challenges of monitoring this elusive species post release.

Key-words: adaptation; breeding; heathland; reintroduction; sand lizard; species conservation.

INTRODUCTION

Species reintroductions are a significant conservation tool for many taxa (Fischer & Lindenmayer, 2000; Soorae, 2016), though such initiatives are often technically and logistically challenging. Understanding the outcomes and experiences of concerted reintroduction efforts is essential for practitioners to plan or refine methodologies (Sutherland *et al.*, 2004; Seddon *et al.*, 2007). Here, we report on a quarter of a century of captive breeding and reintroduction of Sand lizards *Lacerta agilis* in the south of England, sharing knowledge about the biology of the species accrued during this period, and summarizing the challenges and effectiveness of this long-term initiative. The project carried out at Marwell Zoo in Hampshire, UK, contributed to the re-establishment of Sand lizards at 26 locations, and is an example of reintroduction from a small captive founder population maintained in naturalistic conditions within the indigenous range of the species. This was one of a number of parallel and coordinated projects that contributed to successive national action plans to stabilize and reverse the decline of the Sand lizard in the UK.

SAND LIZARDS IN THE UK

There are ten recognized subspecies of Sand lizard found throughout Europe and

across Asia. While the species is patchily distributed in places, its range stretches from the United Kingdom in the west, through Europe and Central Asia to north-western Mongolia in the east (Agasyan *et al.*, 2010). The nominotypical subspecies *Lacerta agilis agilis* occurs in central and western Europe including the now fragmented populations in England and Wales (Cox & Temple, 2009; Andres *et al.*, 2014). The wide distribution of the species means that it is categorized globally as Least Concern in *The IUCN Red List of Threatened Species* (Agasyan *et al.*, 2010), but has declined sharply in north-west Europe and is therefore considered endangered in this part of its range (Edgar & Bird, 2006). Hence, the Sand lizard is included in Appendix II of the Berne Convention on the Conservation of European Wildlife and Natural Habitats, in Annex IV of the EU Habitats Directive (92/43/EEC: Council Directive, 1992), and is a protected species in most of the countries where it is found (Edgar & Bird, 2006).

In the UK, Sand lizards occur on dry heathland and coastal dune systems with sufficient vegetation structure for foraging and shelter, and areas of open sand for egg laying (House & Spellerberg, 1983; Corbett & Moulton, 1998; Blanke & Fearnley, 2015; Spellerberg & House, unpubl.). The species suffered catastrophic declines following habitat loss, degradation and fragmentation as a result of housing and industrial developments, conversion of heathland to conifer plantations, scrub encroachment, livestock and human disturbance, and fires (mainly caused by arson) (Corbett & Tamarind, 1979; House & Spellerberg, 1983; Edgar, 2002; Moulton *et al.*, 2011). By the late 1980s and early 1990s, this had resulted in the disappearance of Sand lizards from north and west Wales and nine English counties. Where the species remained extant, populations are thought to have reduced by over 90% by which time the majority of the UK's Sand lizards were confined to the fragmented Dorset heathlands (Corbett & Moulton, 1998). The Sand lizard became the subject of a national Species Recovery Programme between 1994 and 1997 (Corbett & Moulton, 1998), and a subsequent Species Action Plan led jointly by the statutory nature conservation agencies in England and Wales, and the Amphibian and Reptile Conservation (ARC) Trust (formerly the Herpetological Conservation Trust), Bournemouth, UK, with contributions from other non-governmental partners.

MANAGEMENT OF SAND LIZARDS

Breeding and reintroduction of Sand lizards was one of several interventions that sought to stabilize and enhance the status of the species in England and Wales (Edgar & Bird, 2006; ARC, 2016). Initiated and supported by the British Herpetological Society Conservation Committee (BHSCC), a breeding facility was established at Marwell Zoo in 1989 (Edgar, 1990); one of a number of vivaria set up for this purpose as part of a wider Sand lizard breeding programme pioneered by BHSCC and ARC Trust. The initial aim of the Marwell facility was to re-establish Sand lizards in the nearby New Forest, but the initiative was continued to produce reintroduction stock for heathland and coastal dune sites throughout the south of England.

A 12 m × 5 m Sand lizard vivarium was created using heathland soil and plants rescued from Canford Heath in Dorset, UK, prior to the construction of a housing estate. The sheltered, south-facing aspect, varied topography, vegetation cover (predominantly Heather *Calluna vulgaris*) and areas of open sand were designed to replicate dry heathland features favoured by Sand lizards (Plate 1). This habitat was created over a foundation layer of bricks and other building rubble to help drainage and provide plentiful hibernation cavities. It was then contained within boundaries of transparent acrylic sheets extending 0·4 m above and 0·3 m below ground, and held in place by a timber frame. An overhang was created along the top of the vivarium walls to

prevent lizards climbing out while deterring encroachment by rodents that might enter the area. However, the entire vivarium and immediate surroundings were enclosed within a wider structure of small-gauge wiremesh and nylon netting for protection against rodent and avian predators while allowing invertebrates, the natural prey of Sand lizards, to enter freely and colonize the habitat.

The vivarium was populated with Sand lizards rescued from heathland sites in Dorset either prior to the habitat being lost to development or following catastrophic fires, some of which were started accidentally or naturally occurring, but mainly caused by arson. A simple husbandry routine was followed. From March to September, the Sand lizards were fed during the middle of the day with gut-loaded Black field crickets *Gryllus bimaculatus* occasionally dusted with a vitamin and mineral powder (most recently Nutrobal for Reptiles) to supplement the natural diet. During periods of little or no rainfall, vegetation was sprayed with a fine mist of collected rainwater replicating natural precipitation and providing opportunities for the Sand lizards to drink. The visibility of Sand lizards while drinking made it possible to count the animals regularly and undertake observations of body condition.

The borders of the vivarium were kept clear of vegetation from March to June to ensure areas of open sand remained available for egg laying and the reproductive status of female Sand lizards was closely monitored from mid-May to mid-July. Depending on conditions at the time, egg laying occurred from late May, peaking in June and sometimes extending into July. Eggs were removed from the vivarium with clutches incubated in tubs of vermiculite. Emerging hatchlings were then transferred to outdoor, vegetated rearing tanks where they received a daily diet of micro-crickets (Black field crickets) and were provided with water as per the adults. Following a period of a month to 6 weeks and precautionary health screening, juvenile lizards were collected and transported for release at designated sites.

The breeding population of Sand lizards hibernated naturally in the vivarium during the winter months. During this time excessive vegetation was removed from the vivarium and any maintenance and repairs to the enclosure were undertaken.

Plate 1. Male Sand lizard *Lacerta agilis* **in the breeding enclosure at Marwell Zoo, UK.** *Rachel Gardner, Marwell Wildlife.*

BREEDING AND BIOLOGY

In total, 61 Sand lizards were transferred to the breeding vivarium at various intervals during the 25 year duration of the project. This founder representation began with a group of seven adults [2.5 (♂.♀)] in 1989, although there was in inauspicious start to the project because of encroachment by rats *Rattus norvegicus* and only two hatchlings survived for release. Following some modifications to improve protection of the vivarium, an additional nine adult Sand lizards (3.6) were introduced in 1990 and there were immediate improvements in hatchling survival with 43 juveniles released that year. In 1991, a further 14 adult female Sand lizards were added to the breeding population, resulting in the release of 180 juvenile lizards at the end of the summer. Unfortunately, residual weaknesses in the original structure of the vivarium were again exploited by rats in 1992 and most of the gravid female Sand lizards were lost to predation. Far more robust reinforcement and protection of the vivarium was undertaken in response and, with the exception of five surviving male Sand lizards, a new founder population was effectively created in 1994 with a group of 22 animals (2.20). Further lizards, rescued from degraded heathland sites, arrived in 1996 (2.0), 2000 (3.3) and 2001 (1.0). Apart from isolated cases of rodents entering the area through breaks in the enclosure netting, the protection of the vivarium proved adequate for the next two decades and no further losses to predation were recorded.

Around 20 Sand lizards were typically housed in the vivarium each year (max = 30) with a mean of 2·5 females per male (max = 6, min = 0·7). This resulted in a mean density of 0·32 Sand lizards per m^2 (max = 0·5 m^{-2}, min = 0·13 m^{-2}). With a mean 0·8 (± 0·3) clutches per female, there were some years in which not all females reproduced, but in favourable conditions double clutches were possible. A mean 7·4 eggs were laid per female (max = 17·8, min = 1·8). Eighty-seven per cent (± 13·1) of eggs hatched and 89% (± 9·2) of hatchlings survived until release age (4–6 weeks). These intuitively high figures are likely reflections of protection and husbandry interventions, albeit data on wild Sand lizards for comparison are lacking.

Mean weight of newly hatched Sand lizards was 0·67 g (± 0·13, n = 50) with a mean snout to vent length of 28·58 mm (± 1·85, n = 50). Young lizards increased their weight by up to 30% during the first month, with those maintained at low density (5 m^{-2}) growing at faster rates than their counterparts kept at double this density regardless of food supply not being a limiting factor (Isaacs, 2009; Kain, 2010). Aggression between juvenile Sand lizards was significantly lower when kept at low density (Isaacs, 2009; Kain, 2010), with the cost of additional energy expended by animals maintained at high density the likely cause of observed differences in growth rate.

Wide variations in the weights and snout to vent lengths measured in adult male and female Sand lizards reflected continuous growth with age. Contrary to expectation, weights of female Sand lizards (mean = 15·7 g ± 3·7, n = 38) were often greater than of males (mean = 12·27 ± 3·25 g, n = 25). This may have been an artefact of age, reproductive status, the provision of abundant supplementary food designed to keep female Sand lizards in optimal body condition during the breeding season, and the competitive energy expenditure of males kept at high density. However, there was little difference in snout to vent length of adult male (72·42 ± 23·61 mm, n = 25) versus adult female Sand lizards (73·24 ± 6·00 mm, n = 38).

As reported by Fearnley (2009), sightings of Sand lizards in this closed environment were influenced by sex, reproductive stage and weather. The probability of detecting males was greater before mating when observations were strongly associated with weather and time of day, compared with later in the season when they were most often seen at solar radiation values of

between 300 and 700 W m^{-2}. The probability of detecting females was greater after mating when surface ground temperature was between 17·5 and 27·5°C, compared with earlier in the season when they were often seen basking within an optimum level of ultraviolet light (UV index between 2·00 and 5·00). Further variations in detection of female Sand lizards occurred during and after egg laying.

Sand lizards arriving at Marwell were subject to health screening and a period of quarantine before joining the breeding population. Similarly, health-screening protocols were adopted to mitigate risk of pathogenic disease transmission to wild Sand lizard and other reptile populations during releases (Lloyd & Sainsbury, 2003; Molenaar *et al.*, 2008). Because of the difficulties of practically, safely and humanely testing small and fragile juvenile Sand lizards, oral and cloacal swabs, and faecal samples were taken from adult Sand lizards to assess the health status of the population as a whole. Aerobic and anaerobic cultures isolated *Escherichia coli*, *Klebsiella oxytoca*, *Pasteurella multocida*, *Serratia marcescens*, *Staphylococcus* sp and *Streptococcus* sp in some years, but perhaps surprisingly there were no positive tests for either *Salmonella* or *Campylobacter*. Particular caution was exercised following a positive test for *Serratia marcescens* as the only common finding in the post-mortem testing of four juvenile Sand lizards that died in quick succession in 2005 (Molenaar & Sainsbury, 2009). However, this species of bacteria proved to be present in wild Sand lizard populations in Dorset and, therefore, was not considered a significant risk (Sainsbury, 2012).

OUTCOMES OF REINTRODUCTIONS

During 25 years of captive breeding in a naturalistic environment, the project contributed 1892 Sand lizards for release at 21 reintroduction sites across the south of England (Fig. 1). Animals were released in more than one location within five of the reintroduction sites and so contributed to the attempted re-establishment of 27 discreet populations (Plate 2). This included 758 juvenile Sand lizards released between five locations in the New Forest between 1990 and 1994 representing 91% of the reintroduction stock for that area and completing the original aim of the project. Overall, the majority of Sand lizard release locations were heathland habitats in the counties of Dorset (10), Hampshire (10, including those in the New Forest), Surrey (2) and West Sussex (1), with the remainder coastal dune systems in Devon (1), Dorset (1), West Sussex (2) and Kent (1). With juvenile Sand lizards from Marwell joining other captive-bred stock, a mean 144 (± 64) Sand lizards were released per location (max = 306, min = 13).

Surveys carried out by ARC Trust confirmed the continued presence of Sand lizards at 21 of the release locations during 2016, at two locations in 2015 and at three other locations in 2014. Hence, all but one of the 27 reintroduction initiatives have been assessed within the last 3 years with apparently successful outcomes. The remaining release location was last evaluated in 2000 and thought to have failed because of fire (suspected arson).

Based on the period elapsed between the year of last release at a given location and the most recent survey confirming their presence, seven Sand lizard populations had become re-established for over 20 years; the longest dating back to the beginning of the project with animals being detected 26 years after founders were reintroduced. Nine Sand lizard populations had become established for between one and two decades while a further ten Sand lizard populations were at earlier stages of re-establishment of between 2 and 9 years.

DISCUSSION

Given the precarious status of the Sand lizard in southern England by the late 1980s and early 1990s, captive breeding and reintroduction were seen as a necessity

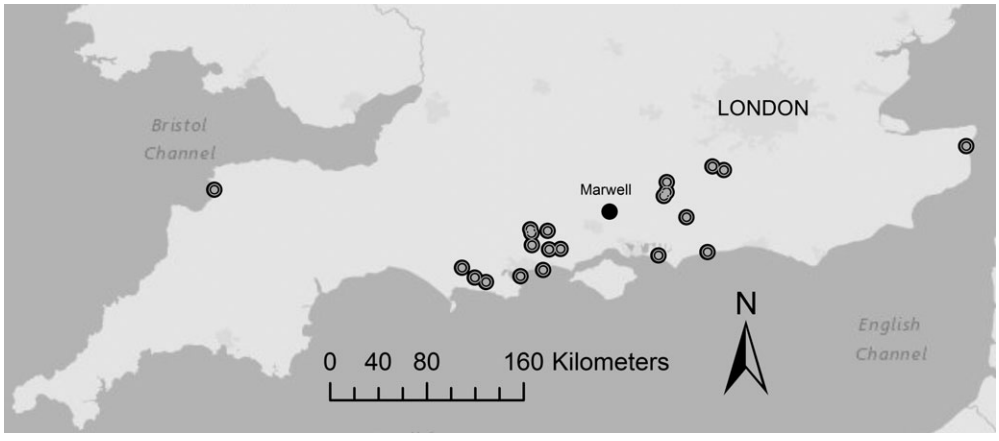

Fig. 1. Sand lizard *Lacerta agilis* **reintroduction sites across the south of England (circles) and the location of Marwell Zoo (black dot) in Hampshire, UK.** *Contains OS data © Crown Copyright and database right (2016).*

Plate 2. Sand lizard *Lacerta agilis* **release site on the dry heathland of Woolmer Forest in Hampshire, UK.** *Tim Woodfine, Marwell Wildlife.*

(Corbett & Moulton, 1998). Common to these sorts of interventions, the successes reported here have not come without risk and cost (IUCN/SSC, 2013). In this case, loss of valuable founder animals to rat predation was a major setback during the early stages of the project and physically protecting the population was a considerable undertaking. Similarly, biosecurity measures and health screening of animals arriving for breeding and of those departing for release (Lloyd & Sainsbury, 2003; Molenaar *et al.*, 2008) required substantial effort while judgements on these matters were hampered by lack of information on the infectious-disease status of wild Sand lizards until very recently (Molenaar & Sainsbury, 2010; Sainsbury, 2012) and even then, this knowledge is limited.

However, there were theoretical benefits accruing from this approach to captive breeding of Sand lizards. Use of transplanted

habitat within the indigenous and climatic range of the species, and allowing natural processes, such as hibernation, foraging for prey and typical social interactions, were designed to minimize mismatch with the evolved biology of the species and retain adaptive traits that may otherwise be lost over time (Frankham, 2008; Bijlsma & Loeschcke, 2012; Schulte-Hostedde & Mastromonaco, 2015). Similarly, juvenile Sand lizards spent a relatively short period of time in captivity, being released within several weeks of hatching and thereafter being exposed to natural selection at the recipient site. Protection of the breeding population from predation and disturbance, plus the artificial incubation of eggs and supplementary feeding, were designed to enhance productivity, hatch rates and survival of juveniles to point of release. This helped to produce the numbers of Sand lizards needed for release but with unknown consequences of relaxing the selection pressures (Lahti *et al.*, 2009; Christie *et al.*, 2012) on young Sand lizards during this critical phase of their development and on subsequent generations.

The Sand lizard reintroduction strategy followed an original recommendation to release cohorts of 50 juveniles (Moulton & Corbett, 1999), and more recently 80 juveniles (Berglind *et al.*, 2015), per year during three consecutive years, taking place any time between mid-April and early September (Plate 3). Evaluating the outcomes of releases is reliant on evidence (sightings of live animals or recently shed skin) that the species persists post release. However, population estimates and, therefore, trends in the status of the species at a given location have not been established because of the cost and logistical challenges of detecting these often elusive animals. Even with improved understanding of detection probability (Fearnley, 2009), coinciding site visits with conditions favourable for seeing Sand lizards on sufficient occasions remains difficult. With this in mind use of indices of Sand lizard abundance, such as egg-burrow counts per unit area over time, are now being assessed, while advances in cost-effective micro-scale tracking technology could improve understanding of post-release dispersal and habitat selection (Brady & Phillips, 2012), and thereby help to focus monitoring efforts.

Despite the challenges of monitoring reintroduced populations, recent records of Sand lizard sightings at 26 out of 27 locations provide some indication of successful outcomes. However, intrinsic pressures on small populations, such as founder effect, inbreeding and genetic drift (Olsson *et al.*, 1996; Frankham *et al.*, 2010), continue to jeopardize the long-term viability of reintroduced Sand lizards. While we lack much of the biological and environmental data needed to predict the probability of persistence, molecular techniques (Russell, 2012a,b) can provide insights into genetic variation found within isolated populations. Unless numbers of Sand lizards at a given site have become large enough to achieve a long-term viable population and evolutionary adaptation (Franklin, 1980; Frankham *et al.*, 2014), genetic augmentation and metapopulation management will become an important component of the next phase of conservation intervention for this species in southern England. Indeed, questions about how distribution and phenotypic traits of Sand lizards may be positively or negatively affected by a warming climate (Urban *et al.*, 2014; Ljungström *et al.*, 2015) place further emphasis on plasticity and genetic adaptation of isolated populations.

The inability of Sand lizards to naturally recolonize suitable habitats or move between fragmented patches means that their dispersal will continue to depend on human-mediated connectivity for the foreseeable future. Captive breeding may continue to have a role in supplying the large numbers of Sand lizards needed to re-establish the species (Berglind *et al.*, 2015) where they became extinct, but with the inherent genetic limitations of small captive founder populations and potential distraction from allocating resources to achieving favourable conservation status for existing wild populations. In contrast, wild to wild

Plate 3. Juvenile Sand lizards *Lacerta agilis* **in Woolmer Forest, Hampshire, UK, following release.** *Tim Woodfine, Marwell Wildlife.*

translocation offers a lower-cost option for genetic augmentation and metapopulation management with access to the full spectrum of available genetic variation. However, these operations may be impractical and there are questions about the robustness of potential donor populations to high levels of offtake. In either case, risks of disease transmission need to be mitigated. Regardless, ongoing restoration and management of heathland and coastal dune sites remains essential because viability of Sand lizard populations will ultimately depend on the size and quality of habitat.

Even for small-bodied species with short generation lengths, the period of time needed to re-establish a reintroduced population can be considerable and difficult to determine from the outset. A long-term strategy with accompanying financial and institutional commitments is therefore needed. This has fortunately been the case for the Sand lizard in the south of England during the last quarter of a century, albeit against a backdrop of considerable statutory change and cessation of governmental funding contributions in more recent years.

The last two decades of this reintroduction programme have seen substantial changes in the structure and function of the UK's statutory agencies responsible for wildlife conservation resulting from policies of successive governments and requirements of devolution. During this period, the original Sand lizard Species Recovery Programme informed and was superseded by the UK Biodiversity Action Plan (UK BAP), with specific interventions for the Sand lizard eventually combined with those for the Smooth snake *Coronella austriaca* in a rare reptile Species Action Plan (ARC, 2009). Since then UK BAP has in turn been replaced by the UK Post-2010 Biodiversity Framework (JNCC & Defra, 2012). In England, this is now delivered through a landscape-scale strategy for wildlife conservation and ecosystem services (Defra, 2011) allied to the Convention on Biological Diversity *Strategic Plan for Biodiversity 2011–2020* and the achievement of the Aichi Targets (CBD, 2010). The Sand lizard is listed alongside other priority species in Section 41 of the Natural Environment and Rural Communities Act, 2006, and linked with Lowland Heathland and Coastal Sand Dune priority habitats. There will be inevitable uncertainty about approaches to Sand lizard conservation in the coming years because of the UK's exit from the European

Union; how this will affect status of European Protected Species in this country, and how the role and resourcing of Natural England and other statutory agencies will be shaped are unpredictable.

The Sand lizard vivarium at Marwell Zoo has in the meantime provided an opportunity to enhance understanding of the reproductive biology, morphology, behaviour and health of this species in a naturalistic setting, as summarized here. As part of parallel and coordinated long-term efforts, the project made a significant contribution to the re-establishment of Sand lizard populations across the south of England.

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PRODUCT MENTIONED IN THE TEXT

Nutrobal for reptiles: vitamin/mineral supplement specifically formulated for insectivorous reptiles and birds, manufactured by VETARK Professional, PO Box 60, Winchester SO23 9XN, UK.

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