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Optimisation of reintroduction protocols for cryptic species: Reintroducing the sand lizard *Lacerta agilis* to a lowland heath site

by

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Thesis for the degree of Doctor of Philosophy

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Abstract

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At a time of high biodiversity loss and in the light of global climate change, some species are at particular risk. Geographically restricted specialists may undergo biotic homogenisation. Less charismatic and smaller taxa are often under-studied, with cryptic species raising additional monitoring challenges. The close links between reptile biological cycles and temperature and moisture leave many populations facing an uncertain future.

Following dramatic population declines of the sand lizard *Lacerta agilis* in the UK due to habitat loss, fifty years of captive breeding and reintroduction efforts have focused on returning populations to sites in their former range. Ongoing presence of the species shows reintroduction efforts are broadly successful, but the cryptic nature of the sand lizard has raised post-release monitoring difficulties. This research addressed the challenges associated with this species, offering a more quantitative assessment of reintroduction practice to determine efforts are best-placed and to inform protocols going forward.

Microhabitat preference of the sand lizard was studied in the light of monitoring difficulties. Detectability of sand lizards varied, with less dominant adults spatiotemporally restricted to less optimal environmental conditions. Juveniles showed aggregative behaviour as a thermoregulation mechanism, the benefits of which may influence post-release movement. Survey methodologies were assessed at the receptor site, Eelmoor Marsh SSSI in Hampshire, UK. Recommendations were made for the sand lizard as well as the wider reptile community, including the more generalist common lizard *Zootoca vivpara* and slow worm *Anguis fragilis*, and predators adder *Vipera berus* and grass snake *Natrix helvetica*. Refugium materials of felt and slate proved favourable to sand lizards and common lizards, both species highlighted as being poor users of traditional tin refugia.

Reintroduction protocols currently focus on hatchlings. Hatchlings, yearlings, and a small number of two-year-olds were released in this study. Releases were undertaken in September 2017 and September 2018, of 80 and 86 lizards respectively, and post-release monitoring occurred for a year following each. Overwinter survival favoured yearlings over hatchlings. Yearlings also showed predictable post-release movement and survival; hatchlings did not. Yearlings displaying higher locomotor performance and those that showed a less exploratory and less active behavioural response to the novel release site, were more likely to survive overwinter. The furthest travelled yearlings pre-winter showed more exploratory and active traits. Many yearling lizards showed release site fidelity, remaining in or returning repeatedly to the area, highlighting the importance of maintaining this as optimal, structurally diverse habitat. Released yearlings were observed having successfully bred within a year, at two years of age; released hatchlings would likely not breed until their third year. Observations of sand lizards in less favourable environmental conditions and beyond the normal active season indicated release may be favourable earlier in the year; this would benefit from further study. Continued monitoring is recommended to observe longer-term trends.

This research highlighted the potential and benefits of developing a rigorous post-release monitoring approach for cryptic species. It showed the importance of building on current understanding of species ecology and biology at demographic and individual levels to aid conservation initiatives.

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decResearch Thesis: Declaration of Authorship

Print name:	Rachel Sarah Gardner
Title of thesis:	Optimisation of reintroduction protocols for cryptic species: Reintroducing the sand lizard *Lacerta agilis* to a lowland heath site

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission.

Signature:

Date:

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Definitions and Abbreviations

AGI Agonistic index

ARC Trust Amphibian and Reptile Conservations Trust

ARG UK Amphibian and Reptile Groups of United Kingdom

ARI Aggressive Index

DI Dominance Index

eDNA Environmental DNA (Deoxyribonucleic acid)

GPS Global Positioning System

Guidelines for reintroductions Guidelines for reintroductions and other conservation translocations (IUCN, 2013)

hPa Hectopascal Pressure Unit

IUCN International Union for Conservation of Nature

LIDAR Light Detection and Ranging

lux International System of Units derived unit of illuminance

N/S/E/W North/South/East/West (and derivatives, e.g. **NW** north-west)

NA Not applicable

NARRS National Amphibian and Reptile Recording Scheme

PIT Passive Integrated Transponder

RFID Radio-frequency identification

Secs Seconds

SSSI Site of Special Scientific Interest

SVL Snout-vent length

UK United Kingdom

UV (Index) Ultraviolet (Index)

Veg Vegetation

W / m2 Watts / metre^2

< / > Less than / Greater than

~ Approximately

Statistical abbreviations

AIC Akaike Information Criterion

ANOVA Analysis of variance

arcsin Arcsine (inverse of sine)

CI Confidence Interval

χ^2 (Test) Chi-Squared (Test)

d.f. Degrees of freedom

F / F value F statistic

FET Fisher's Exact Test

GLM Generalised linear model

GLMM Generalised linear mixed model

KW Kruskal-Wallis Test

log Logarithmic

LRT Likelihood Ratio Test

Lower Q / Upper Q Lower quartile / Upper quartile

M1, M2… Statistical Model 1, 2…

n Sample size

PI Prediction Interval

***P* / *P*-value** Probability value

R^2 Coefficient of determination

sq. rt. Square root

St. dev. Standard deviation

Std. Err. Standard error

***t*-test** Student's t-test

***t* / *t*-value** t-statistic

U Mann-Whitney U statistic

z Z-score

***** Interaction term between two variables

Chapter 1 Literature Review and Project Introduction

1.1 Threats to biodiversity

Current demands on the natural environment for resources to meet the requirements of the exponentially growing and technologically advancing human population are unsustainable. From global climate change to species extinction, the evidence of negative human impact is irrefutable. The threat to biodiversity has been widely acknowledged in recent years as the beginning of a sixth mass extinction (Dirzo and Raven, 2003; Barnosky *et al.*, 2011). The rapidly changing environment, along with species population declines resulting in decreased genetic diversity and bottlenecks, is a perfect storm for an extinction event. These are categorised when the relative roles of extinction and speciation are critically unbalanced over a geologically time window; extinction rates are over several hundred times the rate typically observed, resulting in the loss of ≥70% of all species (Bambach, 2006).

Today, species are struggling to adapt or, if their biological mobility allows, move and re-establish themselves quickly enough to respond to rapid habitat loss and shifting climatic systems (Parmesan and Yohe, 2003; Newbold *et al.*, 2016; Wiens, 2016; Betts *et al.*, 2017). The effects of such changes on community ecology and wider ecosystems as a result of disrupted species interactions are complex (Berg *et al.*, 2010; Staniczenko *et al.*, 2017). Species adaptation to climatic changes that are predicted over the next century would need to occur at rates that have rarely been observed in vertebrates (Quintero and Wiens, 2013). As well as temporal barriers, those species fortunate enough to be mobile also face a greater number of spatial barriers than ever before. Potential pathways between decreasing areas of suitable habitat are blocked (Eigenbrod *et al.*, 2008) and suitable habitat itself is shifting more quickly than dispersal is possible (Radinger *et al.*, 2017).

There is also greater threat to specialist species and those populations at the limits of their species range experiencing comparable ecological restrictions. It is anticipated that biotic and species homogenisation will occur as niche specialists with geographic restrictions are replaced with generalists (Olden *et al.*, 2004; Savage and Vellend, 2015) and there is reduced biodiversity. This has been modelled for a variety of taxonomic and geographical groups, including tropical species (Afonso Silva *et al.*, 2017), anurans (Menéndez-Guerrero *et al.*, 2020) and avian communities (Davey *et al.*, 2012). Although the effects of climatic change will vary regionally, they will typically result in lower diversity in a given area (α-diversity) (Menéndez-Guerrero *et al.*, 2020). Loss of diversity is ultimately loss of ecosystem resilience and sustainability (Olden *et al.*, 2004) and thus the need to better understand the biology and ecology of the specialists within our environment, and to successfully enhance and increase the populations that still remain, has never been greater.

The current surge in public awareness and action is helping progress the drastic societal changes required globally to reduce the scale of damage being caused (Gelcich *et al.*, 2014; Hagerman and Pelai, 2016). At this key time, with conservation closer to the forefront of the scientific, political and public minds, it is most pertinent to ensure the efforts being driven by conservation scientists and practitioners are achieving their aims. Despite the worsening state of the natural world suggesting otherwise, awareness and action within this field has been underway for decades. Systematic and regular review and assessment of actions is essential in order to ensure this remains appropriate, effective and the best use of resources (Brichieri-Colombi and Moehrenschlager, 2016; Orlikowska *et al.*, 2016; Willer *et al.*, 2019).

Conserving species *in situ* within their natural habitat is desirable, to maintain populations and community and ecosystem function that consequently offers stronger resilience to change; for example through habitat management and restoration, and the development and maintenance of protected areas (Oldekop *et al.*, 2015; Newmark *et al.*, 2017; Pringle, 2017; Török and Helm, 2017; Stokes, 2018). However, this approach is not always possible. Populations may decline at unprecedented rates that outweigh countering efforts (Canessa *et al.*, 2015; Powers and Jetz, 2019). In these instances, alternative approaches and interventions may be sought to give populations greater chances of long-term recovery.

1.2 Species reintroduction

Species reintroductions and conservation translocations have been employed to counter many of the emerging issues facing species (Fischer and Lindenmayer, 2000; Armstrong and Seddon, 2007; Ewen *et al.*, 2012). Translocation, generally, embodies measures from the individual to population level to return species to natural ecosystems. The concepts are outlined in detail in the International Union for Conservation of Nature (IUCN) Guidelines for reintroductions and other conservation translocations (IUCN, 2013) (herein termed ‘IUCN Guidelines for reintroductions’) and are included in Table 1. Species reintroduction intends to reinstate a species, leading to improved ecosystem function and biodiversity, where it has previously existed but been lost. Population reinforcement parallels this as the augmentation of a remaining subpopulation in order to demographically and genetically optimise its chances of survival (IUCN, 2013).

Table 1 Conservation translocation definitions, taken from IUCN Guidelines for reintroductions (IUCN, 2013).

	Term	Definition	Purpose
Population restoration *(within indigenous range)*	Reinforcement	The intentional movement and release of an organism into an existing population of conspecifics.	To enhance population viability, for instance by increasing population size, by increasing genetic diversity, or by increasing the representation of specific demographic groups or stages.
	Reintroduction	The intentional movement and release of an organism inside its indigenous range from which it has disappeared.	To re-establish a viable population of the focal species within its indigenous range.
Conservation introduction *(outside of indigenous range)*	Assisted colonisation	The intentional movement and release of an organism outside its indigenous range to avoid extinction of populations of the focal species.	Includes a wide spectrum of operations, from those involving the movement of organisms into areas that are both far from current range and separated by non-habitat areas, to those involving small range extensions into contiguous areas.
	Ecological replacement	The intentional movement and release of an organism outside its indigenous range to perform a specific ecological function.	This is used to re-establish an ecological function lost through extinction, and will often involve the most suitable existing sub-species, or a close relative of the extinct species within the same genus.

Captive breeding is widely employed as an *ex situ* conservation tool that is often used in conjunction with conservation translocation initiatives. It allows a population to be closely managed, in many instances utilising regional or global species studbooks, allowing genetic diversity to be maximised (Wizenberger and Hochkirch, 2011). At the very least, it optimises population size, demographics and health in order that a captive group may one day provide the underlying physical resource to return the species to its range, the resource of the animals themselves.

The Guidelines for reintroductions (IUCN, 2013) outline a methodical approach to this conservation tool, from initial planning and animal selection to release techniques and post-release monitoring and management. The framework highlights potential considerations for project management. However, it is necessarily general in approach in terms of species-specificity. The focal species of such conservation efforts are determined by various priorities, be they ecological, biological, social, political, economic or cultural, and typically a combination of these. Priorities inevitably fall on species considered under threat in a given area, from regional to international scales. Invariably the two scales coincide, however some species may be deemed at risk of only localised extinctions. For example, a species may be of Least Concern on the IUCN Red List of threatened species at global level, however within specific countries populations might be struggling to maintain strongholds (Brito *et al.*, 2010). This is highlighted through the designation of country-specific priority species, for example those included in the UK Biodiversity Action Plan (UK BAP), later succeeded by the UK Post-2010 Biodiversity Framework (Joint Nature Conservation Committee (JNCC) and Department for Environment, Food and Rural Affairs (DEFRA), 2012). By improving habitats and maintaining species populations locally, across the entirety of their range, conservationists and policy-makers improve the species' chances of having the capacity to be resilient and respond in a more natural way to wider global threats, such as climate change, and ultimately optimise biodiversity (Rytteri *et al.*, 2017; Timpane-Padgham *et al.*, 2017; DEFRA, 2020).

The aims and objectives of reintroductions will differ from project to project, from returning the final piece of a historical species assemblage to increasing awareness and understanding of a species among local communities (Ewen *et al.*, 2014). This variety is highlighted in the IUCN Global Reintroduction Perspectives reports, where each contributor outlines their project goals (Soorae, 2011; Soorae, 2016; Soorae, 2018). However, fundamentally each project will aim to create or enhance an established population of the released species at a given site. As with any project involving goal-setting, efforts to return or increase populations must be closely monitored to determine their effectiveness (Fischer and Lindenmayer, 2000; Taylor *et al.*, 2017).

1.2.1 Post-release monitoring

Monitoring species populations following a release is considered "essential" by the IUCN Guidelines for reintroductions (2013). There are numerous studies and reviews highlighting the importance of post-release monitoring in order to assess the release population (Griffith *et al.*, 1989; Nichols and Williams, 2006; Bernardo *et al.*, 2011; Parker *et al.*, 2013; Barata *et al.*, 2017). Bernardo *et al.* (2011) and Parker *et al.* (2013) discuss the importance of monitoring over relevant timeframes and at appropriate intensities for the species and questions in hand, in order to minimise misinterpretations of project outcomes at a given point in time.

Multiple stages have been identified following release at a receptor site. The immediate post-release phase has been termed 'establishment'; the 'persistence' stage extends beyond this (Armstrong and Seddon, 2008). Alternatively, key stages have been identified 'release', 'growth' and 'regulation' (Sarrazin, 2007). Given the immediate challenges faced by a population and the individuals within it when released to a site where no conspecifics currently exist, understanding survivorship and population demographics in the establishment phase of a reintroduction is critical. Without this assessment of the state of the population, predictions going forward as it moves into the persistence stage are difficult. Consequently, tailoring adaptive post-release management of species and habitats to account for changes in the field is made impossible (Seddon, 1999). Making recommendations for future release strategies without this evidence base is also hampered.

Ensuring that the aims and objectives of a reintroduction are defined *a priori* will allow the degree of success of the project to be measured in relation to these. Measures may include evidence of breeding by the first wild-born generation (Sarrazin and Barbault, 1996b), the use of population viability analysis, or the establishment of a self-sustaining population (Fischer and Lindenmayer, 2000). However, only the first of these examples is measurable and objective, with some subjectivity present in the latter two in terms of parameters and definitions; the minimum viable population will depend on the projection model employed. Attempts have been made to provide further quantification of population viability, for example using modelling to suggest whether measurable data, such as population size, might act as a proxy for extinction risk in reintroduced populations that have reached carrying capacity (Robert *et al.*, 2015). Standards have also been recommended by using IUCN Red List Indices (RLIs) to assess regional reintroduction outcomes.

As specified by the IUCN (2013), monitoring at a wider, ecological scale is also imperative. Changes in both habitat, due to the functional role of the species, and effects on the species assemblage *in situ* require consideration in order to detect changes that may have resulted from the return of a species to the ecosystem. This monitoring ought to be considered strategically and systematically in order to ensure it is appropriate and efficient to answer the specific questions in hand (Nichols and Armstrong, 2012). Pre-release monitoring of the wider species community is also highlighted in order to set the baseline for post-release observations and to detect changes that may be attributable to the species released (Osborne and Seddon, 2012).

Post-release monitoring techniques follow comparable surveillance techniques as monitoring wild populations (Nichols and Williams, 2006). The novel aspect of monitoring a released population is that initially it can be quantified to some level. For some species and reintroductions, this may just be the number of individuals and some detail on the cohort demographic. In other instances, genetic profiling may be considered. Identifying individuals may also be achievable, be this through differences in natural markings, or artificial markings such as tags. Where it is possible, the latter offers potential to identify every individual post-release (pre-population recruitment), allowing for observation of individual movement and behaviour. Such mark-recapture methods can consist of either physical observation or capture, or remote survey tools such as camera traps, depending on the species (Royle *et al.*, 2013). In contrast, identifying every individual in a wild population, is difficult to guarantee and particularly so when the species is cryptic.

Undertaking sufficient post-release monitoring to assess project success is a time-intensive and resource-consuming activity. However, in some instances the biology and ecology of the species released raises particular challenges. Wide-ranging animals, such as avian and migratory species like the bearded vulture *Gypaetus barbatus* (Margalida *et al.*, 2013) and European white stork *Ciconia ciconia* respectively (Shephard *et al.*, 2018), raise geographical challenges. If animals are not followed as individuals, where mark-recapture methods can more precisely consider demographic population parameters (Sandercock, 2020), population dynamics are more challenging to assess. It is possible persistence of the release cohort, as opposed to adequate recruitment, could indicate apparent success. Where long generation lengths exist it can also take a long time for reductions in fitness to be observed should the released individuals be of a genetic strain that does not match well with the release environment, therefore delaying the reporting of a successful outcome or otherwise. This is highlighted in the reintroduction of the lake sturgeon *Acipenser fulvescens* to the Mississippi and Missouri rivers (Drauch and Rhodes, 2007).

Technological advancements in recent decades have enabled affordable tracking opportunities, including the use of satellite telemetry, radio telemetry, acoustic telemetry, passive transponders and even drones, to locate individuals (Hardman and Moro, 2006; Margalida *et al.*, 2013; Hodgson *et al.*, 2016; Watkins *et al.*, 2018; Mertes *et al.*, 2019). For example, the satellite tagging of twenty-one rehabilitated and released manatees *Trichechus manatus manatus* in north-east Brazil enabled researchers to determine that reintroductions of this species would have the potential to reconnect distant sub-populations (Normande *et al.*, 2016). The soft release of forty-six red-billed Curassows *Crax blumenbachii* in the Macacu Environmental Protection Area, Brazil was monitored by radio transmitters attached to the birds' backs on release from an acclimatisation area. Data showed that acclimatisation period and size of released cohorts both affected post-release survival and could feed into protocols for reintroduction of this species going forward (Bernardo *et al.*, 2011). Monitoring techniques must be appropriate for the species and habitat.

It is vital that reintroduction project objectives are measurable in order to ascertain success and validate the large quantities of resources being devoted to them, be these animals, equipment, finances, expertise or human hours (Gilbert *et al.*, 2017). The updated IUCN Guidelines for reintroductions (2013) detail a step-by-step approach when considering a reintroduction, in order to anticipate and prepare for challenges throughout the process. However, it is not possible to foresee all eventualities. For every reintroduction initiative, the question remains of whether it has a high potential of meeting its aims and whether it is possible to evidence this.

1.2.2 Conservation and monitoring of cryptic specialists

Lesser observed and less enigmatic taxa suffer particularly from the historical conservation research and publication bias towards more charismatic species, invariably birds and mammals (Bonnet *et al,*, 2002; Fazey *et al.*, 2005). This is replicated in the reintroduction literature (Fischer and Lindenmayer, 2000; Seddon *et al.*, 2005). Both reporting of projects and consequentially review papers follow this bias (Bajomi *et al.*, 2010). The trend is also seen in the species selected for reintroduction efforts (Seddon *et al.*, 2005; Gilbert *et al.*, 2017). In comparison, only a handful of published project reports discuss reintroduction of invertebrates, fish, amphibians and reptiles.

In a recent study of reintroduction projects undertaken by European Association of Zoos and Aquaria (EAZA) institutions (Gilbert *et al.*, 2017), mammals and birds represented 37.2% and 39.7% species and 42.3% and 37.3% projects respectively. In comparison reptiles represented just 6.4% of species (*n* = 156) and 8.1% of projects (*n* = 260). This is despite the estimated numbers of described species of mammals, birds and reptiles totalling 5515, 10 424 and 10 391 respectively at the time of publication (Gilbert *et al.*, 2017). Research efforts into species outside of 'favoured' taxonomic classes therefore hold comparably great weight in their field. Only through persisting with efforts can tools such as reintroduction be assessed and optimised for these species.

Less charismatic species are additionally often small and cryptic, elusive in their behaviour and difficult to observe. Small terrestrial species are further impacted by their limited mobility to move into more favoured, suitable habitat. What is more, the corridors and connections that may have once existed and provided vital linkages across species ranges are now fewer, and water bodies and roads often act as boundaries (Beebee, 2013). In such cases, for populations to survive, translocation of individuals through reintroduction becomes an essential tool.

As species populations decline, more species will likely be brought into the captive environment to offer resilience and a reserve population away from habitat and climatic threats. In a recent review of North American translocations, of 279 species translocated 162 (58%) were captive-bred (Brichieri-Colombi *et al.*, 2019). Small, terrestrial, specialist species will be affected first by such changes, being less mobile and less able to adapt to shifts in resources given their specialist requirements. They are in need of conservation efforts in order to provide community heterogeneity to maintain resilience and for ecosystem function to endure (Olden *et al.*, 2004). Captivity raises additional concerns for species on the individual and population level, for example adaptation over multiple generations and effects on behaviour, morphology, genetics and physiology, as well as ethical and welfare concerns (Lyles *et al.*, 1987; Griffin *et al.*, 2000; McPhee, 2003; Parker *et al.*, 2012). However, options are increasingly restricted and alternatives such as wild-wild translocations must also consider the effects on the source population. Thus focus should be on researching and best managing captive populations to optimise reintroduction practice (Griffiths and Pavajeau, 2008; Harding *et al.*, 2016; Lewis *et al.*, 2019). The captive environment also offers opportunity to broaden species knowledge to inform conservation efforts both *in* and *ex situ*.

Technology is advancing and opportunities for monitoring small and cryptic species are greatly improved from just a few decades ago. One example of this, although not in a reintroduction, is radio-frequency identification (RFID) tags used on honey bees *Apis mellifera* and *Apis cerana* to assess home ranges and behavioural niches of the two species (Xujiang *et al.*, 2013) and mating behaviour of queens *A. mellifera* (Heidinger *et al.*, 2014). The tags are glued to the bees' abdomens and activated on entrance and exit to the hive by two antennae. These log individuals by the different codes assigned to each transmitter and the order of antenna activation determines direction of movement. Due to the passive nature of the tags, cumbersome batteries are not needed, allowing application with this small species. The requirement to monitor only specific locations allows this technique to answer the research questions in hand.

Otherwise known as Passive Integrated Transponder (PIT) tags, RFID tags have also been used extensively to monitor fish species. Tagged fishes either swim through 'gates' encased in receiver antennae that will log their transmitter on passing and allow individuals to be tracked, or surveyors will use portable sweeping antennae above the water to detect individuals below (Cucherousset *et al.,* 2005; Acolas *et al.*, 2007; Cucherousset *et al.,* 2008, 2010). The main limitation of this technique is the proximity required between the receiver and the transponder in order for the information to be transmitted; the maximum detection distance of the sweeping receiver is just 36 cm. PIT tags have been applied more widely across other taxa, such as to amphibians including ambystomatid salamanders (Hamed *et al.*, 2008) and also reptiles including lizards *Crotaphytus collaris* (Santoyo-Brito and Fox, 2015) and *Zootoca vivipara* (Le Galliard *et al.*, 2011) and pine snakes *Pituophus melanoleucus* (Burger and Zappalorti, 2011).

Very High Frequency (VHF) radio tags are an alternative method of locating individuals, with the capacity to emit active signals over wide-ranging distances up to tens of kilometres depending on the tag signal strength. The trade-off, however, is the size of the tag required to enable appropriate battery life. Tag attachment options for lizard species have been assessed, including tape attachment (Dent, 1986), glue mount or harness attachment (Richmond, 1998; Warner *et al.*, 2006).

Studies often bypass the step of assessing tag effect on the study species (Bateman and Gresswell, 2006; Burger and Zappalorti, 2011). Those studies that have been undertaken highlight the importance of this aspect. The use of PIT tags in northern pine snakes *P. melanoleucus* and the associated handling and disturbance are not found to impact their rate of re-sighting. This is therefore a successful method of marking individuals and ensuring their reliability as bioindicators of a healthy food chain (Burger and Zappalorti, 2011). However, the injection of PIT tags in common lizards *Z. vivipara* has negative effects on locomotion for more than seven days following tag implantation (Le Galliard *et al.*, 2011). Therefore, despite progression in tag development and decreasing mass and size, their use must still be approached with caution. Well established direct monitoring and surveying techniques, and the optimisation of these, may continue to offer good methodologies for improving understanding of a species until technologies become more viable.

Research for this thesis trialled radio-tracking of released sand lizards *Lacerta agilis* for the first time, however, following poor tag retention this monitoring method did not prove successful. Further testing is required to ascertain if this method is feasible for this species in heathland habitat.

1.2.3 Considerations for post-release monitoring

Adequately anticipating the response to release by a species, both at population and individual level, allows all other aspects of the reintroduction process to be optimised, both pre-release and subsequently to maximise success. Key aspects of this ideal scenario are discussed below, with particular reference to cryptic species.

1.2.3.1 Post-release movement and survival

A variety of factors must be included when modelling the immediate response of a reintroduced population. Observations that are years and perhaps generation lengths post-release, cannot be used to retrospectively determine details of the immediate post-release dynamics, nor the latter be fully indicative of the population going forward. Factors for consideration include seasonal variation, sexual and age-dependent variation, random individual variation and density dependence including Allee effects (Armstrong and Reynolds, 2012). These should be considered within the release context and monitoring, for example with regards to time frame and survey frequency. Understanding factors influencing post-release movement are crucial to manage the trade-off between site fidelity to the area in which individuals were released and adaptive movement (Le Gouar *et al.*, 2012).

1.2.3.2 Habitat selection

Post-release locational data can indicate how species perceive their environment, move through it and make selections of optimal habitat, and in turn how these favour survivorship (Bennett *et al.*, 2012). This offers insights into best management approach for the habitat prior and subsequent to the release process to ensure it is optimal, offering connectivity between patches and more widely to increase permeability of the landscape for species dispersal (IUCN, 2013; DEFRA, 2020).

Bennett *et al.* (2012) assess home ranges for a partially radio-tagged reintroduced brown treecreeper *Climacteris picumnu* population, quantifying individual movement parameters in terms of foray distance, foray rate, search rate and search area. Although a study with relatively small sample size (43, of which 18 were radio tagged), some unexpected results are seen. There is a preference for both low and high vegetation cover in wooded areas as opposed to the expected medium height, suggesting other factors may be at play, potentially within different social groups. There is also no suggestion that the longer an individual spends in the settling phase, the more at risk its social group is to predation which could result in lower survivorship, as might be expected. Of the 43 individuals released, none use the nest boxes set up as an additional experimental treatment, some opting instead to utilise tree cavities. This highlights the need to address artificial nest boxes as a monitoring tool for this species.

A study by Cote and Clobert (2007) contradicts the assumption that individuals released of the same species will prefer the same habitat at all costs, with reference to the presence or absence of conspecifics. Three dispersal phenotypes covering a full spectrum of social responses are identified in the common lizard *Z. vivipara* – individuals who colonise empty habitat, individuals attracted to areas of high density of conspecifics and those attracted to areas of lower density populations. (This concept is discussed in more detail in section 1.2.3.3.3.)

1.2.3.3 Individual variation

It is important to ascertain the variation in response to release by individuals within a release cohort, and potential key drivers of this, in terms of dispersal, and habitat and home range selection (Le Gouar *et al.*, 2012). The traits resulting in variation in individuals are complex. They are the interaction of genes and environmental factors amounting to observable characteristics. Many factors may affect individual response to release including body size, fitness and behaviour, and factors are unlikely to be independent of each other.

Individuals of the same species are unlikely to respond in an identical and predictable manner following release. For example, post-release monitoring of reintroduced puaiohi *Myadestes palmeri*, a critically endangered thrush, shows that 57% of the population dispersed out of the intended target release area, with two birds travelling over 3 km within one day of release (Foster *et al.*, 2003). It is important to consider the potential implications of individual variation on spatial use of a release site and beyond and to manage that site accordingly.

1.2.3.3.1 Morphometrics

It might be expected that animals that are of larger body size within their age cohort and considered to have an optimal body condition score (also not overweight) are fitter and thus more likely to survive. There are many studies assessing associations between size and survivorship. A lot focus on turtles, with varying results. Following a long-term release and recapture experiment with snapping turtles *Chelydra serpentina* from 1980 to 1993, as well as a series of three shorter studies Congdon *et al.* (1999) find no evidence to support the so-called “bigger is better” hypothesis. However, studies into the red-eared slider turtle *Trachemys scripta elegans* by Janzen *et al.* (2000) show size-dependent recapture probabilities. These are interpreted to represent mortality being biased towards smaller turtles, as was observed during initial release of 356 hatchlings from a seminatural rearing environment where some individuals had naturally perished. The study observes that a significant negative correlation exists between body size and time between release and recapture, suggesting that survivorship is likely linked to improved locomotor performance and thus the increased ability to escape predation.

In 115 neonatal Western rattlesnakes *Crotalus viridis*, survivorship over the first winter is found to be independent of both weight and condition at birth. This suggests that any advantage gained from greater size at birth occurs during the initial active season when food for smaller snakes is harder to obtain (Charland, 1989). Conversely, Nagy *et al.* (2015) find survivorship of 53 juvenile Agassiz's desert tortoises *Gopherus agassizii*, hatched and head-started in predator-resistant natural enclosures, increases with body size and age. They make recommendations that head-started tortoises should reach > 100 mm in carapace length (at least nine years of age) prior to release. A study of Trinidad killfish *Rivulus hartii* show that individual growth is positively correlated with movement in an area with high density of predators, where dispersal is likely to be of high risk; this correlation is not observed in areas where predators are absent (Fraser *et al.*, 2001). The contradictory nature of experimentation between species, across long study periods and with relatively large sample sizes, indicates that the relationship between size and survivorship is species-dependent and therefore requires study at species-level.

1.2.3.3.2 Physical fitness and locomotion

Timing of birth is often correlated with offspring fitness in animals. Parturition date of a viviparous lizard *Niveoscincus ocellatus* is largely driven by female thermal conditions, particularly maternal basking strategies (Wapstra *et al.*, 2010). Females also show repeatability in their parturition date each year, which could indicate consistent basking behaviours between individuals over time. Results show individuals born earlier are more likely to get through their first winter.

Stress and body condition are assessed in dispersal of common lizard *Z. vivipara* juveniles (Meylan *et al.*, 2002), where artificially increased levels of corticosterone and poor body condition in pregnant females results in limited dispersal of juveniles. Good body condition of juveniles also favours dispersal. However, no effect is had following corticosterone treatment on juveniles following birth. The fitness costs of tail autotomy have been widely studied in lizards, given the importance of this appendage for energy (lipid) storage (Doughty *et al.*, 2003). Tail loss has even indicated more cryptic behaviour by individuals (Olsson, Pauliny, *et al.*, 2010) with negative effects on locomotor ability (Guimarães *et al.*, 2014).

1.2.3.3.3 Behaviour

Behaviour is the neural control mechanism coordinating an animal's response to events in its own environment. These environmental stimuli are processed by the nervous system to give a view of the world that is shaped by natural selection. Behavioural plasticity, the change in behaviour resulting from an environmental stimulus, is either contextual where an immediate response is produced, or innate where it has been altered by past experiences and new neural and hormonal pathways have consequently formed. Decision-making by animals is ultimately driven by the need to successfully reproduce.

Individual factors will lead to different response to reintroduction. Natural variation and combinations of behaviours, termed behavioural syndromes or herein personality, must be considered and may be accentuated by duration in captivity due to relaxation or exertion of certain selection pressures (Beck *et al.*, 1994; Le Gouar *et al.*, 2012). Phenotypic plasticity is the ability for a specific genotype to show differing observable characteristics including behaviour, development and physical appearance, depending on its environment.

Behavioural diversity within a population provides resilience to changes in the environment, of which the greatest concern at present is climatic change (Wolf and Weissing, 2012). There are potential implications for community dynamics, evolution and even speciation. Maintaining capacity for a population to respond to threats through behavioural variation is vital and should be a key consideration in conservation efforts (Schindler *et al.*, 2010; Wolf and Weissing, 2012).

Historically, behavioural studies on reptiles have focused on thermoregulation due to its influence on diurnal and annual cycles (Spellerberg, 1972; Christian *et al.*, 2016). The thermal environment during embryonic development is found to affect offspring phenotypic traits in viviparous lizards and thus potentially fitness of individuals (Cadby *et al.*, 2014). Manipulating basking opportunities shows female basking behaviour differs in individuals from different geographic locations, with females in alpine regions being more opportunistic baskers than those in warmer areas. The growth of offspring varies between populations, potentially showing local adaptation to basking opportunities. This may infer that maternal effects and phenotypic plasticity will allow species to cope with changing environmental conditions.

Personality has been linked to fitness across taxa, for example in terms of survival and reproduction in great tits *Parus major* (Dingemanse *et al.*, 2004) and red squirrels *Sciurus vulgaris* (Boon *et al.*, 2007). Dingemanse *et al.* (2004) determine that adult survival rate is related to the behaviour of individuals in a novel environment, but with an opposite effect for each sex. They also establish that survivorship of offspring to breeding age is related to parents' personalities. Within reptiles, Cote *et al.* (2008) find that 'social' female common lizards *Z. vivipara* reproduce better than 'asocial' lizards, while juvenile lizards with low social tolerance have greater survivorship in populations of lower density. Social tolerance is also found to be constant regardless of situation. In a further common lizard study, some juveniles are found to be attracted to, and others repulsed by, the odour of male lizards (Cote and Clobert, 2007). It follows that social and asocial lizards may choose microhabitats based on the proximity and density of the population present. Different selective pressures will invariably lead to phenotypes with differing morphology, physiology and behaviour, and complex interactions between these (Le Galliard *et al.* 2003; Cote and Clobert 2007).

Dispersal is an ecological and evolutionary process, regulating movement of individuals, populations and species to an area deemed more suitable, be this natal movement, movement away from poor habitat, movement associated with the presence of conspecifics, or movement away from a point of release in a reintroduction (Cote and Clobert, 2007; Michelangeli *et al.*, 2017). Factors affecting demographic differences in response to reintroduction have been addressed. Ryckman *et al*. (2010) find elk *Cervus elaphus* calves disperse mostly in a south-easterly direction whereas adults disperse south-west. However, beyond this, there are individual differences and propensities to disperse within species.

The dispersal syndrome exhibited by an individual is typically linked to specific morphological, behavioural and physiological characteristics (Cote *et al.*, 2010; Debeffe *et al.*, 2014). In the context of dispersal, bolder, more exploratory personality types are seen to be typically linked with greater dispersal tendencies (Dingemanse *et al.*, 2003; Michelangeli *et al.*, 2017). Dingemanse *et al.* (2003) suggest that the positive correlation between high exploratory behaviour of parents and greater dispersal of great tit *P. major* offspring is due to the heritability of exploratory behaviour. However, they acknowledge that environmental effects may affect behavioural phenotypes and different dispersal techniques are often associated with other behavioural phenotypic traits, such as individuals dispersing shorter distances being more able to cope with social stress. Potential differences between sex are also highlighted. Phenotypic-dependent dispersal may thus underly much of a population's spatial ecology post-release, including in response to species interactions and distribution of a reintroduced cohort across a release site.

There is caution raised around making direct inferences on dispersal post-release based on natal dispersal tendencies, which have typically been more commonly studied. Reintroduced hihi *Notiomystis cincta* are found to make very different habitat selection, depending on whether they are natal or post-release dispersers, with natal dispersers being heavily influenced by conspecifics (Richardson and Ewen, 2016). It is also important not to assume comparable behaviour at repeated releases at a site, where for the first release no conspecifics will be present, yet for subsequent releases they will (Richardson and Ewen, 2016). Increased heterogeneity between habitat patch quality at a site is found to require a greater dependence on habitat selection strategies by the released population if it is to persist; strategies being ‘random’ versus ‘quality’ habitat selection, and social cues involving ‘avoidance’, ‘presence’ or ‘reproductive success’ of conspecifics (Mihoub *et al.*, 2009). When selection is based on patch quality and conspecific reproductive success, there is lower risk of project failure than for random habitat selection and presence/avoidance of conspecifics more generally. Interestingly, the authors also find that irrespective of this, the release of adults rather than juveniles is more effective, particularly for short-lived species (here, modelled on the barn swallow *Hirundo rustica* with a 4 year life expectancy; with the long-lived griffon vulture *Gyps fulvus* as its comparison with a > 40 year life expectancy, where results proved less conclusive). Therefore, releasing adults is found to reduce extinction probability regardless of habitat selection strategy (Mihoub *et al.*, 2009). Aggregation is also found to exist regardless of environmental conditions where strategies are more focused on social cues, as documented more widely (Citta and Lindberg, 2007; Mihoub *et al.*, 2009). Post-release dispersal and movement tendencies based on individual behaviour and habitat selection strategies, as well as the specific habitat of the release site itself and the age of individuals released, will therefore have likely consequences for population recruitment and abundance and distribution of the population.

Outward traits associated with captivity may influence an animal’s ability to fulfil its original ecological function, or fundamentally survive in its indigenous range habitat (Frankham *et al.*, 2010). Behavioural traits around boldness are often linked to a captive environment and in particular a loss of fear is invariably poorly suited to living in the wild. Bremner-Harrison *et al.* (2004) find that bolder swift foxes *Vulpes velox* that leave dens more quickly and approach novel stimuli more closely are those that die within the first six months post-release. The study is limited by a small sample size of 16 juvenile foxes, however, all effects are significant ($P < 0.05$). Many studies on hatchery versus wild fish have found that bolder and more aggressive individuals show poor antipredator behaviour leading to low survival rates (Conrad *et al.*, 2011).

Reintroduction practitioners may consider training of animals pre-release in order to attempt to reduce this potentially detrimental effect. Azevedo and Young (2006) implement anti-predator training on 15 greater rhea *Rhea americana* and find birds in the experimental group behave, as hoped, more shyly following the training and compared to the control group. Haage *et al.* (2017) observe an opposite trend. Bolder personality types in European mink *Mustela lutreola* during an Estonian reintroduction result in few predation attempts. This shows the inter-specific inconsistency of survival consequences for individuals along the behavioural spectrum. It also suggests that training, if undertaken, may be required to have the opposite effect for some species.

Few studies until recently have addressed reintroduction in the context of personality (Seddon *et al.*, 2007). The opposing outcomes across different taxonomic groups and species highlight the risks of making species-dependent assumptions based on previous research; this reflects research into size and survivorship assumptions. It is in practitioner interest to observe variation in individuals and assess how it may influence post-release movement, including density of conspecifics. Such knowledge allows *a priori* and continued site management to optimise microhabitat areas suitable for the variety of phenotypes observed. Response to a novel environment and initial reaction to release, in terms of personality syndromes displayed, could be a key predictor of ultimate dispersal, or rate of movement away from the release site, as well as survivorship. The links found between all key personality types and dispersal highlight the applicability of assessing this in a reintroduction context (Cooper *et al.*, 2017; Michelangeli *et al.*, 2017). There is also potential for animal selection for release dependent on personality (Haage *et al.*, 2017).

1.3 The sand lizard *Lacerta agilis*

The sand lizard *L. agilis* is a model small, specialist, cryptic species at risk of localised extinctions, and with a long history of reintroduction from captive populations.

1.3.1 Species distribution

Sand lizards are oviparous, diurnal green lizards widespread across Europe and northwest Asia. They are a member of family *Lacertidae* and there are ten recognised subspecies (Andres *et al.*, 2014).

The sand lizard exists in 36 European countries and into western Asia (Blanke and Fearnley, 2015). Severe declines have occurred across northern France, Belgium, the Netherlands, northern Germany, Denmark, Sweden, Estonia and the UK over recent decades (House and Spellerberg, 1983; Edgar and Bird, 2006). The subspecies present in northwest Europe, *Lacerta agilis agilis*, is found at its northerly limit within the UK, in well-managed heathlands and protected dune localities in southern England, west Wales and Merseyside (Jackson, 1979; Edgar and Bird, 2006).

The sand lizard occurs in the UK in three geographically and genetically distinct populations: Merseyside, Wealden, and Dorset (Beebee and Rowe, 2001; Russell, 2012). In southern England, the Wealden heathlands of south-west Surrey formerly acted as one of the species’ strongholds. However, only a few isolated natural populations now survive in this area.

1.3.2 Species biology

The sand lizard still harbours many unknowns in terms of its biology and ecology and improved understanding will help better inform conservation efforts. Much existing literature has a strong natural history element.

Sand lizards are ectotherms, requiring heat from an external source in order to raise their body temperature and thus perform essential biological functions such as defending territories and breeding. They have been described as 'shuttling heliotherms', obtaining heat by basking in solar radiation (heliothermy) as well as through contact with a heat source (thigmothermy) (Spellerberg, 1976). Optimal locations within their habitat may offer both options, along with proximity to sources of prey, predominantly arthropods, and shelter from predators both ground-dwelling such as the smooth snake *Coronella austriaca*, and aerial such as the kestrel *Falco tinnunculus* (Edgar and Bird, 2006). A preferred body temperature of 23-38°C has been reported (Spellerberg, 1976; Bischoff, 1984). Critical maximum and minimum body temperatures also exist, beyond which lizards will seek alternative space (Blanke and Fearnley, 2015). Research has been undertaken into the complex thermoregulatory energy balance of reptiles and relationships between body temperature, air temperature and an animal's 'operative temperature', and temporal changes in these (Castilla *et al.*, 1999; Christian *et al.*, 2016).

The spatial response to fluctuations in temperature by reptile species will also be a response to the surrounding and adjacent microhabitats. Thermal factors requiring consideration are namely solar radiation, reflected solar radiation, air temperature, soil/ground surface temperature and wind speed (Christian *et al.*, 2016). Anecdotal evidence discusses locations where lizards are seen basking (Blanke and Fearnley, 2015; Moulton, N. pers comm.; Poland, J. pers comm.); however, their preference of such spots and the exact mechanisms driving this are widely undocumented. Sand lizards have been observed selecting tree trunks and stones as basking spaces; they will additionally align their body to the sun's angle of incidence and their selection of a basking spot may also reflect its aspect (Blanke and Fearnley, 2015). The use of basking spots and microhabitats deemed most favourable is also affected by variation between individuals. For example, territoriality during breeding season may result in defence of basking locations of optimal temperature, be this due to its composition, angle or shading. Research into this concept has a history within tropical lizard species such as the bronze anole *Anolis aeneus* (Stamps, 1977; Stamps and Eason, 1989) and interspecific competition has been examined, for example in montane skink species in Australia (Langkilde and Shine, 2004). However, studies into this are limited for the native UK lizard assemblage (House *et al.*, 1979; Govier, 2017).

Sand lizards are considered sexually mature once brumated two to three times, maturity being more dependent on size than age (Bischoff, 1984). Brumation is a state of torpor, similar to hibernation in endotherms, allowing reptiles to survive extreme environmental conditions. Unlike hibernation, where mammals transition between torpidity and sleep regularly, during brumation ectotherms are dependent on environmental temperatures to dictate their emergence from this state (Wilkinson *et al.*, 2017). Literature often uses 'hibernation' interchangeably with 'brumation' for reptiles. Following overwintering male sand lizards will establish and defend territories prior to mating. Females begin trial burrowing and finally egg-laying between June and the start of July (Spellerberg and House, 1980; Beebee and Griffiths, 2000). Clutches exceeding ten eggs are not uncommon for sexually mature lizards; however, a mean of 7.4 eggs per female is reported from Marwell Wildlife's twenty-five-plus years of captive breeding of the species (Woodfine *et al.*, 2017). Males display a stockier appearance than females, with a broader head and thicker tail, although females often have a longer snout-vent length (SVL), the standard length metric in reptiles (Bischoff, 1984; McDiarmid *et al.*, 2012). In the UK, SVL for female sand lizards has exceeded 90 mm and for males 80 mm (Nicholson, 1980). Adults can weigh 15 g (The Wildlife Trusts, 2020).

In the UK, sand lizards overwinter any time between the months of October and March (Spellerberg and House, 1980; Beebee and Griffiths, 2000). Males have been found to enter brumation and emerge in the spring up to two weeks to a month before females (Olsson and Madsen, 1996; Inns, 2009).

Overwintering is a key period for reptiles and they can lose up to 10% of their body mass during this time (Gregory, 1982). It is therefore of great importance that they enter their brumation period in optimal body condition. Individuals have been noted to enter it particularly late and either perish or emerge particularly early when in poor condition to attempt to maximise hunting opportunities (Blanke and Fearnley, 2015). A wide body of literature and anecdote documents final and first sightings of sand lizards at the end and start of the season respectively across their range, highlighting variability even within the same population in the same year (summarised by Blanke and Fearnley, 2015). Broadly speaking, sand lizards have been found to enter brumation earlier following warmer summers with optimal feeding opportunities (Peters, 1970).

Little is documented regarding locations in which reptiles choose to overwinter, termed 'hibernacula', and little is known regarding survivorship and how this may vary with age and hibernacula selected. Juveniles appear more social than their adult counterparts and are often seen aggregating (Blanke and Fearnley, 2015) – basking alongside and on top of each other – and utilising the same burrow space (R. Gardner, personal observation); this indicates a benefit from overwintering socially. Hibernacula have been quoted at varying depths, dependent upon the substrate present, natural cavities available to exploit and the climes in which the lizard is located. In the UK a depth of around 30 cm has been suggested (Simms, 1970). Pre-existing sandy burrows and hollows will often be exploited; however, sand lizards will excavate to expand or create new burrows as required. Preferred cavity characteristics are reasoned as providing good isolation and drainage, in sloping ground and with vegetative or litter ground cover (House and Spellerberg, 1983).

1.3.3 Habitat requirements

The sand lizard's range extends from Wales and West France to Lake Baikal in Siberia (west to east), and Sweden and Karelia in Russia to Greece (north to south). Towards the centre of its global distribution the sand lizard occupies a broad spectrum of habitats, from boreal forest to the subtropics (Blanke and Fearnley, 2015). At the outer limits of its range more fragmented and specialised populations exist; in Great Britain the sand lizard is restricted to habitats with sandy substrates (Edgar and Bird, 2006). Here, the species occupies lowland heath and coastal dunes as well as secondary habitats such as railway embankments (Moulton and Corbett, 1999).

Within these sandy habitats the sand lizard favours localised topographical areas, termed *foci* (Corbett and Moulton, 1998). These are areas including south facing slopes and features and bare interfaces adjacent to the cover of vegetation; they are crucial in allowing this more specialist reptile species to meet its fundamental physiological needs. Ultimately there must be microhabitat offering adequate basking and shading opportunities allowing thermoregulation, potential for burrowing and oviposition in sandy soils, and opportunity for hunting and predator avoidance in often structurally diverse understorey vegetation (Edgar and Bird, 2006; Blanke and Fearnley, 2015). Such characteristics will be driven by factors such as underlying geology and soils, topography, vegetation and plant litter and associated microclimatic variables. Conservation efforts have sought to manage existing *foci* and upgrade those areas with potential to become highly favourable (Corbett and Moulton, 1998; Moulton and Corbett, 1999; Edgar *et al.*, 2010).

1.3.4 Threats and management

Habitat destruction, damage and fragmentation are key drivers of animal population extinction in a wide variety of taxa and across the world (Tanentzap *et al.*, 2012; Horvath *et al.*, 2019). Such detrimental environmental impacts over recent centuries, invariably in the form of development of urban and industrial areas as well as the creation of agricultural land (Underhill-Day, 2005), have resulted in many species remaining extant in small pockets of satisfactory habitat where former range has become uninhabitable (Merriam and Wegner, 1992).

Destruction and fragmentation of habitat in nineteenth and twentieth century Britain resulted in sand lizard populations being isolated and ultimately lost from the counties of Berkshire, Cheshire, Wiltshire, Sussex, Hampshire and Kent, and presumed former range in Devon and Cornwall (England) as well as Flint and Denbigh (Wales) (Moulton and Corbett, 1999; Blanke and Fearnley, 2015). Former species distribution is inferred from known loss of habitat, as historical records of sand lizard presence are inconsistent. The degree of habitat loss reflected across the south of the UK is illustrated in the example of Poole Basin where heathland habitat declined from 30,400 ha in 1811 to 832 ha in 1978 (Rose *et al.*, 2000). A combination of both natural and reintroduced populations now exist in the counties of Surrey and Dorset and reintroduced populations are present in Berkshire, Hampshire, Kent, West Sussex, Devon and Cornwall, occupying predominantly heathland but also sand dune sites (Moulton and Corbett, 1999; Edgar and Bird, 2006). Heathland is considered to be one of the habitats most impacted by urbanisation with increased risk of fire and disturbance through high human and domestic animal presence (Hayhow *et al.*, 2019).

Even when protected, mid-successional lowland heath favoured by sand lizards in the UK requires continual management in order to retain 'favourable' condition. This is achieved by preventing succession to a climax woodland vegetation, involving: diversifying neglected habitats, removing invasive trees and vegetation, turf cutting, and maintaining scrapes and ponds from year to year (Edgar *et al.*, 2010; Marwell Wildlife, 2015). Conservation grazing is one such method of stalling succession. Grazing, trampling and dunging at low-density stocking levels may offer benefit to reptile assemblages through the structural differences in vegetation created (Nature, 2005; Rose, 2010; Reading and Jofré, 2015; Broom, 2018). However, meeting this balance is challenging and if misconducted can be detrimental to some species (Reading and Jofré, 2015). Some heathlands are managed by controlled burning as an alternative to, or in addition to, grazing. However, there is belief that these controlled burns are often inappropriate for lowland heath management, having detrimental effects on species assemblages (Jofré and Reading, 2012). The intensity and spatial and temporal aspects of such activities require careful consideration and monitoring to ensure that there is not a conversely damaging impact (Reading and Jofré, 2015). When managed successfully, such an environment can support an array of vertebrates and invertebrates, as well as a variety of flora (Crofts and Jefferson, 1999; English Nature, 2005; Marwell Wildlife, 2015).

Evidence-based recommendations are vital for habitat management (Day *et al.*, 2003). Maintaining a site to encourage optimal biodiversity is often challenging. Many species will be more generalist, such as the slow worm *Anguis fragilis* and common lizard *Z. vivipara*, both of which can inhabit many habitat types. However, specialists such as the sand lizard will have more specific requirements, such as sand for oviposition, restricting its range (Simms, 1970; House *et al.*, 1979; Nemes *et al.*, 2006; Saveliev *et al.*, 2006; Blanke and Fearnley, 2015). Therefore, habitat restoration and ongoing maintenance must be undertaken with an understanding of the ecological requirements of the species assemblage spatiotemporally (Broom, 2018).

Other threats include introduction of the non-native western green lizard *Lacerta bilineata*, and likely non-native wall lizards *Podarcis muralis*, in the south of the UK, in terms of both competition and risk of disease transfer in contact zones with sand lizards (Wilkinson *et al.*, 2011; Williams, R. pers comm.). The nature of potential risks is being examined (Williams, R. pers. comm.).

1.3.5 Species protection

Sand lizards are considered of Least Concern on the IUCN Red List of Threatened Species due to their extensive range (Agasyan *et al.*, 2010). However, decline in numbers in the twentieth century in the north western part of this range has left populations threatened here (Edgar and Bird, 2006). This has led to an intense input of conservation effort over most recent decades. Legislation in Britain and Europe currently provides high protection for the sand lizard through The Bern Convention (1979); it is a European Protected Species (EPS) and is also listed on Annex IV of the European Habitats Directive (92/43/EEC). Additionally, in the UK the sand lizard is protected under Schedule 5 of the Wildlife and Countryside Act (1981), has been part of a Species Recovery Programme (SRP) project and subsequently subject to a Species Action Plan (Edgar and Bird, 2006), as well as being identified as a national priority under the UK Biodiversity Action Plan (BAP) (UK steering group 1995). A programme of captive breeding for reintroduction has formed a great part of conservation efforts, to attempt to halt decline and re-establish populations in areas of restored and protected habitat. This has just reached its fiftieth year (ARC-Trust pers. comm.).

1.3.6 The wider UK reptile assemblage

There are six native terrestrial species of reptile in the UK comprising three snakes, the adder *Vipera berus*, grass snake *Natrix helvetica* and smooth snake *Coronella austriaca*; and three lizards, the common lizard *Z. vivipara*, sand lizard *L. agilis* and legless slow worm *A. fragilis*. All species are at the north-westerly limit of their geographical range in the UK and are at particular risk of rapid population decline for the same reasons as outlined for the sand lizard above (Spellerberg and House, 1980; Edgar *et al.*, 2010).

Biological and ecological overlap of the sand lizard and common lizard has received research focus over recent decades (Dent, 1986; Graham, 2017), these being the only native legged-lizard species in the UK, with occupation of heathland and dune habitats by both species. Sand lizard prey primarily consists of invertebrates including spiders, grasshoppers, crickets and insect larvae (Nicholson, 1980; Edgar *et al.*, 2010). However, they will typically hunt anything that catches their attention through movement and that is smaller than them, including butterflies and dragonflies (R. Gardner, own obs.). Such prey is also the primary component of common lizard diet in a heathland environment; however, across the more diverse habitats occupied by this species soft-bodied prey forms a larger proportion (Edgar *et al.*, 2010). Where sand lizard home ranges may extend between just tens of square metres to several hundred (Nicholson, 1980; Strijbosch *et al.*, 1983; Blanke and Fearnley, 2015), common lizards have more limited movements to the lower end of this scale and much overlap is seen between the home ranges of conspecifics (Strijbosch *et al.*, 1983; Edgar *et al.*, 2010). Common lizards are typically seen from February through to November in southerly parts of the UK whereas sand lizard activity, especially of adults, is more restricted between March/April and September, although hatchlings have been observed as late as November (Edgar *et al.*, 2010; R. Gardner, personal observation). The species differ in terms of reproductive biology, the sand lizard being oviparous and the common lizard viviparous (Strijbosch, 1988; Beebee, 2013). Requirements during the summer months for sand lizard oviposition are a sandy substrate, offering areas of exposed sand and adjacent denser vegetation ideally facing south/south-west. In comparison, common lizards can actively seek a wider variety of microhabitat types that will regulate their body and embryo temperatures accordingly. This gives the common lizard its more generalist habitat requirements and an advantage over the sand lizard in the northern limits of its range (Edgar *et al.*, 2010). Basic reproductive characteristics for both species are determined by climate conditions in previous years; the sexually active period depends on climatic conditions of the current year (Saveliev *et al.*, 2006).

The third lizard native to the UK, the slow worm, occupies a broader range of habitats than even the common lizard and is able to tolerate less varied vegetation structure, making it even more generalist. Individuals are often found in the same location with home ranges of a similar size to common lizards; however, their prey is predominantly soft-bodied invertebrates such as slugs (Edgar *et al.*, 2010). Their thermoregulatory requirements cover a wide range, found to be between 9.8 and 33.2 ℃ in individuals utilising refugia at sites on the Isle of Purbeck, Dorset; this highlights their ability to be more generalist in habitat selection (Smith, 1990). However, even within broadly suitable habitat, densities have been found to differ during spatial capture-recapture research, possibly as a result of social interactions, resource availability or habitat quality (Schmidt *et al.*, 2017), having consequences for surveying techniques and predicting population sizes.

Along with the sand lizard, the smooth snake is a rare reptile in the UK, with habitat also restricted. Adders and grass snakes are generalists, with grass snakes the most mobile of all reptile species, found with home ranges of > 20 ha and able to travel up to several kilometres throughout their active season between the most favoured egg-laying, foraging and brumation locations (Madsen, 1984; Reading, 1997; Wisler *et al.*, 2008; Edgar *et al.*, 2010). The sand lizard, common lizard and slow worm are all potential prey for the UK's three native snake species, also commonly found inhabiting dry heath environments (Edgar *et al.*, 2010).

There is growing acknowledgement that reptiles collectively are increasingly under threat in the UK, including the widespread, more generalist species (Amphibian and Reptile Conservation Trust, 2020). Initiatives are being introduced to raise public awareness and understanding of their plight and even encouraging engagement through citizen science. Concerning trends in adder populations, for example, suggest shifting annual cycles in the light of warmer springs and significant population declines (Gardner *et al.*, 2019). Dry heath is the only habitat type in the UK in which all native reptile species occur in sympatry. Research into the sand lizard within this assemblage would therefore have beneficial implications across this reptile community.

1.3.7 Monitoring standards for reptiles in the UK

Reptile monitoring techniques are species and habitat-dependent at a global scale (McDiarmid *et al.*, 2012). Use of artificial refugia (or artificial cover objects) alongside walking transects in reptile surveys is applied widely in the UK. Refugia are attractants within the wider habitat, exploiting reptile reliance on the environment for thermoregulation. Consistent refugium types and dimensions enable repeat surveys to be standardised at a site and between sites. They minimise bias, offering locations of focus that reduce the effect of surveyor experience. Refugia are less disturbing than surveying natural coverage of sensitive habitats. Their ability to concentrate reptiles in known locations minimises time in the field, lowering disturbance further. Herpetologists recommend they are 100 x 61 cm (using standard industry measurements of corrugated iron), suggesting larger refugia perform better than smaller (Langham, 2011). They should be cleared of underlying litter and the longer they have been present at a site, the more likely their use by reptiles (Willson, 2016). These recommendations are predominantly based on field experience.

1.3.7.1 Challenges for reptile monitoring in the UK

Given the prominence of the refugia technique, determining the most advantageous use of materials would greatly aid its justification. A monitoring method for dry lowland heath is recommended with multiple hexagonal arrays of 37 steel refugia best visited in May, June and September (Reading, 1996; Reading, 1997). However, this does not seem to have been fully adopted by practitioners in subsequent monitoring advice (NARRS, 2011; Langham, 2012). It also has limitations in terms of its broader applicability to other habitat types, practicality of deployment of refugia at such high density, and the limited use of traditional corrugated metal refugia by legged lizards (Reading, 1997). There is a need for further research into this area and how it could be adapted and applied to species surveys taking into account fluctuating habitat and weather variables (Grant *et al.*, 1992). Some ecologists opt for (roofing) felt refugia in addition to, or instead of, tins (Sewell *et al.*, 2012; Gleed-Owen, 2017; Cathrine, 2018; Poland, J., pers. comm.). The quantifiable benefit of felt over tin, or potential alternatives, is not determined. It would be of great benefit to further optimise formal reptile surveying methodologies in the UK using field-based evidence to aid researchers and practitioners going forward (Sewell *et al.*, 2013; ARC Trust, 2019).

1.4 Reintroduction protocols for sand lizard in the UK

Efforts for fifty years in captive breeding and reintroductions of sand lizards in the UK have provided hope for their survival (Corbett and Moulton, 1998; Moulton and Corbett, 1999). Just over 10,000 lizards have been released across 79 sites in England and Wales at the time of writing (Moulton, N. pers comm.). The project was initiated by the British Herpetological Society Conservation Committee and ARC Trust with support from the government via Natural England (formerly English Nature), as well as private breeders and institutions with breeding facilities. Suitable receptor sites are assessed and reintroductions planned around five years in advance. Probability of absence of the species at a receptor site must reach > 95% prior to release (ARC Trust, pers comm.).

The protocols broadly followed have been to release ~ 50 captive-bred juveniles per site each year for three consecutive years, ideally early to mid-September but occasionally falling back to mid-April if weather proves unfavourable (Corbett and Moulton, 1998; Moulton and Corbett, 1999; ARC Trust, 2016). Following research conducted in Sweden, this number was raised to ~ 80 juveniles a year in 2016 (Berglind *et al.*, 2015, Moulton, N. pers.comm.).

Currently, reintroductions are considered successful at a given time following confirmation of the presence of live individuals, recently sloughed skin, or signs of breeding (Moulton and Corbett, 1999; Woodfine *et al.*, 2017). Detectability of sand lizards is notoriously low. This is taken into account in the guidance literature for conserving this species, where both qualitative (presence or absence) and quantitative assessment are referred to (Corbett and Moulton, 1998; Moulton and Corbett, 1999). Transect surveys are highlighted as a key tool for reptile surveyors across many habitats, offering comparable inter-survey data when standardised to assess abundance and distribution spatiotemporally (McDiarmid *et al.*, 2012). For sand lizards, it is recommended transects are of pre-determined length along key features where sand lizards are most easily observed, such as sandy traces and paths; this offers the highest observation likelihood. However, it does not allow for thorough spatial, systematic cover of the site, focusing instead on the areas in which the lizards were released. It also makes comparison between sites difficult. Surveys are recommended ~ 4 times in the spring and again in the autumn to give an indication of broad population trends (Corbett and Moulton, 1998; Moulton and Corbett, 1999).

Efforts have been made to suggest optimal time of year and conditions during which to survey, including PhD research that began with support from Marwell Wildlife in 2005 to assess factors that may be affecting detectability of sand lizards. Sex and reproductive stage are found to influence detection rate seasonally. Localised climatic conditions on any given day are also highlighted a key consideration when monitoring populations in order to maximise detectability (Fearnley, 2009). Despite this, population estimates have not been achievable due to insufficient sightings over enough survey instances. Typical capture-mark-recapture (CMR) techniques have returned too few recaptures of lizards in wild populations to estimate population size (Fearnley, 2009).

More recent development of techniques to use egg test burrows along transects to estimate abundance of females, such as explored with gopher tortoises *Gopherus polyphemus* (Stober and Smith, 2010), may allow progress to be made in this area (ARC Trust, pers. comm.; Woodfine *et al.*, 2017). However, typical reptile survey techniques involving tin refugia have thus proved suboptimal in use for the UK's legged lizards, attracting predominantly snakes and slow worms, as discussed previously (Reading, 1997; Sewell *et al.*, 2012).

1.4.1 Shortcomings, challenges and quantifications needed

Sand lizard breeding groups are kept and managed in deemed appropriate male : female ratios and densities, with husbandry and egg/juvenile rearing carried out as outlined in guidelines developed for this purpose (Langford, 1985; Davis, 2004). Captive populations are supplemented from wild populations under license (by ARC Trust) as breeding adults are lost by natural causes over time.

It is not possible to establish full parentage of clutches laid within breeding groups; females may breed with multiple males rendering establishing the intra-clutch male parentage impossible (R. Gardner, personal observation.) without genetic sampling (Olsson *et al.*, 2010). It is feasible to link females with the clutches they have laid, but only if they are observed in the laying process and the eggs are excavated immediately, prior to any possible adjacent clutch-laying by other females. This is highly time-consuming and not guaranteed conclusive (R. Gardner, personal observation.).

Animals released from captive populations are juveniles that have been bred and supplementary fed in naturalistic outdoor vivaria. Juvenile lizards are not genetically profiled prior to release, nor are they selected for release in any specific manner, for example, based on their morphometrics or body condition. This could be argued to reflect what would be observed in the wild, such that natural selection can operate on the released cohort, as it would on all juveniles hatched *in situ*. However, two factors will have already acted against this. Firstly, breeding occurred in a population at unnaturally high density, where, although a number of different females parent clutches, it is possible that only one or two of the more dominant males will have contributed to the offspring's genetics, outcompeting their conspecifics for territorial and breeding rights (Blanke and Fearnley, 2015; Govier, 2017). Secondly, head-starting the lizards in captivity, instead of transferring them as eggs to sites to hatch naturally as has been carried out in other countries (Berglind *et al.*, 2015), is intended to optimise body condition to improve survival rate over winter. This may result in some individuals surviving beyond the point they would have in the wild, improving their chance of reaching adulthood in an environment with completely different selection pressures. Therefore, there is an artificial element included in the genetic pool of the F1 (first filial) post-release generation with unnatural selection already occurring pre-release. If genetic selection is not feasible, selection of the release population by other means may still be beneficial in reintroductions of this species to optimise success (IUCN, 2013) .

One of the greatest challenges of any reintroduction initiative is determining its success or failure through post-release monitoring. Research into favourable breeding conditions and dispersal has been undertaken for the sand lizard (Olsson and Shine, 1996; Olsson *et al.*, 1997). Studies have also been conducted into microhabitat use by this and other native reptile species (Langton and Beckett, 1995; Amat *et al.*, 2003; Vanhooydonck and Van Damme, 2003; Grozdanov *et al.*, 2014), but such studies invariably generate as many questions as they set out to answer (e.g. de Bont, van Gelder and Olders, 1986; Sacerdote-Velat *et al.*, 2014).

Little is known regarding survivorship and movement of juveniles once released in their cohort in both the short- and long-term. Post-release movement may involve the departure of individuals away from the release site, either permanently or temporarily (Clobert *et al.*, 2001). Further research into juvenile use of microhabitats and *foci* and temporal changes in this would aid monitoring efforts post-release. While some level of survivorship or breeding has deemed reintroduction a success at many sites of rare reptile release, the effect on the ecosystem and communities already in place also warrants due consideration. A thorough, robust surveying methodology must therefore be integrated into any reintroduction project in order to assess such impacts alongside monitoring the release population (Beck *et al.*, 1994; Taylor *et al.*, 2017).

Of the 27 sites to which Marwell Wildlife has contributed lizards (prior to this research), 26 have evidence that the species is still present between 2014 and 2016; the final site is thought to have failed due to fire damage from suspected arson (Woodfine *et al.*, 2017). Resource limitations such as personnel, time and underlying these funding, have meant quantifications regarding the release population in terms of movement or survival have also been limited.

To date, just over 10,000 lizards have been released as part of the wider reintroduction program, but the numbers that have survived to breeding age are unknown. It is from this key life stage that the population is subsequently augmented through entirely natural recruitment processes. Across the three years of a release initiative, lizards released could be the offspring of as few as eight to ten captive females and likely fewer different males, due to dominance hierarchies existing within captive populations (Govier, 2017). If a very small percentage of these individuals survive to breeding age in the wild post-release, the population will enter a greater genetic bottleneck than that created through captive breeding. Conversely, when survival to breeding age is unknown, the 'three annual releases' approach to reintroduction of sand lizards and the recent increase in release numbers are difficult to either support or argue against, without an evidence-base. If the release approach is surplus to what is necessary, it is possible that resources would be best focused elsewhere to establish the species across more sites and the metapopulation within the wider landscape (Armstrong and Seddon, 2008; Taylor *et al.*, 2017). Conversely, it is possible that recently revised numbers would benefit from still further increase. Without quantitative data it is difficult to make objective assessments of protocols.

1.5 Project introduction

This research employs current UK sand lizard reintroduction protocols, from captive breeding (Davis, 2004) to release (Corbett and Moulton, 1998; Moulton and Corbett, 1999) and then makes the first detailed efforts to monitor sand lizards intensively in the wild post-release. It makes monitoring recommendations for the sand lizard and sympatric widespread reptile species. The focus is on the establishment, or release-growth, stages of reintroduction (Sarrazin, 2007; Armstrong and Seddon, 2008). Released sand lizards are assessed at both population and individual level incorporating biological, ecological and behavioural variables to assess and quantify post-release movement, habitat and microhabitat use, and immediate post-release survival. The potential application of such findings is considered within the wider reintroduction biology field.

The initial study focus was around detectability; specifically, how it may be improved to help answer the key questions in this research, but also to suggest a best practice approach for post-release monitoring of sand lizards following reintroduction. There is a "lack of up-to-date formal reptile survey and monitoring guidance for conservation and ecological consultancy purposes" (ARC Trust, 2019) (Sewell *et al.*, 2013). This research hopes to offer evidence-based recommendations for sand lizard reintroduction and post-release monitoring, whilst incorporating assessment of the wider reptile assemblage (Reading, 1997; Fearnley, 2009; Edgar *et al.*, 2010; Santos and Poquet, 2010; IUCN, 2013).

1.5.1 Research aims

Each data chapter details its specific aim and objectives. The following are overarching aims (with corresponding chapters specified):

1. To assess spatiotemporal behaviour of sand lizards, including optimal times, conditions and methods for surveying, highlighting demographic and social differences (Chapter 2).

2. To determine microhabitat and microclimate preferences in the field and inform survey methodologies for sand lizards post-release and wild widespread reptile species in the UK, focusing on species-specific variables of refugium type, placement and surveying conditions (Chapters 3 and 4).

3. To determine demographic and individual factors affecting immediate post-release movement and post-winter survival of sand lizards, in order to make recommendations for reintroduction protocols going forward (Chapters 5 and 6).

1.5.2 Site descriptions

1.5.2.1 Marwell Zoo

Marwell Wildlife’s captive sand lizard population is held as part of their collection at Marwell Zoo in Hampshire, UK. It has provided several opportunities for research over recent years (Fearnley, 2009; Isaacs, 2009; Kain, 2010; Govier, 2017; Jordan, 2018). The captive breeding programme was set up at the zoo in 1989 (Edgar, 1990) for lizards of the genetically distinct Dorset descent. The breeding adult population has been supplemented as required over the last three decades, following natural loss, with wild-caught individuals obtained under license by ARC Trust. Juveniles are released as part of the national reintroduction programme that ARC Trust coordinate.

Sand lizards were kept in vivaria in an off-show area of the zoo to minimise disturbance for breeding and overwintering; the outer enclosure was upgraded in winter 2018-19 (Figure 1; enclosure further described in 2.2 and Figure 3). The sand lizard population was managed as per the Captive Husbandry Manual (Davis, 2004), with wild food supplemented by gut-loaded and vitamin dusted black crickets *Gryllus assimilis.* Every year, sand lizard eggs were laid in pre-prepared sand patches within the adult breeding vivarium, upon which they are excavated as soon after laying as possible before being artificially incubated to optimise hatching rate, approximately 6 weeks later. Twenty-four hours after the complete hatching of each clutch, hatchlings were put outside into rearing vivaria until their release. The enclosure also included a research vivarium for behavioural studies.

Figure 1 The sand lizard enclosure at Marwell Zoo, showing the adult breeding vivarium in the foreground, research vivarium to its right, and a juvenile rearing vivarium top left; *R Gardner*.

1.5.2.2 Eelmoor Marsh SSSI

Eelmoor Marsh is a 79 ha site located adjacent to Farnborough airport. It is owned by QinetiQ, managed in conjunction with Marwell Wildlife and entirely fenced and protected. It is bounded on all sides by Farnborough Airfield to the south and east, Cody Technology Park to the north and the A323 and Basingstoke Canal to the west. Beyond these lie residential and industrial areas, large Ministry of Defence sites, as well as parks, golf courses, woodlands and Nature Reserves.

The marsh has undergone grazing management since 1995 with highland cattle *Bos taurus* and Przewalski's horses *Equus ferus przewalskii*. This, alongside routine clearing of invasive species and the creation of scrapes and ponds, enabled the ultimate designation of its condition as 'favourable' by Natural England in 2013, with this status retained in 2020. It comprises wet, dry and humid heath, grassland, mire and woodland communities (Figure 2). The livestock is of a low stocking density (four horses and eight-ten highland cattle total). The horses do not have access to the dry heathland habitat; the cattle were also limited in grazing this area, spending short durations here through only the late autumn, winter and early spring period (with little crossover with dry heath reptile surveys). Eelmoor holds over 400 species of conservation concern including 32 grassland indicator species and over a third of Britain's dragonfly and damselfly species. It hosts four of Britain's seven native amphibians and all four widespread reptile species. It is also part of the Thames Basin Heaths Special Protected Area (SPA) (Marwell Wildlife, 2015).

Figure 2 Mature dry heath habitat at Eelmoor Marsh SSSI; *R. Gardner*.

Species reintroduction had not been carried out at Eelmoor Marsh prior to this research, the site instead being allowed time to respond to its restoration. This resulted in the return of many species that had disappeared as a result of traditional land use decline, exotic tree species introduction, and extensive drainage (Marwell Wildlife, 2015). With favourable conditions recently declared this research came at an optimal time to consider the reintroduction of species whose biological constraints in terms of mobility would not allow them to repopulate the site of their own accord, to encourage a more complete assemblage of lowland heath ecosystem species.

Reptile surveys already conducted at Eelmoor (using long-standing tin refugia) determine the presence of the four most common reptile species (Hutchins, 2004; Langham, 2015). Natural England require that there is at least 95% probability of absence of sand lizards at a site prior to considering it for a reintroduction. In order to meet this criterion, the survey effort required is calculated, accounting for the detectability of the species, and then undertaken. The probability level was surpassed at Eelmoor by 2017, reaching 99% (Langham, pers. comm.).

1.5.3 Animal welfare and ethical considerations

The sand lizard population at Marwell Zoo is part of a wider breeding programme coordinated by ARC Trust, with support from Natural England. Optimal body condition is encouraged for both the breeding population of adults and their offspring for release by supplementary feeding and misting with rainwater. Population density was maintained following husbandry guidelines for the species (Davis, 2004). Handling and human presence was limited as far as possible to minimize unnatural behaviours and more long-term adaptation to captivity. This was especially important for the animals being released. The nature of this research endeavoured to balance the fundamental practical reintroduction of this species to Eelmoor Marsh, with pre- and post-release experimental testing and monitoring that in its design limited human interaction, whilst maintaining sufficient scientific robustness to offer results with statistical validity. It used routine, standard assessments of the captive population at Marwell (health screening, morphometric recording) to obtain additional data pre-release as far as possible. All aspects of data collection were ethically approved in advance by the University of Southampton's ethical review process, following Ethics and Research Governance Online (ERGO approved Submission IDs 23009 and 27600).

1.5.4 Application of research to species conservation

Justification for the current sand lizard reintroduction framework is supported by signs of ongoing species presence over subsequent years (Corbett and Moulton, 1998; Woodfine *et al.*, 2017; Moulton, N. pers comm.). Outcomes of semi-comparable conservation efforts made in other parts of Europe, such as the translocation of sand lizard eggs to a forest plantation in Sweden and subsequent post-release monitoring (Berglind *et al.*, 2015), also support this broad approach. However, it is clear that limitations will exist when assessing a species in different countries, in different broad habitat types, and with a differing release approach; therefore the predictability of outcomes may not be entirely transferable. This research aims to address the situation for sand lizard reintroduction in the UK.

There will likely be limitations around application of recommendations following the research. For example, ARC Trust are dependent on different institutions and private breeders to supply lizards for release. The capacity of these organisations and individuals (often volunteers), their ability to alter capacity, available time and the finance involved, will require due consideration. However, anticipating potential challenges for recommendations should not deter assessment of protocols.

The applicability of this research to other species reintroductions are diverse. The study addresses a common situation within the field of reintroduction biology, whereby a longstanding project has only partial, detailed, measured understanding of success. This is often due to insufficient capacity to undertake monitoring to an optimal level, through various constraints, be they temporal, biological or financial (Seddon, 1999; Nichols and Armstrong, 2012; Taylor *et al.*, 2017). This research is an example of how qualitative and anecdotal findings from efforts to date can be more quantitatively tested and consequently, recommendations made. This will highlight the importance of comparative studies across the field of reintroduction biology. With reintroduction a prominent conservation tool globally, this study aims to explore the dynamic approach that should be taken towards such conservation efforts. Scientists and practitioners must continue to work together to ensure methodologies and approaches are as applicable now as they have previously been.

It is through persistence in research, maximising the success of conservation projects and in disseminating their key messages – particularly in a fast-changing world – that we are constantly reminded of the recovery capacity of species and biodiversity. Besson and Cree (2010) highlight the ability of the tuatara *Sphenodon punctatus* to adapt to a thermally variable environment (by quality and duration) to retain its body condition. Viviparous lizards too have been seen to alter their basking regime in order to optimise development of their young (Wapstra *et al.*, 2010). This gives hope that in the face of climate change, one of the greatest modern-day threats to biodiversity, the species that may struggle in terms of mobility to keep up with shifting local climates, hold capacity to adapt in an alternative way. It is possible that sand lizards and other cryptic species may possess capacities science is still to observe. It is only through research such as this that we can elevate our conservation output and species understanding to make predictions for capacity to adapt to changing climes and habitats. With positive intervention and improved understanding, ecological resilience has potential to persist.

1.5.5 Chapter overview

Following on from the context provided in the literature review and project introduction of **Chapter 1**, **Chapter 2** lays the foundation for post-release monitoring of sand lizards, assessing their spatiotemporal behaviour in naturalistic vivaria in captivity. This assessment is taken forward into a field setting for sand lizards post-reintroduction at Eelmoor Marsh in **Chapter 3**, as well as assessed at this site for the widespread reptile species native to the UK in **Chapter 4**. Through applying the surveying techniques developed, demographic trends in sand lizard response to reintroduction and survival are examined in **Chapter 5**, before this is examined at the individual level in **Chapter 6**. Conclusions and conservation recommendations are discussed in **Chapter 7**.

All data relating to this thesis have been deposited in the University of Southampton Research Repository and are accessible here: https://doi.org/10.5258/SOTON/D1797 .

Chapter 2 Optimising survey protocols for the sand lizard *Lacerta agilis* using artificial refugia: demographic, social and environmental considerations

2.1 Introduction

Successful species monitoring, such as that required following reintroduction, relies on sufficient understanding of the biology and ecology of the species concerned, as well as the use of physical and conceptual tools (IUCN, 2013). In the case of many cryptic species, which are often less charismatic, less observed and lesser studied (Seddon *et al.*, 2005; Bajomi *et al.*, 2010), such optimal tools and methods may be challenging to determine. There are many reasons why conservation interventions have often been broadly experience-based. Often these centre on a lack of opportunity or resource to formally assess methods. In instances where scientific evaluation has occurred, there is often limited access to results in a suitable, applicable form for practitioners, and engagement between scientists and decision-makers can be limited (Pullin *et al.*, 2004; Rose *et al.*, 2019). In the field of reintroduction biology, and conservation more widely, there are continued calls for scientific learning to be more comprehensively integrated into practice to support management decisions with an evidence base, and to ensure that key issues are addressed (Sutherland *et al.*, 2004, 2012; Pressey *et al.*, 2017; Taylor *et al.*, 2017). Such scientific approaches can inform future protocols throughout the reintroduction process, for example from the selection of a suitable receptor site (Draper *et al.*, 2019) through to post-release monitoring (Canessa *et al.*, 2016; Normande *et al.*, 2016; Baker *et al.*, 2018; Gooley *et al.*, 2019).

Understanding key predictors of species detection can help to optimise and focus survey efforts and hence improve monitoring methods. This maximises the quantitative data that can be collected, makes best use of resources, and ultimately feeds this back into practitioner protocols. It can be challenging to obtain data from cryptic species in a wild, uncontrolled environment, where failing to detect individuals does not mean they are not present; different approaches may be required. Remote monitoring of species is becoming increasingly common due to rapid technological developments and their improved cost-effectiveness (Rowley and Alford, 2007; Hamed *et al.*, 2008; Bennett *et al.*, 2012). However, this is not always appropriate or possible, particularly for smaller, cryptic species in a complex habitat.

Access to captive populations of species offers the opportunity for more specific experimental design, in a controlled environment and with identified individuals. There may be very direct applications to populations within the captive environment, for example, assessing spatial behaviour in response to differing light and heat conditions by reptile species inform most appropriate husbandry practices (Wheler and Fa, 1995; Dickinson and Fa, 1997). The *ex situ* environment also offers the opportunity to assess behaviours that directly impact *in situ* research, monitoring and management. These studies are of particular importance for cryptic species that cannot be studied easily in the wild; for example assessing temporal variation in behaviour (Bagilet *et al.*, 2017), breeding behaviour (Thomas *et al.*, 2018) and social behaviour and use of space and resources (Miura, 1984; Halliwell *et al.*, 2017). Miura (1984) finds that dominance hierarchies in secretive muntjac deer *Muntiacus reevesi* mean high-ranking males hold well-defined, protected areas throughout the year; this is reflected in captivity by the deer partitioning the enclosure. Halliwell *et al.* (2017) find that habitat structure is fundamental to social and mating systems of White’s skink *Liopholis whitii,* using wild-caught individuals in an experimental outdoor setup.

It must be considered that wild and captive populations may not show wholly comparable behaviour, for example captivity may affect social structure within a group. However, when a species is difficult to observe in the wild and where captive populations exist for conservation purposes, the benefits of such studies have great value when considered, with their limitations, to both captive and wild populations (Miura, 1984; Mäekivi, 2016). Captive settings, often breeding facilities, commonly hold a range of demographic groups for study.

2.1.1 Thermoregulation and consequent spatiotemporal behaviours

Reptiles rely on their environment to meet optimal body temperature and their spatiotemporal behaviour and metabolism reflects this (Huey, 1982; Huey, 1991; Besson and Cree, 2010; Edgar *et al.*, 2010). They are in a comparably vulnerable position to endotherms at a similar trophic level because thermoregulation is not a goal in its own right, but a step to other behavioural and physiological functions that ultimately influence fitness and survivorship (Huey, 1982).

The requirement to maintain optimal body temperature, which varies between reptile species (Brattstrom, 1965), will physiologically drive the spatiotemporal behaviour of an individual, in a behaviour called positive thermotaxis (moving towards warmth) (Harshaw *et al.*, 2017). Some species regulate their temperature more precisely than others (Christian *et al.*, 2016). The niches that are used by species are affected by a multitude of thermal and energetic influencing factors, driven by underlying natural cycles and linked to their ecosystem, potentially within a social context (Harshaw *et al.*, 2017). As optimal body temperature is neared priorities may shift in order to meet other physiological needs, such as foraging or seeking a mate. Thermoregulation can vary not just interspecifically but also intraspecifically. It temporally changes both diurnally and seasonally, due to differences in prevailing weather and thus microclimatic conditions (environmental indicators) (House *et al.*, 1980; Fearnley, 2009), and seasonal shifts in behavioural priorities such as juvenile/egg-development in females and food availability (Christian *et al.*, 2016). Predator avoidance is another essential behaviour that must be constantly re-evaluated and re-prioritised. When in the open, reptiles may be at greater risk of predation, and basking sites are often adjacent to higher vegetation offering an escape and shelter (Castilla *et al.*, 1999; Meister, 2008). However, the ability to flee from predation once optimal body temperature is reached is much enhanced. It is this trade-off and continual risk assessment and decision-making that drives behaviour.

Sand lizards *L. agilis* are described as 'shuttling heliotherms'; they obtain their required heat through both heliothermy, basking in direct solar radiation, and thigmothermy, direct body contact with something that holds heat (Spellerberg, 1976). Their preferred operative temperature is between 23° C and 38° C (Spellerberg, 1976; Bischoff, 1984) and critical thresholds either side of this will cause sand lizards to shift locations. The reliance on thigmothermy and heliothermy causes the sand lizard to carefully select basking locations to account for optimal incidences of both. Areas that offer a high temperature gradient, invariably through surrounding heterogeneous vegetation, are selected. The substrate should respond quickly to a heat source, be quick-drying and provide insulation. The opportunity for lizards to align themselves to the sun's angle of incidence is also preferred (Blanke and Fearnley, 2015). A study observing up to five sand lizards and common lizards *Z. vivipara* in a captive setup for 34 days from May to August finds wood (logs) is used preferentially over dry vegetation, sand or bricks for basking (65.9 % basking time compared to 22.5 %, 5.9% and 5.3 % respectively) (House *et al.*, 1980). The temperature of the wood is between 15° C and 39° C when being utilised. This preference for wood is also noted in observations in wild populations (Blanke and Fearnley, 2015). A study into an urban population of sand lizards in Bonn, Germany, finds the majority of basking time is spent on living or dead vegetation (Meister, 2008).

Sand lizard use of space will shift both diurnally and annually, in response to changing season and reproductive cycle. In comparison to the sympatric common lizard in the UK, sand lizards are found to reach preferred temperatures more slowly and have overall shorter diurnal active periods; the sand lizard is particularly reliant on a variety of thermal conditions in heterogeneous vegetation structures and higher temperatures through solar radiation (House *et al.*, 1980; Blanke and Fearnley, 2015). Such temporal patterns may be the effect of a higher body surface to mass ratio being advantageous in the common lizard, the smaller of the two species, meaning its emergence can be profitable in lower temperatures than the sand lizard (House *et al.*, 1980). These differences are further demonstrated in the more generalist habitat requirements of the common lizard and its geographic range extending as far north as the Arctic Circle (House *et al.*, 1980; Edgar *et al.*, 2010). The daily activity patterns of sand lizard individuals are found to consistently vary from conspecifics, such as basking in higher temperatures in the same area; however, broad trends exist (Blanke and Fearnley, 2015). Seasonal shifts result in observation likelihood being higher either with clear skies and temperatures up to 15° C, or when overcast in higher temperatures; this infers that in spring and autumn observations will be increased in the middle of the day, and in warmer summer months, at the start and perhaps end of the day (Inns, 1996). Male sand lizards in the UK are more active in March and April, and females May to October (Fearnley, 2009). Predicting how climate change may impact such seasonal and diurnal patterns would be advantageous to anticipate its effect on this species, as well as improving population modelling and survey protocols (Cruz *et al.*, 2015; Rytteri *et al.*, 2017; Préau *et al.*, 2019).

Selection of space within an environment is also influenced by social factors and individual differences in response to these. This may involve particularly social or asocial individuals that consequently select microhabitat based on density of conspecifics (Cote *et al.*, 2008). It may also be linked to demographic and time-dependent tendencies, such as territoriality, direct competition and dominance hierarchies, both intra- and inter-specifically between sympatric taxa (Langkilde and Shine, 2004). These concepts are considered below in specific reference to the sand lizard.

2.1.2 Dominance in adult sand lizards

Dominance hierarchies within species are invariably correlated with fitness measures, such as breeding success (Neumann *et al.*, 2018). Persistent selection by females of the same secondary-sexual traits in males, despite their having no tangible benefit to offspring fitness, should eliminate variance in these traits over time. However, this is evidently not the case as such traits persist. This is known as the lek paradox (Taylor and Williams, 1982; Kirkpatrick and Ryan, 1991). Partial explanations have been offered in recent years, for example traits being condition-dependent with great genetic variance in condition (Kotiaho *et al.*, 2001) and recognising the importance of indirect genetic effects such as maternal phenotypes and consequent offspring provisioning (Miller and Moore, 2007). However, a solution is not wholly concluded.

Hierarchy and sexual success has been observed in captive sand lizards, specifically in the form of a single dominant male consistently monopolising mating opportunities and holding large, priority territory in the population (Govier, 2017). Where genetic profiling is not possible on a captive-bred population intended for reintroduction, understanding such social hierarchies in the adult source population indicates genetic diversity among offspring and informs captive management and husbandry approaches, such as number and density of individuals held. In the captive context, dominance - often associated with the personality trait of boldness (Bremner-Harrison *et al.*, 2004; Govier, 2017) - has a positive relationship with breeding success. Such personalities and behavioural syndromes, known to be heritable (Smith and Blumstein, 2008), may however have negative consequences for released individuals in a wild environment. This has been observed in a number of species across taxonomic groups (Bremner-Harrison *et al.*, 2004; Conrad *et al.*, 2011) where increased risk of predation is apparent in bolder animals, and animals that are more detectable and monopolise habitat. However, it does lack complete consistency, with some taxa showing an opposite effect (Haage *et al.*, 2017); this highlights the importance of studying this on a species-by-species basis. Dominance has also been correlated with exploratory behaviour, which has shown contrasting effects depending on both context, such as whether a stable hierarchy exists or is still developing (Verbeek *et al.*, 1999), and life stage (Dingemanse and De Goede, 2004).

Dominance determines distribution of ecological resources for sand lizards, such as optimal microhabitats and *foci* for foraging and basking, whereby conspecifics are displaced (Govier, 2017). Sand lizards bask near each other on the same surface comparably less than common lizards; when this does occur for sand lizards, it is either two females or a male and female, indicating a lack of tolerance by males to other males (House *et al.*, 1980). In comparison, common lizards are observed basking next to two or more conspecifics over three times as often. Such habitat and resource partitioning driven by dominance hierarchies is observed in a variety of species (Miura, 1984; Nakano, 1995; Taylor and Lattanzio, 2016; Borgmans *et al.*, 2020). It is likely affected by context, such as density of conspecifics and available resources locally, as well as geographic range and corresponding resource opportunities such as to aid thermoregulation (Strickland *et al.*, 2016; Taylor and Lattanzio, 2016). Dominance may drive interactions and resource division initially; additional behavioural variation can influence longer term outcomes (Taylor and Lattanzio, 2016).

Context-specific dominance behaviour, as well as its relationship with behavioural traits, will often affect resource and habitat use. Better understanding dominance on a species-specific basis will help to establish the drivers behind spatiotemporal behaviour within habitat. It is important in the context of breeding programs for reintroduction to understand this in captivity and to infer its relevance to a wild, post-release situation. It also informs monitoring and habitat management.

2.1.3 Aggregative behaviour in juvenile sand lizards

Aggregation, observed and studied in a variety of ectothermic species (Espinoza and Quinteros, 2008; Khan *et al.*, 2010; Chapperon and Seuront, 2012; Rabosky *et al.*, 2012), offers both opportunity and risk. It can provide enhanced foraging efficiency, greater predator avoidance, and warmth. But, depending upon phenotype, can it also increase resource competition, risk of predation and disease risk (Jullien and Clobert, 2000). In thick-tailed geckos *Nephrurus milii*, aggregative behaviour is suggested to primarily occur to allow individual control of thermal exchange rates; this species inhabits microhabitats of highly variable thermal properties, in a climate that is also highly variable (Shah *et al.*, 2003). In the desert night lizard *Xantusia vigilis* winter huddling disproportionately benefits smaller individuals and may cause delayed dispersal and the formation of kin groups; in warmer temperatures this aggregatory behaviour could decline and cause a complete shift in social behaviour of populations of this species (Rabosky *et al.*, 2012). Competition for optimal thermal resources is assessed in neonatal bearded dragons *Pogona vitticeps*. Mutual tolerance to conspecifics is observed when a light resource is present, with aggregation towards this when set to lower temperatures and aggregation away from it when set to higher temperatures. The study highlights likely mutual attraction to the same microclimate features and conspecific tolerance, however, whether individuals aggregate prior to or after assessing conspecific temperatures is not known (Khan *et al.*, 2010).

Social aggregation is observed in sand lizards, typically in groups of their own age demographic. It occurs sometimes in adults, typically on cooler days, and more commonly in juveniles (Blanke and Fearnley, 2015). The latter is particularly evident in the captive environment where high densities mean easy access to conspecifics (R. Gardner personal observation). Whether this is driven predominantly by thermoregulatory benefits or other factors, such as the presence of a mutually beneficial resource, or a combination, has not been studied for this species.

Sharing of thermogenesis between two or more individuals, termed kleptothermy, is an additional thermoregulatory mechanism to heliothermy and thigmothermy. If kleptothermy is a driver of aggregation in juvenile sand lizards, aggregation would occur primarily within specific weather or environmental bounds and with an underlying temporal pattern. For example, it would be expected typically in early mornings to speed up reaching optimal body temperature, and in evenings to maintain optimal temperature for longer prior to seeking overnight shelter.

2.1.4 Suggested stages in monitoring sand lizards

Poor detectability of sand lizards is the greatest challenge underlying their monitoring. Three stages are suggested for successful observation and form the structure of this chapter's analyses. Firstly, whether sand lizards have the potential to be observed at a given point in time; this equates to being above ground and not obscured from view by vegetation.

The second stage is the use of space in relation to microhabitat at a given time; whether there are microhabitat features that would be indicative of where lizards are more likely to be when visible and therefore where surveyors may choose to concentrate their efforts. Use of microhabitat is determined by the behaviour being undertaken; an optimal place for basking may not equate to an optimal location for foraging (Huey, 1991; Blanke and Fearnley, 2015).

The third stage involves the use of artificial refugia, a technique long established for reptile monitoring (Froglife, 1999; Langham, 2011; Sewell *et al.*, 2013). Reptiles are commonly observed in the UK when raising their body temperature either through openly basking or refuging in a warm location; this is the behaviour surveyors utilise to observe them most easily. Refugia are a form of attractant. Attractants intend to increase encounter probability beyond that expected by random chance, used in conjunction with the normal sampling method or device (Stewart *et al.*, 2019); in the case of reptiles the latter is transect walks. Refugia mirror, for example, attractant techniques of baiting live mammal traps and areas in front of camera traps that utilise the physiological need for food in order to monitor a species. The value of such methodologies is debated. They are species- and context-specific and may influence animal movement resulting in habituation to an area. This may bias data collected on species density and distribution, resource and habitat use, and behaviour. Reptile transect routes and refugia placement often focus on specific habitat features rather than using random or systematic sampling methods to try to improve species detection (Cusack *et al.*, 2015). This may cause further bias in sampling methodology. However, where cryptic species are concerned, such negative effects may be outweighed by the importance of optimising detection probability to obtain enough data for species distribution and population estimates in a timely and cost-effective manner (Preez *et al.*, 2014; Stewart *et al.*, 2019).

Refugium materials deployed have thermal properties that offer greater thermoregulation opportunities than the surrounding environment (Froglife, 1999; Joint Nature Conservation Committee, 2004). These refugia offer thigmothermy potential, as well as heliothermy through openly basking or using ambient heat underneath the material in a more protected and sheltered location. Placement of refugia intends to improve detectability by encouraging reptiles to a location that can be systematically surveyed. Following the stages of theoretic visibility and use of specific microhabitats, the final stage in successful observation therefore considers refugium preference and associated spatial and temporal variables.

2.1.5 Aims

This initial study within the captive population of sand lizards at Marwell Zoo allowed all three suggested stages of monitoring sand lizards to be examined. The space potentially utilised was finite and fully observable, and a known number of lizards were within that space (be they visible or not) – this context is not possible in a field setting. Therefore, the captive study provides a unique opportunity for insight into detectability as a concept for this species, which could not be examined in the same context in the wild.

It is anticipated that the proportion of time spent visible in a given location may differ according to microhabitat and refugium type (as discussed above) but also by individual, sex and temporally as previously discussed. Time-dependence is further indicative of wider seasonal and environmental conditions, such as diurnal weather changes. Aggregation and dominance also require consideration. By quantifying this in a small captive population in controlled conditions, inferences can be made to optimise surveying for this species in the field, and context offered for inter-lizard differences observed. Chapter 3 goes on to take the second and third stages of monitoring into a field setting.

The overarching aim of this chapter is: **to assess the effect of social, demographic and environmental factors on sand lizard detectability and use of space, suggesting implications for surveying**. Its objectives are:

1. To assess social and temporal factors affecting adult and juvenile sand lizard visibility and use of space in late summer, including
    i. territoriality in adults,
    ii. aggregation in juveniles.
2. To determine microhabitat and refugium use by adult and juvenile sand lizards.
3. To consider environmental factors in relation to 1 and 2 for individual adult sand lizards.

2.2 Methods

Behavioural observations of sand lizards took place between mid-August and mid-September 2016 in the sand lizard enclosure at Marwell Zoo. Three males, three females and a clutch of eight juveniles were studied, within their demographic groups.

The south-facing outer enclosure measured approximately 20 m x 9 m (180 m^2) in area and 2.5 m in height. It consisted of a wire and nylon mesh fruit-cage supported by a timber frame and with ~ 80 cm high aluminium sheet panelling at its base with an external overhang to deter rodents; these panels were buried an additional 30 cm underground. The panels were renovated to partially buried breeze blocks with aluminium overhang, the mesh was renewed and an external electric fence was added to improve security in winter 2018-19 (Figure 1); the previous structure had been in place for 25 years and showed signs of degrading. The fruit-cage enclosure contained a series of vivaria, surrounded by gravel walkways: a large vivarium for captive breeding adults (area ~ 12 m x 5 m); a research vivarium (5 m x 5.65 m); two smaller vivaria for rearing hatchling lizards pre-release (~ 6 x 2 m); and two glass tanks (0.9 x 0.9 m) for temporary quarantine or additional juvenile rearing capacity. The research vivarium allowed the study of selected animals in an experimental set-up and could be subdivided (Figure 3).

The adult, research and juvenile rearing vivaria were constructed with Perspex sheeting, at a depth and height of 30 cm below and above ground, overlain by inverse drainpipes in order to deter escape. The tank glass extended 0.6 m in height and 0.1 m below ground and had wooden-framed mesh lids. The habitat in the adult and research vivaria was soil and vegetation recovered from Canford Heath, Dorset, prior to development, on top of a rubble and clay piping underlay offering ample hibernacula opportunities for overwintering lizards. The rearing vivaria and glass tanks constituted a coarse sand and sandy-soil mix with planted heather and grass habitat.

For this study, the research area was subdivided by translucent twin-wall polycarbonate roofing sheets to create smaller vivaria for male and female observations (Figure 3). As with the Perspex, the polycarbonate was buried ~30 cm below ground; it reached a height of ~25 cm above ground, 5 cm below the outer perimeter Perspex and inverted piping to prevent escape. Each of the male and female vivaria measured 1.15 m x 5.65 m. A walkway between the two areas of 0.5 m in width allowed each to be observed from all sides. The juveniles were observed in one of the glass tanks, which could also be viewed from all angles (Figure 3).

Habitat within each study vivaria for adults and juveniles consisted of ~20 % sand and 80 % grasses and heather *Calluna vulgaris*. Each then offered twelve refugia, consisting of four material types, each across three vegetative/microhabitat variants. Materials were: plywood (5 mm thick); Cembrit Ltd. natural Spanish roofing slate (4-6 mm thick); Marley® dark red plain clay roofing tile (25 mm thick); and flat steel 'tins' (0.9 mm thick). Materials measured approximately 20 x 15 cm each in the adult vivaria and approximately 10 x 7 cm in the juvenile tank; this was roughly proportionate to body size of the lizards. (See Figure 4). Microhabitat conditions were: surrounded by $\geq$ 95% bare sand ($\geq$ 2cm border around material); surrounded by $\geq$ 95% vegetation ($\geq$ 2cm border); and on partial sand, partial vegetation (within 40%-60% split). Heating properties of refugia that are substantially smaller than those deployed in the field will differ. However, this study is concerned with comparing material types within a given scenario (and including all properties that may affect use by reptiles); each was a comparable size within each scenario to account for this. The file study in subsequent chapters enabled the approach to be studied further.

Observations were undertaken in conjunction with L. Donovan (University of Southampton). Inter-observer reliability in recordings was determined by clear definitions of use of space, determined collaboratively during a two-day pilot study (see Appendix A.1). The observer stood to the north of the male and female vivaria to avoid casting a shadow over the individuals being studied; observer movement needed to better observe individuals was slow and minimal. The juvenile vivarium was observed from overhead and the west or east, according to time of day, ensuring again that no shadow crossed the study subjects; observer movement was minimal.

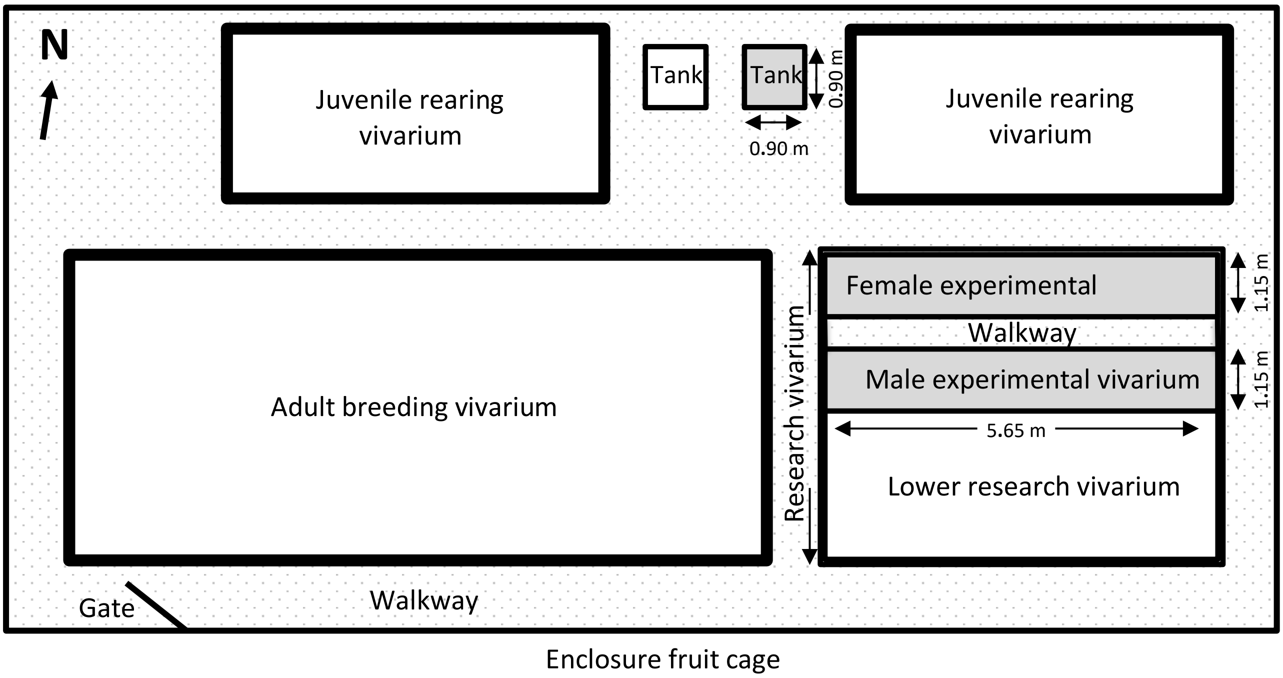


Figure 3 Plan view of sand lizard enclosure at Marwell Zoo, including locations and dimensions of experimental vivaria for behavioural observations, shaded grey.

Analysis of material use by adult lizards needed to consider individual use of space and potential dominance hierarchies and social responses, and whether these may affect the assumption of equal ‘access’ to all materials by all individuals, such as with regard to the ideal free distribution concept of habitat selection (Fretwell and Lucas, 1970). The three male and three female adult sand lizards were approximately three years old. The females were wild-caught; the males were captive-bred and recently transferred to the research vivarium. The adult design needed to assess dominance and its effect on spatial behaviour, as well as offer each lizard ample suitable microhabitat for welfare reasons and in order to observe natural behaviours. Therefore, each lizard needed access to microhabitats offering refuging and basking opportunities that were artificial (refugia) and natural (vegetation, sand). This limited the set-up of the vivaria in anticipation of individuals establishing themselves in areas within it. For example, one end could not be predominantly bare sand and the other densely vegetated. Refugium placement therefore did not match an ideal nested design across the adult vivaria but was a randomised design as far as possible within the heterogeneous microhabitat necessary.

The juvenile sand lizards studied underwent the same process of hatching in an artificial incubator, having morphometrics recorded, and then being placed in the same rearing area as the rest of its clutch. They had the same microhabitat and microclimate conditions, foraging opportunities and exposure to potential predatory threats i.e. human presence and overhead avian presence. The process of hatching and captive rearing was as per the standard sand lizard husbandry guidelines (Davis, 2004). The eight juveniles studied were approximately three weeks old when observations began and two months old when they ended – this being the typical timing of release into the wild. There was less concern over territorial hierarchies and resource and patch competition among juveniles, that may restrict older individuals to certain areas (Blanke and Fearnley, 2015; R Gardner, personal observations). Figure 4 shows the vivaria set-up.

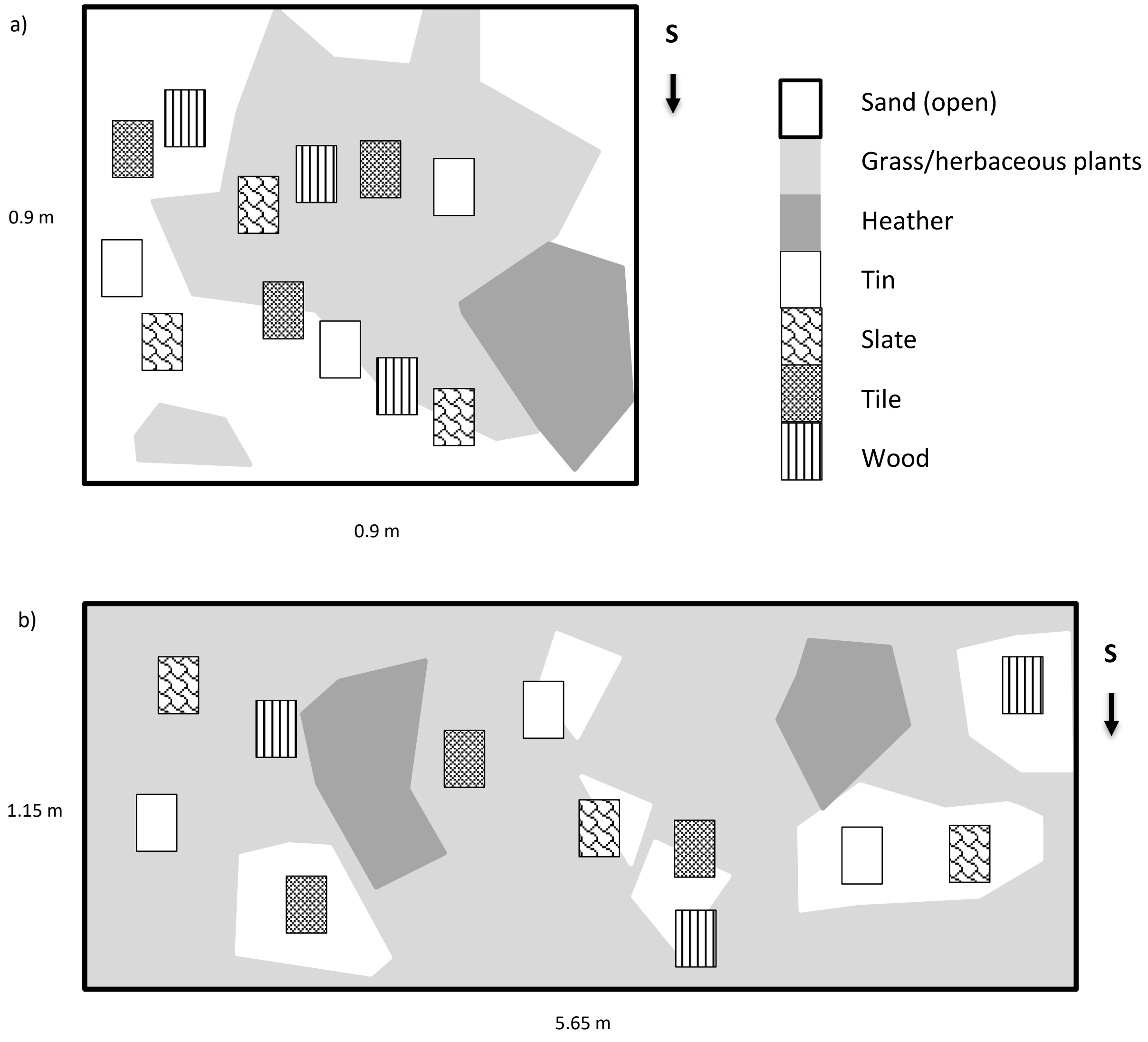


Figure 4 Diagram of a) juvenile and b) adult (female) sand lizard vivarium set-up.

Observations were undertaken on weekdays, Monday-Friday between 16/08/2016 and 17/09/2016. Occasionally intended days or observation sessions were missed due to observer illness or heavy rain (when lizards would be sheltering and unlocatable). Each demographic group was observed in succession for 30 minutes each (90 minutes total) and four of these 90-minute sessions were defined across the day. Twenty full days of observations were obtained (120 hours in total). Male and female observations were continuous focal studies; juvenile observations involved one-minute interval scan sampling. The four daily sessions were time blocks of 2.5–3 hours, occurring between 08:30 and 19:40 and divided into early morning, late morning/early afternoon, mid-afternoon and early evening observations. The order in which observations were made (males, females, juveniles) during each of the four blocks of observations rotated daily to ensure each had comparable spread and was subject to intra-session shifting environmental characteristics (Table 2). A short break (~ 10 minutes) was taken in between each 30 minutes observation period to maintain observer focus and concentration. Each adult vivarium was visually divided into a three by six grid (width x length) of equal parts (numbered A1-C6) by measuring and marking the Perspex and polycarbonate. The juvenile tank was divided into a three by three grid (numbered 1-9). The six adults, three males and three females, were individually identified by their unique dorsal markings (ocelli) and their identities learned prior to the study beginning, during the two days of pilot observations.

Table 2 Sand lizard Session timings and example of daily rotation of order of observations.

	Order of observations		
Session Time	**Day X**	**Day Y**	**Day Z**
08:30-11:00	Males	Females	Juveniles
(3 x 30 mins)	Females	Juveniles	Males
	Juveniles	Males	Females
11:00-13:30	Males	Females	Juveniles
(3 x 30 mins)	Females	Juveniles	Males
	Juveniles	Males	Females
13:30-16:30	Males	Females	Juveniles
(3 x 30 mins)	Females	Juveniles	Males
	Juveniles	Males	Females
16:40-19:40	Males	Females	Juveniles
(3 x 30 mins)	Females	Juveniles	Males
	Juveniles	Males	Females

2.2.1 Adult sand lizards

Location of each adult individual was continuously observed throughout the 30-minute observation sessions and noted to the nearest second using a stopwatch. During each session, the individual's location was detailed by its location in the vivarium and also its use of grass/heather/sand/material.

It was noted in coded abbreviations whether the lizard was moving or stationary and if moving whether this was fast or slow; movement was only separately recorded if it lasted for ≥ 2 seconds (i.e. a small body shift lasting 1 second was recorded as two adjacent 'still' observations). Locations were assigned based on the positioning of the majority of the lizard's main body, classed as from neck to tail-base. Lizards were assigned an 'Out of Sight' category for any period during which they could not be seen. Additional details noted any further activity of interest, for example 'hunting' or 'chased by lizard X'. Any recordings of shared space utilisation by an individual between a vegetated and open habitat (i.e. half body in each) was classed the vegetated category when ≥ 3 'obstructions', for example blades of grass, passed between the overhead observer and the lizard. For analyses, microhabitat use was grouped as follows, using stationary data only; open microhabitat use consisted use of tin/tile/slate/wood refugia, bare sand, on heather/grass; covered microhabitat use consisted under heather/grass and vegetated sand. Out of sight and burrow use were grouped separately as they infer a fully 'hidden' behavioural state / use of the microhabitat. See Appendix A.1 for a full ethogram and definitions.

2.2.2 Juvenile sand lizards

The eight juveniles was observed by instantaneous scan sampling from 0 to 30 minutes (31 observations total) by a tally of the locations of all individuals in sight. This included their location within the tank and use of grass/heather/sand/material and whether they was under or on top of these. Locations were again assigned based on the position of most of each lizard's body; see Appendix A.1. This was a collective study of the collective clutch of lizards, therefore individuals were not identified; this would have been impractical for scan sampling of eight animals of this size at once. All eight juvenile lizards had full access to all areas of the tank, including to their conspecifics, for the duration of the study. Any aggregation behaviour was defined as physical contact between lizards. This was sub-divided into 'touching' and 'piling' behaviour, where 'piling' involved some vertical as opposed to just horizontal alignment of lizards; this was viewed as a stronger aggregatory behaviour. Aggregation was quantified as a count of the behaviour itself, for example two lizards in contact would have a score of 1, three in contact would have a score of 2, and so on.

2.2.3 Weather and microclimate variables

Local weather data were logged at minute intervals for the duration of all observations, including air temperature (°C), barometric pressure (hPa), relative humidity (%), windspeed (m/s) and rainfall (mm/hr), by an Oregon Scientific WMR200 Professional Weather Station positioned just outside the sand lizard enclosure. UV data were obtained from the base station at Reading University (publicly available, via DEFRA) and solar radiation data (hourly) from a recording station in Odiham, Hampshire (MIDAS Open, 2019); these localities offered the geographically closest, consistent data available for the time period of the study. Soil temperature readings (°C) were taken during each block of three observations in the research vivarium and an adjacent comparable juvenile rearing tank, at depths of 2 cm and 10 cm, in the shade. The lower research area (Figure 3), containing no lizards) was used to deploy twelve Thermochron™ iButton thermal dataloggers to obtain data on air temperature (°C). These were placed directly i) under dense vegetation above ground, ii) under sparse vegetation above ground, iii) on bare sand and iv) under one of each refugium material type. A thermal datalogger was also placed under dense vegetation in a tank adjacent to the juvenile study tank to assess its comparable microclimate.

2.3 Analysis

2.3.1 Dominance metrics

Four measures were used to assess social metrics of male sand lizards. They are defined below, as calculated for each individual. Each has its benefits and shortcomings; these have been assessed in detail elsewhere (Langbein and Puppe, 2004). The calculation of a variety of metrics allows corroboration of any single measure. Behaviours included were any interactions between two individuals whereby one responded in a submissive manner to another. This ranged from biting and active chasing, to displacement from a resting/basking place.

The Dominance Index (DI) (Bowen and Brooks, 1978) incorporates each interaction between the individual in question and all other individuals to produce an index between -1 and +1 in the following equation:

$$\text{DI} = \frac{\text{wins} - \text{defeats}}{\text{wins} + \text{defeats}}$$

A second method considers the performance of an individual against all other individuals (i.e. each individual is either ultimately subordinate or dominant to every other), without including the number of interactions. This method focuses on all dyadic relationships (between two individuals) without allowing any one particular dyad that may have a disproportionate number of interactions to potentially bias the index (Lamprecht, 1986). It produces an index of dominance for each individual as a percentage:

$$\text{DI}_{\text{dom}} = \frac{\text{subordinates}}{\text{subordinates} + \text{dominants}} \times 100\%$$

Agonistic interactions are also calculated per time unit to produce the agonistic index (AGI). In this context this is calculated per individual however can be used to provide information at group level over time to assist in monitoring any changes in, for example, season or habitat (Puppe and Tuchscherer, 1994). The AGI produces an integer per designated time unit (e.g. per study session / for the whole study duration):

$$\text{AGI} = \text{agonistic interactions} / \text{time unit}$$

The AGI can be further developed into an aggressive index, taking instead of the number of agonistic interactions an individual is involved with, the number of agonistic interactions it initiated, per time unit (Araba and Crowell-Davis, 1994):

$$\text{ARI} = \text{agonistic interactions initiated} / \text{time unit}$$

2.3.2 Statistical analyses

All statistical analyses were undertaken in RStudio 1.1.463 and 1.2.1335 (R versions 3.5.3 and 4.0.2). All analyses assumed significance at $P \leq 0.05$. Power analyses, as discussed in Results, were run in R package ‘pwr’.

Optimal time(s) of day are recommended in guidelines for reptile surveying, shifting with seasonality (Inns, 1996; Froglife, 1999; Moulton and Corbett, 1999; Fearnley, 2009; National Amphibian and Reptile Recording Scheme - NARRS, Reptile Surveys, 2011). In this regard, time of day is a proxy for expected environmental conditions. As such, the four daily Sessions (early morning (1), late morning/early afternoon (2), mid-afternoon (3) and early evening (4) (see Table 2) underlie initial analyses. Specific environmental variables are subsequently considered in later analyses. Exploratory analyses into relationships between environmental variables and each other, and environmental variables and Session are found in Appendix A.10 (rainfall was not included, only occurring in 5/240 Sessions). An example of thermal datalogger readings across a day in different microhabitats can be found in Appendix A.11.

2.3.2.1 Spatial fidelity of adult sand lizards

Sand lizard spatial fidelity was analysed using Chi-squared. This followed methods employed in captive reptile studies of comparable design (Wheler and Fa, 1995; Dickinson and Fa, 1997); the consequent temporal pseudoreplication is acknowledged and results likely exaggerated. Frequency of cell use was the dependent variable, pooled for each lizard across all observation sessions (40 hours). All eighteen vivarium grid cells were considered separately in male lizard analysis. However, low frequencies of female sand lizard observations resulted in cells being grouped vertically in the grid in order to still obtain results on space use (i.e. pooling of cells A1, B1 and C1; A2, B2 and C2; etc.), and Female C being excluded due to particularly limited observations.

2.3.2.2 Predictors of detectability and use of space by adult sand lizards

Initially a binomial generalised linear mixed model (GLMM) was run to ascertain the significant predictors of proportion of time spent in a given microhabitat, incorporating categorical fixed effects of Vegetation/Refugium use, Vegetative state, Refugium type, Session number, Individual lizard and Sex (Individual was nested within Sex, Refugium type was nested within Refugium-Wider habitat use); Session number within Date was included as a random effect to account for the repeated Sessions over multiple days (time). AIC values and Likelihood Ratio tests were used to compare model iterations. Interactions between variables could not be included due to over-parameterisation with insufficient replication (see Appendix A.1 for initial model results). Exploratory analyses suggested important interactions were present, therefore the variables were explored in simpler models with fewer variables, as now described and presented in the Results.

Binomial GLMMs were used to analyse the effect of Session (indicative of time of day) and Individual on sand lizard visibility as a proportional dependent variable, for females and males separately. Interactions were included in the maximal model and subsequently removed for model comparison. AIC values and likelihood ratio tests were used to confirm the optimal model. Categorical fixed effects were Session (1-4) and Individual; Session number within Date was included as a random effect.

Preferred microhabitat type surrounding refugia was modelled with binomial GLMMs for each sex, with Individual and Microhabitat type (Open/Semi-vegetated/Vegetated) as fixed effects, an interaction between the two, and Session number within Date as a random effect. Preferred refugium material type and refugium use by Session was also modelled for all lizards collectively with a binomial GLMM, with the same dependent variable of proportion of time, fixed effects of Refugium type and Session, and Session number within Date as a random effect. All results were visualised using bar charts and an interaction plot, with supplementary detail and analyses in Appendices where stated.

2.3.2.3 Environmental variables and adult sand lizards

Series of stepwise regressions were run to create minimum adequate models predicting visibility and collective refugium use by individual adult sand lizards, additively assessing the predictive value of the environmental variables collected (summarised in Appendices A.10, A.11, A.12). Models fitted were multiple logistic regression models utilising a binary response variable of presence/absence per session and viewing each session as an independent data point. The additive analysis process utilised Chi square statistics of all explanatory variables and in order to select those having a significant effect on the dependent variable; significant variables were selected additively by lowest AIC value. This process also enabled correlations of predictor variables to be ascertained.

2.3.2.4 Aggregatory behaviour of juvenile sand lizards

A Poisson GLMM assessed Session and frequency of aggregatory behaviour (touching, piling). The dependent variable was count data, with fixed effects of Session (1-4) and Aggregatory behaviour (touching/piling) as categorical variables; Session number within Date was a random effect to account for repeatability of sessions over multiple days. Boxplots display the data.

2.3.2.5 Predictors of detectability and use of space by juvenile sand lizards

Visibility was assessed as a proportional response variable using a binomial GLMM for all eight lizards collectively, with Session (1-4) as a fixed effect and Session number within Date as a random effect. The frequency of use of refugia by Session was modelled using a Poisson GLMM, with Session number within Date as random effect. A bar chart and interaction plot display the data.

2.4 Results

2.4.1 Adult sand lizard detectability and use of space

2.4.1.1 Dominance hierarchies and spatial fidelity

Only one interaction, chasing, was observed between two females across the entire observation period (Female B chasing Female C). This was alone insufficient to determine a dominance hierarchy within the group.

All dominance metrics showed Male A was most dominant, with Males B and C either of equal position, or B slightly more dominant than C (Table 3). (See Appendix A.2 for raw interaction data.)

Table 3 Sociometric measures at the individual level. All time units are 40 hours.

	Social metric			
Male	Dominance Index (f-1 to +1)	Dominance % (0-100%)	AGI (# interactions/ time unit)	ARI (# interactions initiated/ time unit)
A	1.00	100	13	13
B	-0.45	50	9	3
C	-0.67	50	9	2

All females were visible for less time than all males across the study period (see Appendix A.3). (Time discussed in this section is time spent visible and stationary.) There was greater overlap between utilisation of vivarium cells by females, for example the cell most used by Females B and C was the same (Figure 5). Females showed greater cell fidelity than males, with each female having three or more grid cells they didn't enter during the study. Female A split time similarly across 4-5 adjacent cells, whereas Females B and C had a single much preferred cell (67% and 47% of time respectively) and 1-2 other cells where 10-30% of their time was spent.

Males made greater use of the whole vivarium, with only Male C having a cell that was not entered during the study (Figure 6). The Dominant male (Male A) spent most of his visible time in the west end of the vivarium – over 50% in two cells, with Males B and C spending very little time in this area. Male A did however still utilise space across the whole vivarium and spent >11% of his time collectively using the three cells at the far eastern end. Males B and C showed more overlap and closer proximity in their use of space, with Male C's most favoured cell being Male B's second most favoured. Approximately 30% of each of Male B and C's time was spent in one cell, with 5-6 additional cells then utilised between 5 and 20% of the time.

There were opposing relationships between cell use by females (A and B) (χ^2 (5, n = 226) = 121.53, $p < 0.001$) and males (A, B and C) (χ^2 (34, n = 728) = 285.13, $p < 0.001$) (see Appendices A.4, A.5 for full results); although as noted, this baseline analysis includes temporal pseudoreplication across sessions. Females A and B, although showing no agonistic interactions, were still strongly dissociated in terms of their use of the vivarium (see also A.6). Although Females B and C shared greatest proportion of their visible time in B3, Female C's comparably low visibility meant that this was not that great an overlap in actual time. Notably, the one interaction that did occur between females was the chasing of Female C by Female B, and it is Female C that could not be included in these analyses due to her low frequency of use of cells, representing low visibility (activity). The dissociation of Males B and C to those cells favoured by Male A is confirmed (see also Appendix A.6).

Female A

Vivarium cell	1	2	3	4	5	6
A	00:08:29 2.83 %	00:01:51 0.62 %	00:01:03 0.35 %	00:10:00 3.34 %	00:00:00 0.00 %	00:03:39 1.22 %
B	00:04:01 1.34 %	00:03:15 1.08 %	00:38:15 12.76 %	01:16:22 25.48 %	00:35:38 11.89 %	00:58:03 19.37 %
C	00:01:24 0.47 %	00:00:00 0.00 %	00:00:00 0.00 %	00:04:18 1.43 %	00:04:12 1.40 %	00:49:15 16.43 %

Female B

Vivarium cell	1	2	3	4	5	6
A	00:03:54 0.55 %	00:00:00 0.00 %	00:29:47 4.22 %	00:01:29 0.21 %	00:00:00 0.00 %	00:00:00 0.00 %
B	00:14:10 2.01 %	00:00:00 0.00 %	07:50:16 66.56 %	00:44:42 6.33 %	00:08:56 1.26 %	00:02:21 0.33 %
C	00:00:00 0.00 %	00:00:00 0.00 %	01:54:54 16.26 %	00:00:00 0.00 %	00:16:02 2.27 %	00:00:00 0.00 %

Female C

Vivarium cell	1	2	3	4	5	6
A	00:04:19 2.40 %	00:08:54 4.94 %	00:00:00 0.00 %	0:00:00 0.00 %	00:02:49 1.56 %	00:01:14 0.68 %
B	00:52:14 29.00 %	00:21:31 11.95 %	01:25:22 47.40 %	00:02:40 1.48 %	00:01:004 0.59 %	00:00:00 0.00 %
C	00:00:00 0.00 %	00:00:00 0.00 %	00:00:00 0.00 %	00:00:00 0.00 %	00:00:00 0.00 %	00:00:00 0.00 %

Key (percent of time visible and stationary):

0.00 %	0.10-1.99 %	2.00-4.99%	5.00-9.99 %	10.00-29.99%	30.00-49.99 %	>50.00 %

Figure 5 Actual (hh:mm:ss) and percent (%) time that female sand lizards spent stationary while visible in each grid cell of the vivarium (Female A time stationary visible = 04:59:45; Female B time stationary visible = 11:46:31; Female C time stationary visible = 03:00:07).

Male A

Vivarium cell	1	2	3	4	5	6
A	00:57:14 3.97 %	00:09:15 0.64 %	00:07:52 0.55 %	00:08:43 0.61 %	00:18:36 1.29 %	04:58:13 20.70 %
B	00:53:03 3.68 %	00:41:04 2.85 %	00:28:42 1.99 %	00:31:08 2.16 %	00:43:45 3.04 %	07:30:17 31.26 %
C	00:57:50 4.01 %	00:22:09 1.54 %	01:04:05 4.45 %	02:15:49 9.43 %	00:58:30 4.06 %	00:54:22 3.77 %

Male B

Vivarium cell	1	2	3	4	5	6
A	00:16:48 1.64 %	01:12:15 7.07 %	05:10:01 30.33 %	00:09:03 0.89 %	00:14:20 1.40 %	00:07:11 0.70 %
B	00:09:42 0.95 %	02:21:23 13.83 %	02:02:12 11.96 %	00:08:42 0.85 %	00:27:17 2.67 %	00:21:24 2.09 %
C	00:40:05 3.92 %	00:08:43 0.85 %	01:03:22 7.21 %	01:03:22 6.20 %	00:43:51 4.29 %	00:31:59 3.13 %

Male C

Vivarium cell	1	2	3	4	5	6
A	00:45:07 5.14 %	00:13:33 1.54 %	00:53:35 6.10 %	02:27:02 16.74 %	00:07:25 0.84 %	00:00:00 0.00
B	00:30:27 3.47 %	04:14:32 28.98 %	00:52:27 5.97 %	00:18:54 2.15 %	00:06:28 0.74 %	00:29:36 3.37 %
C	01:11:36 8.15 %	01:15:37 8.61 %	00:04:58 0.57 %	00:33:03 3.76 %	00:32:39 3.72 %	00:01:15 0.14 %

Key (percent of time visible and stationary):

0.00 %	0.01-1.99 %	2.00-4.99%	5.00-9.99 %	10.00-29.99%	30.00-49.99 %	>50.00 %

Figure 6 Actual (hh:mm:ss) and percent (%) time that male sand lizards spent stationary while visible in each grid cell of the vivarium (Male A time stationary, visible = 24:00:07; Male B time stationary, visible = 17:01:59; Male C time stationary, visible = 14:38:14).

2.4.1.2 Adult sand lizard visibility

Power analyses show > 0.8 power for medium effect sizes ($f^2 = 0.15$) at a 0.05 significance level existed for the sample size (observations, individuals) included and using the analyses discussed. However, data were zero-inflated, particularly for female sand lizards, thus tests were interpreted with caution and some analyses re-run with no interactions to maximise power, where stated.

When considering individual lizards and Session as categorical factors predicting visibility, Female B was significantly more visible than A or C ($p = 0.020$). Session itself did not significantly affect female visibility (Figure 7a; see Appendix A.8 for full statistical models). Male sand lizards were collectively significantly more visible in Sessions 2 and 3 ($p = 0.027$; $p = 0.044$). However, Male B (mid-rank) and Male C (subordinate) had significantly negative associations with Sessions 2 / 3 ($p = 0.037$; $p = 0.012$) and Session 3 respectively ($p = 0.031$), where Male A (dominant) was most visible (Figure 7b; see Appendix A.8 for full statistical models).

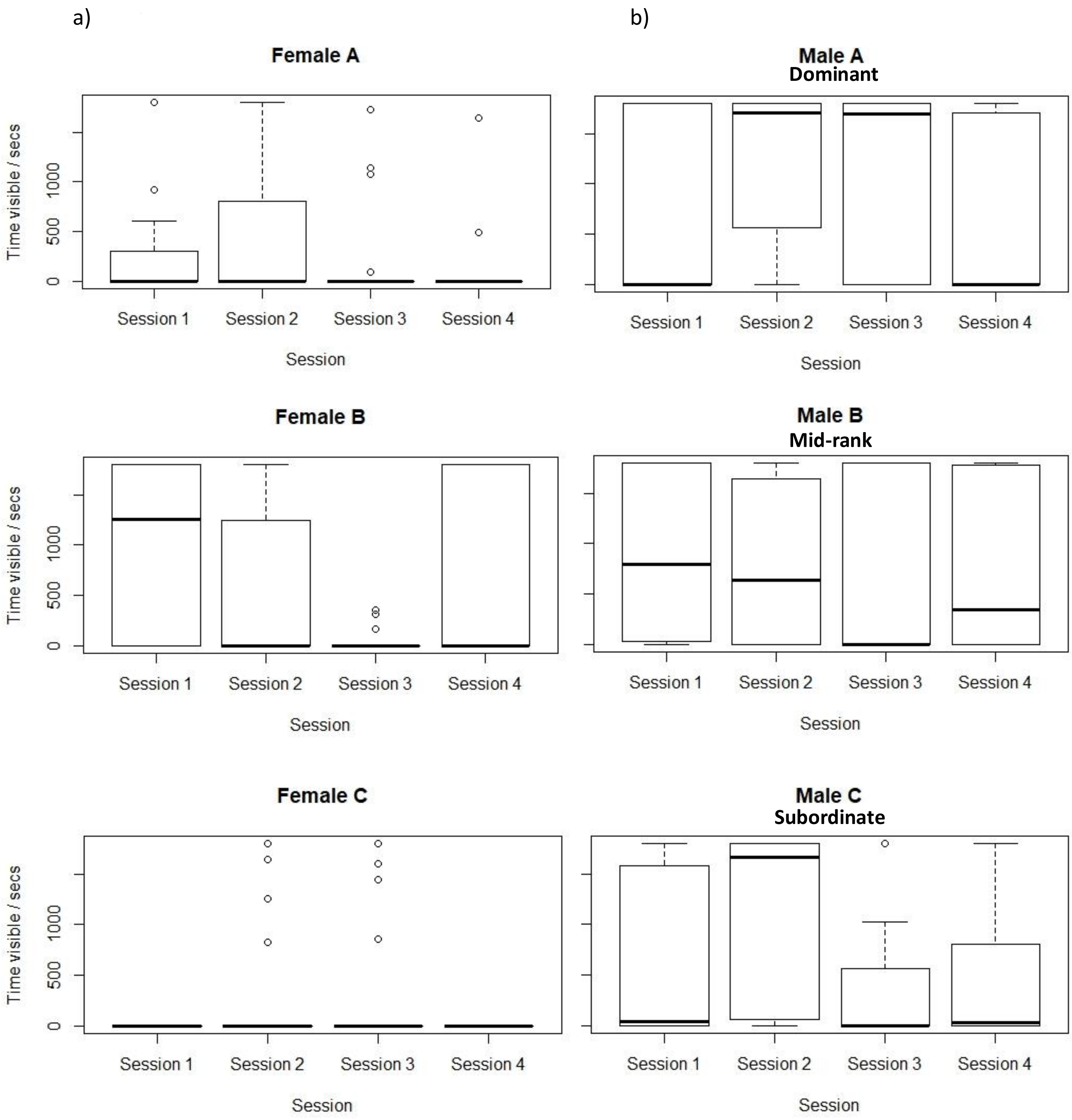


Figure 7 Boxplots showing the effect of Session number (time of day) on visibility of a) female and b) male sand lizards (seconds). Plots highlight the median, interquartile range, extreme values and outliers.

2.4.1.3 Adult microhabitat and refugia use

Females B and C spent the majority of time using refugia in partially vegetated microhabitat, with Female A splitting her refuge-use time almost equally between this (66 minutes) and vegetated microhabitat (68 minutes). Male A (dominant) spent the majority of refuge-use time in partially vegetated microhabitat, whereas Male B (mid-rank) spent more time using sand-surrounded (open) refugia, and Male C (subordinate) using fully vegetated (Figure 8).

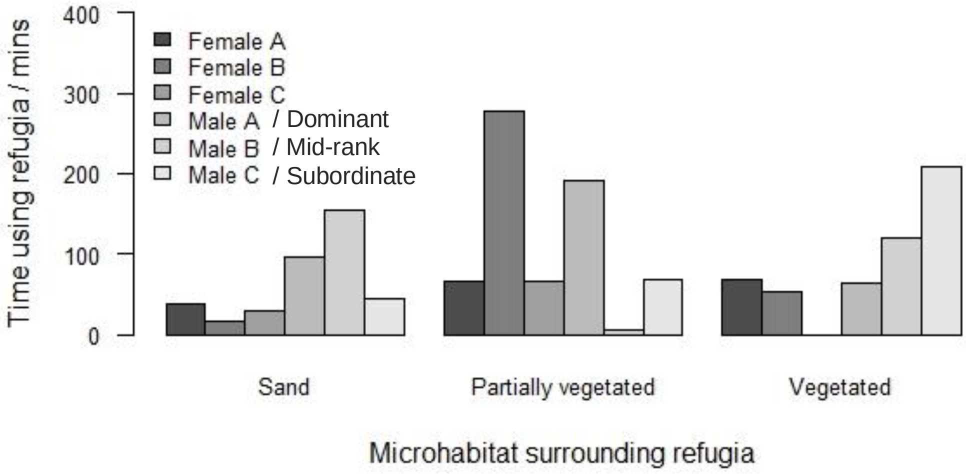


Figure 8 All refugia use (Tin, Tile, Slate and Wood) by female and male sand lizards in three different microhabitat types during 40 hours (2400 minutes) of observations.

When considering female lizard individuals, microhabitat type and an interaction between the two, lizard visibility could be adequately predicted by microhabitat type alone. Partially vegetated refugia were significantly more utilised (p = 0.045) than fully vegetated, and sand-surrounded refugia used the least (Table 4a).

Neither male individual lizards, microhabitat type nor an interaction between them significantly predicted male sand lizard visibility. However, nor could any of these predictors be excluded. The comparable lack of use of semi-vegetated microhabitat by the subordinate male (Male C) was very nearly a significant factor in the model (p = 0.054) (Table 4b).

Table 4 a) Proportion of use of microhabitat type surrounding refugia per session (sand/open, semi-vegetated, vegetated) by individual female sand lizards (‘Vegetated’ is reference level for Microhabitat; ‘Female A’ for Individual); ‘Session number within Date’ was included as a random effect; model was a binomial GLMM. Model 1 included interaction term; Model 2 removed interaction; Model 3 removed Individual. AIC values and Likelihood Ratio tests showed Model 3 was optimal (minimum adequate), with Microhabitat the only significant fixed effect.

	Model 1				**Model 2**				**Model 3 (optimal)**			
Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***	**Estimate**	**Std. Err.**	**z value**	***P***	**Estimate**	**Std. Err.**	**z value**	***P***
(Intercept)	-5.070	0.710	-7.144	**<0.001**	-5.831	0.688	-8.476	**<0.001**	-5.765	0.578	-9.970	**<0.001**
Female B	-0.694	1.227	-0.565	0.572	0.475	0.573	0.829	0.407				
Female C	-12.509	366.976	-0.034	0.973	-0.514	0.733	-0.702	0.483				
Microhabitat: Sand	-0.694	1.227	-0.566	0.572	-0.406	0.914	-0.445	0.657	-0.407	0.914	-0.445	0.657
Microhabitat: Semi-veg	0.002	1.003	0.002	0.998	1.309	0.653	2.004	**0.045**	1.308	0.653	2.003	**0.045**
Female B : Sand	-7.197	51.598	-0.139	0.889								
Female C : Sand	12.506	366.979	0.034	0.973								
Female B : Semi-veg	1.961	1.468	1.336	0.181								
Female C : Semi-veg	12.507	366.978	0.034	0.973								
AIC	204.8				203.1				201.5			
Likelihood Ratio test		**Deviance**	**d.f.**	***P***								
Model 1 : Model 2		6.246	-4	0.182								
Model 1 : Model 3		8.660	-6	0.194								

Table 4 b) Proportion of use of microhabitat type surrounding refugia per session (sand/open, semi-vegetated, vegetated) by individual male sand lizards ('Vegetated' was reference level for Microhabitat; 'Male A / Dominant' for Individual); 'Session number within Date' was included as a random effect; model was a binomial GLMM. Model 1 included interaction term; Model 2 removed interaction. AIC values and Likelihood Ratio tests showed Model 1 was optimal (minimum adequate, as well as maximal), with the removal of any terms causing significant deviance.

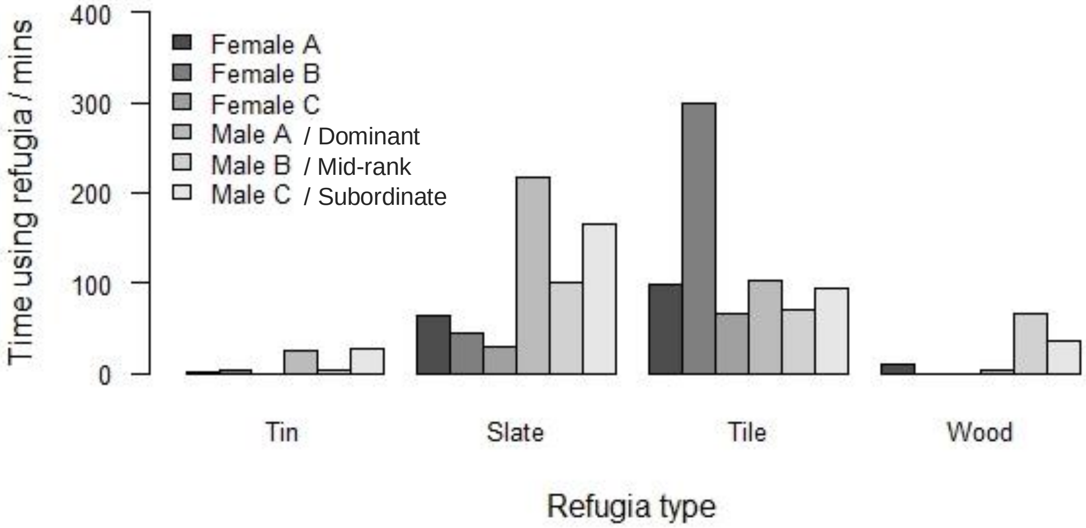
	Model 1 (optimal)				Model 2			
Coefficients	**Estimate**	**Std. Err.**	**z value**	**P**	**Estimate**	**Std. Err.**	**z value**	**P**
(Intercept)	-5.780	1.008	-5.737	**<0.001**	-4.687	0.483	-9.698	**<0.001**
Male B / Mid-rank	-0.012	1.412	-0.009	0.993	-0.564	0.629	-0.896	0.370
Male C / Subordinate	1.954	1.066	1.833	0.067	0.360	0.495	0.728	0.467
Microhabitat : Sand	0.690	1.221	0.565	0.572	-0.409	0.530	-0.772	0.440
Microhabitat : Semi-veg	1.383	1.115	1.240	0.215	-0.409	0.530	-0.772	0.440
Mid-rank : Sand	0.424	1.681	0.252	0.801				
Subordinate : Sand	-2.643	1.620	-1.631	0.103				
Mid-rank : Semi-veg	-7.932	25.703	-0.309	0.758				
Subordinate : Semi-veg	-2.645	1.374	-1.924	0.054				
AIC	257.5				261.0			
Likelihood Ratio test		**Deviance**	**d.f.**	***P***				
Model 1 : Model 2		11.487	-4	**0.022**				

Tile and then slate were used significantly more than wood and then tin when considering type of refugium and session at predicting sand lizard visibility collectively (Table 5). Lizards were significantly more visible in Session 2, over Sessions 1 and 4. The greater use of slate and tile is evident in Figure 9a. Figure 9b shows an interaction between sex and refugium type, with females using tile more than slate, and males using slate more than tile. This preference was the case across all individuals of both sexes (Figure 9a) and can be seen in more detail, and by Session, in Appendix A.9.

Table 5 Preferred Refugium and Session presence by sand lizards collectively. 'Session 1' was designated reference level for Session; 'Wood' was reference level for Refugium type; 'Session number within Date' was included as a random effect; model was a binomial GLMM. Both Session and Refugium type were retained for best model fit.

	Model 1 (optimal)			
Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
(Intercept)	-5.986	0.805	-7.440	**<0.001**
Slate	2.118	0.753	2.813	**0.005**
Tile	2.353	0.745	3.160	**0.002**
Session 2	1.249	0.493	2.531	**0.011**
Tin	-0.699	1.226	-0.570	0.568
Session 4	0.159	0.563	0.283	0.777
Session 3	-0.011	0.607	-0.018	0.986
AIC	367.80			

a)



b)

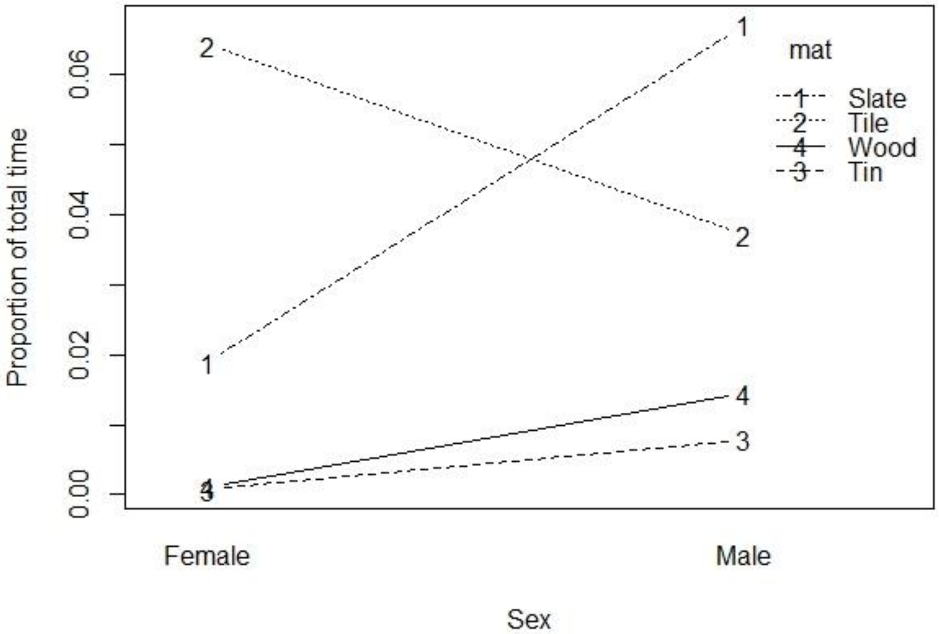


Figure 9 a) Time spent using refugia and b) comparable proportion of time spent using refugia by female and male sand lizards, during 40 hours (2400 mins) of observations.

2.4.1.4 Individual differences in relation to weather

The relationships between weather and microclimate variables, refugia heating properties and temporal diurnal trends in are shown in Appendices A.10, A.11 and A.12. In summary, air temperature, wind speed and soil temperature (at 2 cm depth in shade) were highest during Sessions 2 and 3, humidity was lowest during Sessions 2 and 3, and UV Index and solar radiation peaked in the morning sessions before dropping off rapidly in Sessions 3 and 4. Temperatures below slate and tile peaked the highest and these materials also retained their heat for longest when air temperature dropped.

Considering the following environmental analyses, power was > 0.8 for medium effect size (f^2 = 0.15) at a 0.05 significance level for visibility analyses. Models of female sand lizard refugium use had little power; the only female retained was Female B, still with limited power (*italicised in table*) (power = 0.79, with large effect size (f^2 = 0.35), at 0.05 significance). Models of male sand lizard refugium use had power > 0.95 with large effect size (f^2 = 0.35) at 0.05 significance level. The success of models at predicting visibility and refugium use is shown in Appendices A.13 and A.14 respectively.

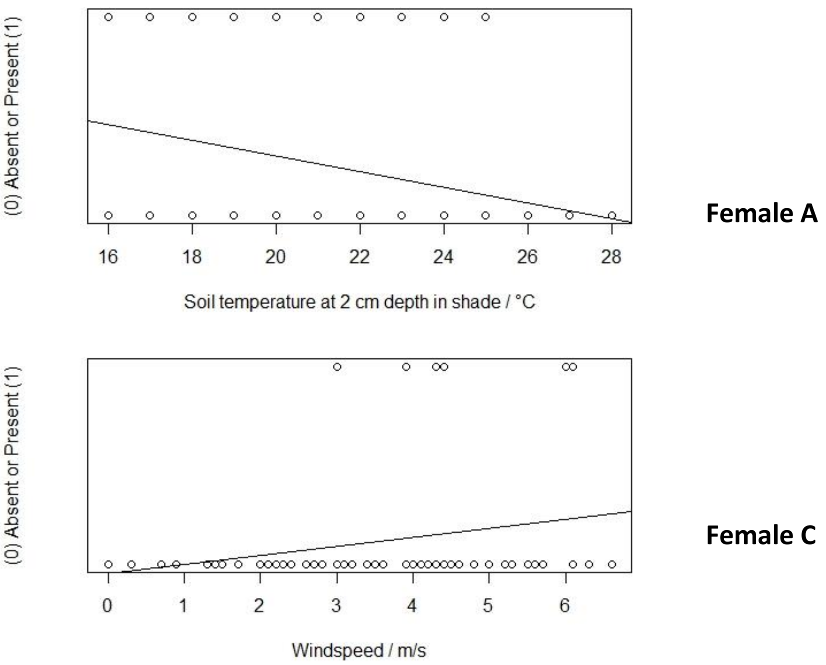
Visibility of Female B and Male B were not significantly predictable by any environmental variables (Table 6). Visibility of Female A was best significantly predicted by a negative relationship with soil temperature; visibility of Female C was significantly predicted by a positive relationship with windspeed (Table 6, Figure 10a). Visibility of Male A was significantly predicted by a negative relationship with humidity and positive relationship with temperature (both factors were required in the minimum adequate model, despite a significant negative correlation between them); visibility of Male C was predicted by a negative relationship with humidity (Table 6, Figure 10b).

Refugium use (versus surrounding microhabitat) by Female B was significantly predicted by a positive relationship with windspeed and soil temperature, although these lost significance once other variables were removed from the model, despite its deviance from the full model not being significant (NB very limited power as discussed) (Table 6, Figure 11a). Use of refugia was not predictable by any environmental variables for Male A. Refugium use by Male B was significantly predicted by a positive relationship with UV; and refugium use by Male C was significantly predicted by a negative relationship with humidity (Table 6, Figure 11b).

Table 6 Final minimum adequate models for ‘Individual adult lizard Visibility’, and ‘Refugium use during sessions when visible’, following model reduction using multiple stepwise logistic regression from including all environmental variables per session (Visibility model session n = 80; Refugium use model session n = variable). Dependent variable was binary presence/absence. There were several significant environmental predictors, varying between lizards; some lizards’ responses were not significantly predictable by any variables. Where insufficient power was present, this is stated.

	Visibility					**Refugium use**				
	Coefficients	Estimate	SE	z value	*P*	Coefficients	Estimate	SE	z value	*P*
Female A	Intercept	3.731	2.076	1.797	0.072	Little power				
	Soil temp / °C	-0.233	0.101	-2.296	**0.022**					
	D²		0.066							
	Null deviance		89.974		79 d.f.					
	Residual deviance		84.026		78 d.f.					
Female B	No significant independent variables					*Intercept*	*-20.866*	*11.129*	*-1.875*	*0.061*
						Windspeed m/s	*2.477*	*1.279*	*1.936*	*0.053*
						Soil temp / °C	*0.817*	*0.440*	*1.857*	*0.063*
	D²								*0.508*	
	Null deviance								*23.842*	*30 d.f.*
	Residual deviance								*11.728*	*28 d.f.*
						Variables had no significance once incorporated in model				
Female C	Intercept	-4.219	1.165	-3.623	**<0.001**	Little power				
	Windspeed / m/s	0.540	0.260	2.078	**0.038**					
	D²	0.094								
	Null deviance	52.013			79 d.f.					
	Residual deviance	47.140			78 d.f.					
Male A	Intercept	1.284	4.078	0.315	0.753	No significant independent variables				
	Humidity / %	-0.089	0.031	-2.831	**0.005**					
	Temperature / °C	0.259	0.124	2.097	**0.036**					
	D²	0.310								
	Null deviance	109.650			79 d.f.					
	Residual deviance	75.667			78 d.f.					
Male B	No significant independent variables					Intercept	-0.889	0.541	-1.644	0.100
						UV Index	0.434	0.203	2.133	**0.033**
	D²								0.085	
	Null deviance								59.587	42 d.f.
	Residual deviance								54.527	41 d.f.
Male C	Intercept	2.691	1.362	1.976	**0.048**	Intercept	-5.816	2.716	-2.142	**0.032**
	Humidity / %	-0.035	0.018	-1.933	0.053	Humidity / %	0.087	0.039	2.246	**0.025**
	D²	0.036							0.110	
	Null deviance	110.700			79 d.f.				57.364	41 d.f.
	Residual deviance	106.770	78 d.f.			51.054	40 d.f.			

a)



b)

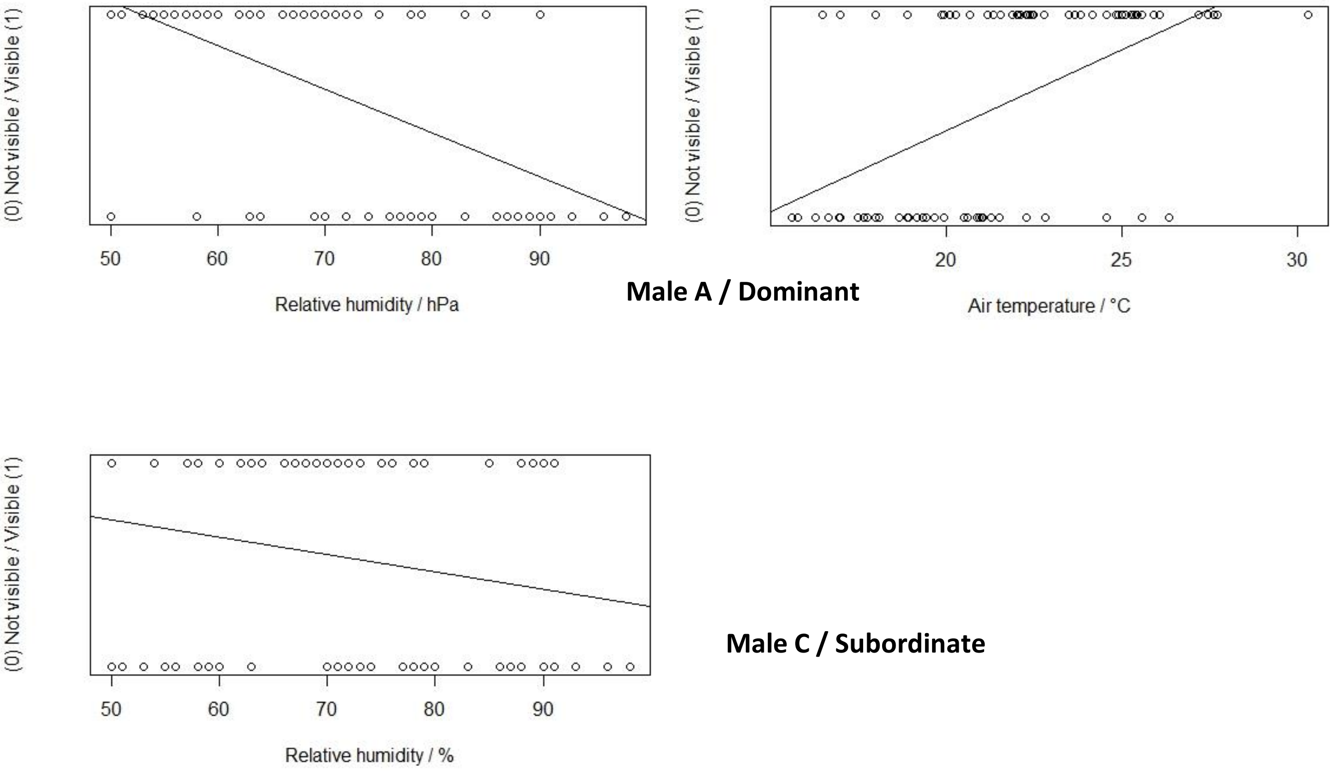
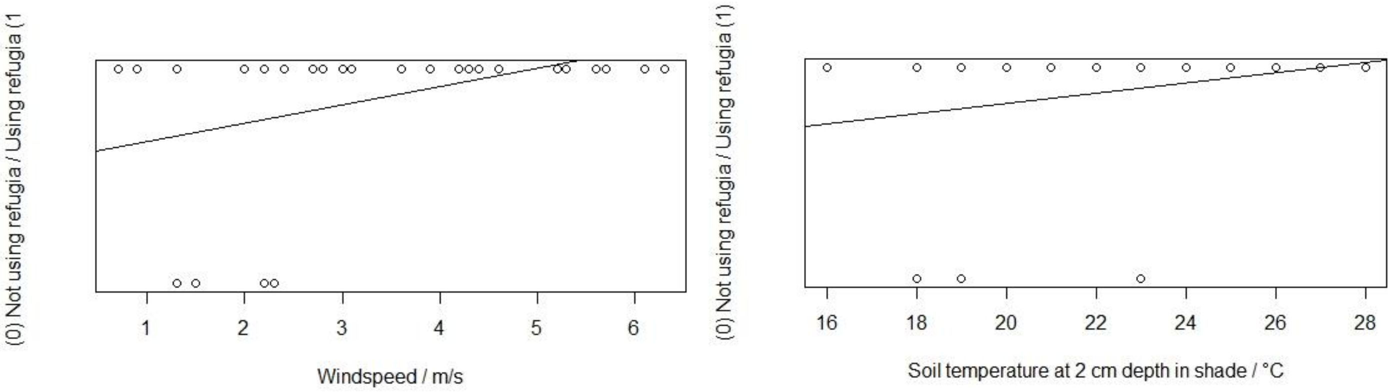


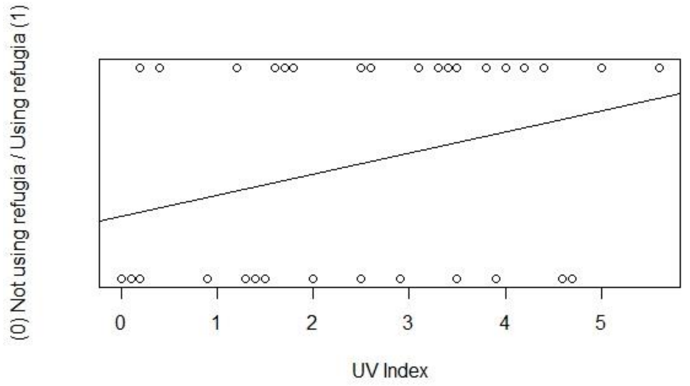
Figure 10 Significant predictor variables of individual a) female (A and C) and b) male (A and C) sand lizard visibility.

a)

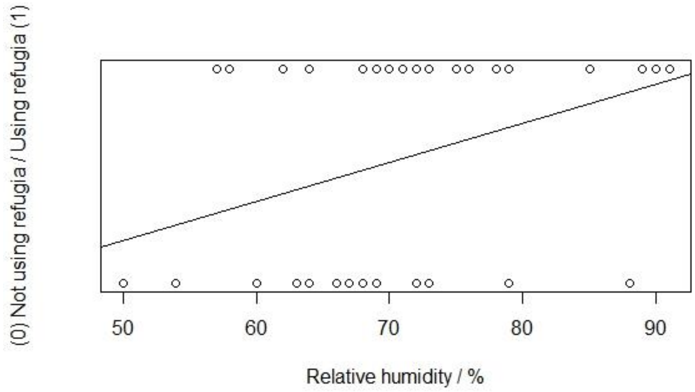


Female B

b)



Male B



Male C

Figure 11 Significant predictor variables of individual female a) Female B and male b) Males B and C sand lizard use of refugia when visible.

2.4.2 Juvenile sand detectability and use of space

2.4.2.1 Juvenile aggregatory behaviour

Piling behaviour was observed more than touching behaviour by juvenile sand lizards, although this relationship was not significant. Session 1 included significantly more aggregation than Session 4, and (insignificantly) more than Sessions 2 and 3. There was significantly less touching than piling behaviour in Sessions 2 and 3, and significantly more in Session 4 (Table 7, Figure 12).

Table 7 The effect of Session (time of day) on frequency of Aggregatory behaviour (Touching and Piling) shown by eight juvenile sand lizards (session n = 80); model was a Poisson GLMM. 'Piling' was the reference level for Aggregatory behaviour; 'Session 1' was the reference level for Session; 'Session number within Date' was included as a random effect.

	Model 1 (optimal)			
Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
(Intercept)	0.740	0.729	1.015	0.310
Session 2 : Touching	-1.280	0.148	-8.664	**<0.001**
Session 3 : Touching	-0.421	0.141	-2.980	**0.003**
Session 4 : Touching	1.468	0.216	6.782	**<0.001**
Session 4	-2.977	0.975	-3.052	**0.002**
Session 2	-2.148	1.496	-1.435	0.151
Session 3	-2.790	1.524	-1.830	0.067
Touching	-0.085	0.089	-0.959	0.338
AIC	1137.3			

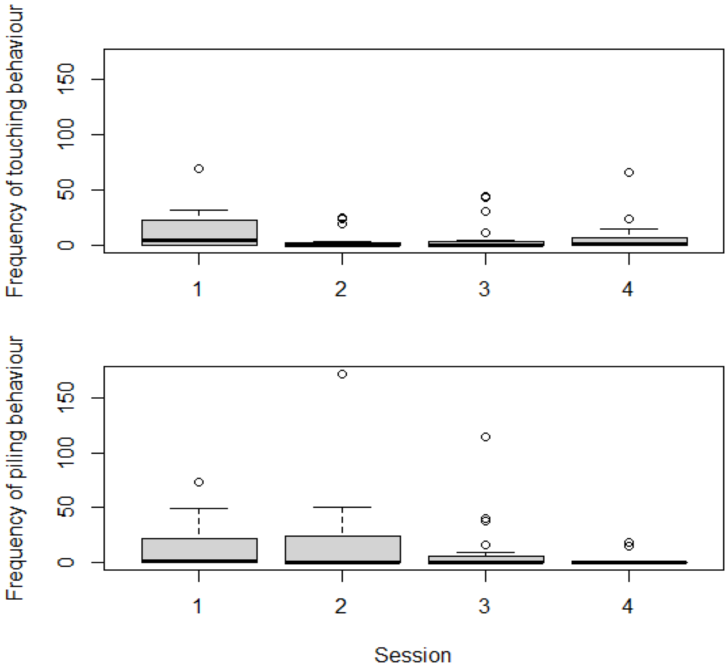


Figure 12 The effect of session (time of day) on juvenile sand lizard aggregatory behaviour (touching and piling).

2.4.2.2 Juvenile sand lizard visibility

Juvenile sand lizard visibility did not vary significantly between Sessions (Figure 13; see Appendix A.15 for full model). Generally, visibility was greatest in Session 1, followed by Sessions 3, 2 then 4.

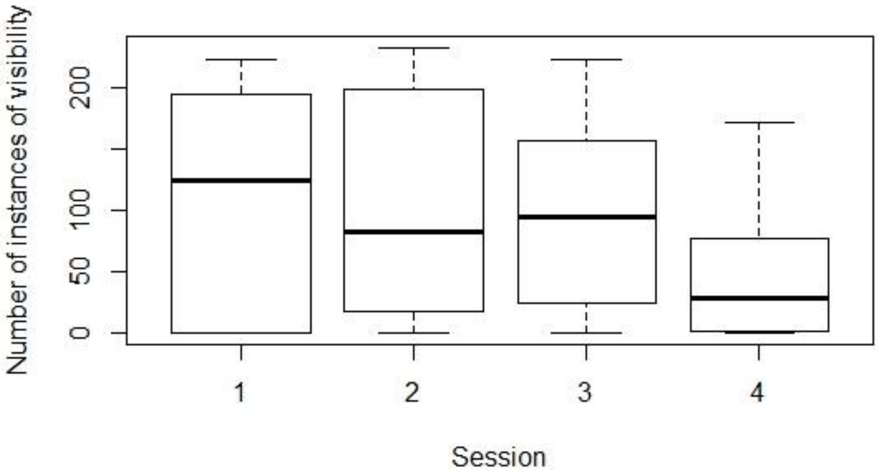


Figure 13 Juvenile sand lizard visibility throughout the day. Plots highlight the median, interquartile range, extreme values and outliers.

2.4.2.3 Juvenile microhabitat and refugium use

Juvenile sand lizards predominantly used slate refugia, followed by tile, tin, then wood; refugium use occurred predominantly in partially vegetated microhabitat (Figure 14). Refugia in sand/open microhabitat were rarely utilised.

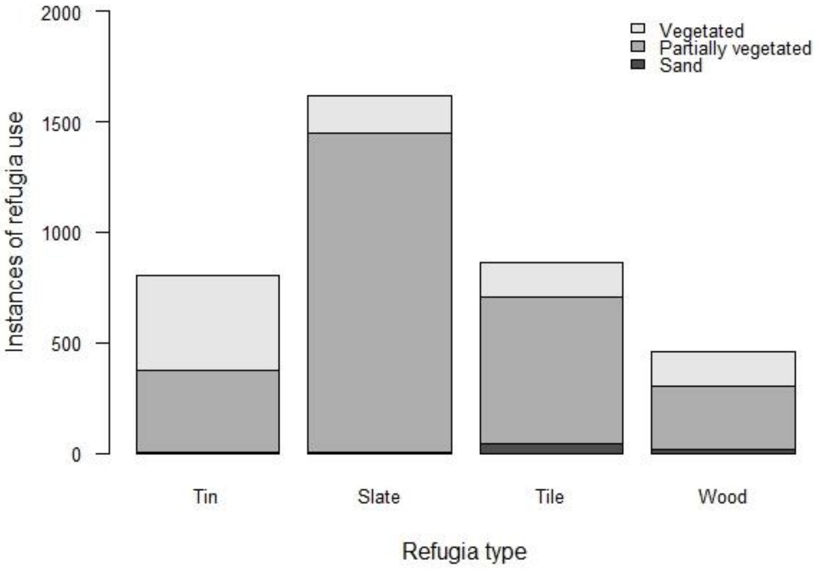


Figure 14 The number of instances eight juvenile sand lizards were observed using each refugium type within each microhabitat type across 80 sessions of 1-minute scan observations, 0-30 minutes (total n = 19,840).

Refugium use collectively was greatest in Session 3, followed by Sessions 2, 4 and then 1. Sessions 2 and 3 had significantly greater frequencies of refugium use than Session 1; Session 4 had almost significantly greater refugium use than Session 1 (Table 8).

Figure 15 highlights the greater refugium use in Sessions 2, 3 and 4. It also shows interactions between Refugium type and Session; slate is favourable in the middle part of the day (Sessions 2 and 3), however its use falls beneath tile and tin in the evening. Wood is utilised poorly, and the least of all refugium types in the afternoon and evening.

Table 8 The effect of Session (time of day) on combined refugium use frequency by 8 juvenile sand lizards (session *n* = 80). 'Session 1' was the reference level for Session; 'Session number within Date' was included as a random effect; the model was a Poisson GLMM.

Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
(Intercept)	-1.641	0.912	-1.800	0.072
Session 2	3.259	1.006	3.241	**0.001**
Session 3	3.647	0.960	3.798	**<0.001**
Session 4	1.960	1.010	1.941	0.052
AIC	4622.9			

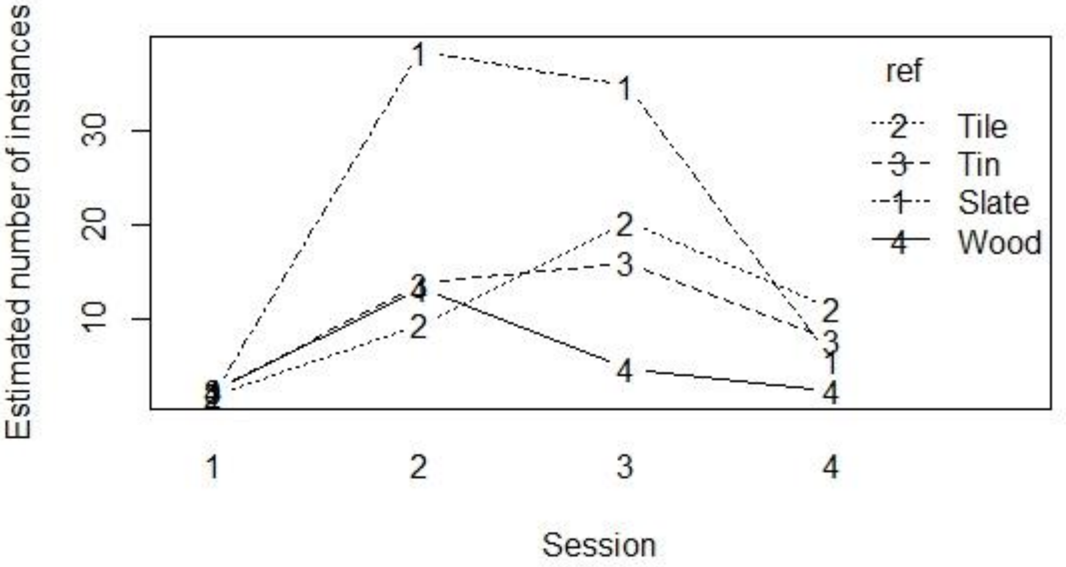


Figure 15 The estimated number of visible instances of eight juvenile sand lizards by Session (time of day) and Refugium type, showing interactions between the two.

2.5 Discussion

2.5.1 Dominance hierarchies and spatial fidelity in adult sand lizards

Dominance hierarchies are observed in many species to be context specific, based on factors such as resource availability and density of conspecifics (Strickland *et al.*, 2016; Taylor and Lattanzio, 2016). The male agonistic behaviours observed between male sand lizards suggest that hierarchy was retained outside of the breeding season where they are commonly observed (Blanke and Fearnley, 2015; Govier, 2017). This counters the suggestion that male sand lizards broadly co-exist peacefully (Nicholson and Spellerberg, 1989; Blanke and Fearnley, 2015), or at least indicates it is not the case for captive populations.

The nature of the dyadic relationships observed indicate hierarchy was not linear between all individuals with some being more tolerant, or of equal status, than others. This was evident by both the social metrics calculated and the use of space across the vivarium by different individuals. This trend corresponded with previous research on the species, where it is also shown that the exact ranking of individuals of similar middling status shifts across the season (Govier, 2017). Given the high levels of dominance seen by Male A over both Males B and C, as well as the comparable spatial fidelity and relative conspecific proximities observed, it can be inferred that Male A would likely maintain his ranking across the year, whereas rankings of B and C may be interchangeable.

The nature of the relationships observed can suggest implications for this species in the context of conservation and reintroduction. Firstly, it is assumed that the most dominant individual will be defending the most optimal territory and microhabitats. In the context of this study, this means that Males B and C could be restricted in access to less ideal territories (Kishani *et al.*, 2019) and also less optimal refugium options. The potential effect of this must be considered when drawing conclusions regarding collective preferred microhabitat and refugium use. Male A spent more of his time mobile than Male B or C, indicating increased exploratory, active behaviour, utilising space at both ends of the vivarium, likely linked to his dominant position in the hierarchy. Although formal exploratory behavioural tests were not undertaken, these observations reflect studies that have shown increased exploratory behaviours in more dominant individuals in groups that are in novel situations, either through novel resource provision or novel space (Verbeek *et al.*, 1999; Dingemanse and De Goede, 2004). In line with this, the male sand lizards had been recently moved to the vivarium in which they were studied. It is hypothesised that if the study were conducted over a longer time period the dominant male would become less exploratory than the subordinate males, as the hierarchy stabilises and the environment loses its novelty (Nakano, 1995; Verbeek *et al.*, 1999; Fox *et al.*, 2009).

The implications for having one particularly dominant male in a captive environment that is defensive of large areas, are that this individual may monopolise breeding opportunities (Govier, 2017), resulting in limited genetic diversity of offspring for reintroduction. Male sand lizards are observed to guard females for several hours to a few days following copulation (Olsson, 1993), further limiting the opportunity for additional males to sire a female's offspring. In a study of a captive population of sand lizards with low genetic variability in Sweden, it is found that when "quality" of males that have mated with a female is more variable, clutches have less multiple paternity, suggesting enhanced cryptic female choice ("quality" based on male coloration/ornamentation, likely linked to genetic quality) (Olsson *et al.*, 2010). However, fewer males contributing to clutch paternity increases risk of malformed offspring, which is of concern in small populations (Olsson, Wapstra, *et al.*, 2010). Dominance hierarchies would be an obvious additional consideration in this sand lizard mating system, along with the dynamics of mate-guarding with regards to this hierarchy, and specifically how this operates within a high-density captive population such as those that support reintroductions. There is suggestion that some sand lizards (typically smaller and with less colouration) show a "sneaky" mating tactic (Olsson, 1994) as observed in other species (Villalobos and Shelly, 1991). This also requires consideration in a captive context, with regards to opportunity for sneaky mating to occur and in the context of mate guarding, possibly affecting genetic variability and potential for malformation of offspring. Were the release population of low genetic variability, this could have negative implications post-release. There may be limited adaptability to the selection pressures that act on it in the wild such as limitations in resources and shifts in climate (Schindler *et al.*, 2010; Wolf and Weissing, 2012). There is some indication that higher temperatures increase sand lizard mating rate and within-clutch paternity with consequent positive effects on offspring fitness (Olsson *et al.*, 2010), which may be a silver lining for this species in the light of climate change.

Another implication from this study is the link between dominance and visibility, with lizards that are more dominant and more visible being at likely higher risk in a wild setting. They will defend larger areas and consequently be more vulnerable to predation. Although not examined within this study, there are also positive correlations between dominance and boldness of sand lizards (Govier, 2017), and boldness has been shown to correlate both positively and negatively with survival for different species following reintroduction (Bremner-Harrison *et al.*, 2004; Conrad *et al.*, 2011; Haage *et al.*, 2017). Personality syndromes are also known to be heritable (Smith and Blumstein, 2008; Sih *et al.*, 2010), which could have ongoing consequences for populations persisting post-release.

It might be suggested that hierarchies observed in captivity would be comparably observed in the wild among the same conspecifics. However, the high density of the population in a captive environment will affect this, resulting in increased overlap of territories and male and female space, leading to unnaturally high breeding opportunities for males that are more dominant (Alberts, 1994). As mentioned, it must be considered that rank may fluctuate temporally and hierarchies, both in terms of their very existence and individuals within them, will shift in different contexts (Strickland *et al.*, 2016; Govier, 2017). Over a study period of three months, three of nine male sand lizards in a captive population were observed to retain rankings, three were observed to shift only one position either side of their original ranking, and three were observed to jump rankings greater than this, showing marked variability amongst some individuals over time, but little variation for the majority. The individuals ranked first, second and most subordinate retained their positions (Govier, 2017).

The sand lizard in the UK has no specific genetic captive breeding recommendations, aside from limitation to the Dorset, Weald and Merseyside races. There is also little ability to control the parentage of offspring within current husbandry guidelines. The presence of a dominance hierarchy and a limited number of particularly dominant individuals (Govier, 2017), now confirmed to also exist outside breeding season, highlights the necessity of maintaining genetic variability in the captive population in another manner. For sand lizards this is currently undertaken through augmentation from wild populations under licence. Maintaining variation within the captive breeding population is essential to optimise reintroduced offspring resistance to release site stochasticity and adaptability to selection pressures.

Beyond the remit of the study herein, causes of hierarchical positioning have been explored for sand lizards. For example, body size (not measured here) is shown to influence the outcome of agonistic interactions. Eight of nine interactions between various males result in the smaller male retreating (Nicholson and Spellerberg, 1989), and larger individuals show significantly fewer submissive behaviours than smaller individuals (Govier, 2017).

Female sand lizards showed only one agonistic behaviour over the study duration, which corresponds to previous studies stating that females seldom exhibit aggressive behaviour and are less territorial (Fearnley, 2009). A dominance hierarchy could therefore not be confirmed in this way. However, there did appear to be strong spatial fidelity at differing locations within the vivarium, at least between Females A and B. Female C was not visible for much of the study period; remaining elusive by sheltering in vegetation or underground. There was indication that she was subordinate to at least Female B, as the single agonistic interaction was Female B chasing her out of their shared preferred grid cell. Female B was visible for more than double the amount of time than either Female A or C were, which corresponds with the dominant male's greater visibility, if some form of dominance were to exist between the females. If that is the case a degree of hierarchy could therefore be suggested, with Female B being most dominant, followed by Female A, and Female C being subordinate. This may have implications for breeding opportunities, as has been shown with male lizards (Govier, 2017), but would require further study.

It should be considered that male and female sand lizards were sourced from different locations prior to this study, as mentioned. Male lizards were recently transferred from the adult breeding vivarium, females were recently wild-caught to supplement the captive population. Male habituation to captivity and response to human presence was likely an additional influencing factor on visibility. This was perhaps underlying the overall comparable lower visibility of females in relation to males, which was contradictory to trends typically observed at this time of year where females are more active later in the season than males (Inns, 2009).

2.5.2 Aggregatory behaviour in juvenile sand lizards

Aggregatory behaviour was predominantly vertical ‘piling’ behaviour of lizards, as opposed to individuals just being in contact (‘touching’). This suggests a thermoregulatory benefit of this activity as opposed to grouping for other reasons, such as an anti-predatory tactic (Shah *et al.*, 2003) or grouping in a small area of preferred microclimate that happens to result in aggregation with conspecifics (Khan *et al.*, 2010). Were the latter the case, individuals could have chosen to bask separately in comparable thermal conditions. Piling and touching behaviour decreased on the whole throughout the day, along with juvenile visibility overall, with a slight rise in touching behaviour in the evening. This indicates that this behaviour is mostly associated with start of day thermoregulatory requirements as well as some, more limited, requirement at the cooler close of day. The sociality of juvenile lizards in the light of their thermoregulatory behaviour, particularly in high density captive conditions, may impact their post-release movement and dispersal in relation to conspecifics (Rabosky *et al.*, 2012).

2.5.3 Visibility of adult and juvenile sand lizards

The fact that individual adult sand lizards significantly varied from each other in terms of their visibility is of importance. There were very clear differences between sex, which will vary seasonally (Fearnley, 2009), and also variances within sex between individuals in terms of the amount of time they spent visible. This indicates individuals respond to their environment, in terms of their spatial and temporal use of it, as a result of underlying differences such as behavioural syndromes.

The most common methods for estimating population size, as likely undertaken to determine population growth in the persistence stage following reintroduction, are capture-mark-recapture (CMR) methods. Population size based on recaptures in the field makes assumptions about the species being monitored, such as seasonal variation in capture probability, variation in capture probability due to previous capture experience, and variance among individuals (Pollock *et al.*, 1990; Williams *et al.*, 2002).

In a field environment, the increased visibility of more dominant individuals of a population will increase the likelihood of their being observed. This will affect post-release estimates of demographics, survivorship and ultimately project success. Conversely, it could also result in their loss from the population due to predation (Bremner-Harrison *et al.*, 2004; Conrad *et al.*, 2011) and consequent underestimates of population size going forward due to other individuals being more elusive. There is growing recommendation for the inclusion of behavioural differences in animal population studies (Singh and Kaumanns, 2005; Durant *et al.*, 2019). Dominance and associated behavioural traits will influence visibility and thus detectability (individual detection heterogeneity). In turn, this impacts capture rates and population size and distribution estimates (Marescot *et al.*, 2011; Carter *et al.*, 2012; Biro, 2013; Merrick and Koprowski, 2017). Determining individual differences in visibility and spatiotemporal behaviour as a result of underlying behaviour can improve monitoring recommendations to account for more accurate species estimates (Carter *et al.*, 2012; Biro, 2013; Merrick and Koprowski, 2017). In small, cryptic species, where remote monitoring options such as tagging and camera trapping are less feasible, this is particularly important.

Current UK reptile surveying guidelines highlight key times of day to survey throughout the season (Froglife, 1999; Moulton and Corbett, 1999; NARRS, 2011). These are broad recommendation and do not account for individual differences resulting in activity outside of the limits suggested. In this study 50 % of adult lizards observed did not show significant temporal variation in visibility. Should this be at all representative of wild populations of sand lizards, it indicates that surveying on the recommendation of time of day alone is not wholly optimal. The differences between adult and juvenile trends in visibility throughout the day also suggest that in order to observe the full demographic range of a population, for example to determine breeding (Sarrazin and Barbault, 1996a), time of day is not a sufficient indication of best survey opportunities. Other measures would greatly enhance survey success and observations across the population demographic.

2.5.4 Microhabitat and refugium use of adult and juvenile sand lizards

Both open and covered microhabitat types were utilised by male and female sand lizards within the wider vivarium, highlighting that surveying for sand lizards should continue to incorporate transect walks as well as refugium checks (Reading, 1997; Edgar *et al.*, 2010; NARRS, 2011; Sewell *et al.*, 2013).

Partial vegetation is the far preferred microhabitat for Male A (dominant). There is a high use of fully vegetated refugia, a low use of open refugia, and almost significantly low use of refugia in partial vegetation by Male C (subordinate). This suggests increased sheltering behaviour with decreased ranking in the dominance hierarchy in male sand lizards. Male C was selecting refugia that offered visual cover (if not olfactory), which could indicate lower boldness and hierarchical position. The spatial fidelity and territoriality observed in earlier analyses could also mean refugium-microhabitat selection is a consequence of what is available within the space the lizard has adopted as its primary location, as an effect of the dominance hierarchy (Nakano, 1995; Taylor and Lattanzio, 2016). This would reflect the principle of ideal free distribution, whereby individuals occupy overall lower quality sites as a response to the best sites being occupied already by conspecifics (Fretwell and Lucas, 1970). Female B notably utilised partially vegetated refugia more than sand or vegetated refugia, indicating a strong preference for this habitat type. The same ranking of microhabitats were observed in the other female lizards, leading to refugia in this microhabitat type being used significantly more than open or fully vegetated microhabitats. Refugia within a partially vegetated microhabitat were also preferred by juvenile sand lizards. The overall preferred use of refugia within partial vegetation by sand lizards (including juveniles, females and the top-ranking male) indicates structural diversity of vegetation is key to lizards' choice of microhabitat, offering basking, fleeing and foraging opportunities (Spellerberg, 1976; Blanke and Fearnley, 2015). Surveying protocols and refugium placement should therefore be considered in light of this.

All adult lizards showed a significant preference for tile and slate over tin and wood, with females preferring tile and males preferring slate. This highlights that previous studies that have observed wild sand lizards preferentially utilising wood for basking over surrounding microhabitats (House *et al.*, 1980; Blanke and Fearnley, 2015) may have had the potential to improve detectability by adding other materials, such as tile and slate, to the environment. Juveniles also preferred slate and tile, with slate utilised the most. Given the high frequency of deployment of tin refugia by surveyors in the field, this is of great significance for reptile monitoring recommendations for sand lizards (Froglife, 1999; Langham, 2012).

The heating properties of different materials showed that slate and tile were much quicker at heating up than the other refugia and retained elevated temperatures over other refugia and microhabitats. The high use of tile in session 4 may be indicative of this refugium type retaining its heat for longer than slate (both of which lose heat rapidly from around 16:00, before levelling off slightly, and then again dropping from around 18:00). This offers more optimal thermoregulation opportunities into the evening than any other habitat or refugium type.

2.5.5 The effect of weather variables on adult sand lizards

There were significant differences between sessions of all weather variables except atmospheric pressure. However, sand lizard variability in visibility and refugium use across sessions suggests that underlying causes of space use were not the same for all individuals and age groups. Possible drivers may be environmental, a result of individual differences, an effect of conspecific presence and dominance hierarchies, or most likely a combination.

Weather variables were modelled in relation to adult lizards individually. Soil temperature, humidity, air temperature and windspeed were predictors of visibility (presence or absence per session) across adult lizards, although Female B and Male B visibility were not predictable by any environmental variables. Other than wind speed, these predictors correlated strongly; air and soil temperature positively, and humidity negatively against these. Air and ground temperature are discussed as key determinants of sand lizard behaviour in the open, with warmer conditions providing better thermoregulating opportunities utilising solar radiation (Fearnley, 2009; Blanke and Fearnley, 2015). At hotter times of year, it would be anticipated a more quadratic relationship would be seen, with sand lizards retreating to shade in the highest temperatures and thus reducing visibility (Spellerberg, 1976; Blanke and Fearnley, 2015). An unusual observation is that whereas high temperature and low humidity led to increased visibility (as expected) of male lizards, a decrease in soil temperature significantly predicted an increase in visibility of Female A; also an increase in windspeed significantly predicted the visibility of Female A, although this environmental variable showed no correlation to any others. Female lizards therefore acted against expectation. It is possible that the natural burrowing/sheltering location of Female A, who was most visible of all females, was located in a position that caused her to seek thermoregulatory opportunities elsewhere once a critical lower temperature threshold was reached. Detailed determinants of burrow use and key thresholds for emergence have not been studied for sand lizards. Female C’s negative relationship with windspeed could be indicative of her selecting to be active when others were less likely to be so; this would further indicate a form of spatial fidelity and some form of dominance hierarchy within the female sand lizards.

Refugium use was significantly predicted by environmental variables for Female B and Males B and C. The models for females A and C had insufficient power; Male A refugium use did not show any predictive variables. The significant variables for Female B lost significance once the minimum adequate model was reached, indicating their limited overall effect on refugium use. Key drivers for Male B and Male C refugium use were increased UV and increased humidity respectively. Lower humidity correlated with higher temperatures, therefore lizards were utilising refugia when humidity was higher, temperatures were lower and UV was higher (this could be indicative of cool clear mornings). This corresponds with previous studies and knowledge of the biology and ecology of this species and its thermoregulatory requirements as previously detailed (Fearnley, 2009; Blanke and Fearnley, 2015).

2.5.6 Conservation monitoring implications

This study has assessed the ecophysiological and social behaviour across a demographic range of sand lizards. Its results can be used to suggest how monitoring protocols may be improved to incorporate spatiotemporal behaviour, in turn reflecting social and individual differences, to improve detectability for this species. It also allows an insight into factors that may affect general habitat use by this species, and others. From this known population in a controlled environment, principles can be adapted and tested in a wild setting.

It is of great importance in the light of habitat and other environmental changes that the underlying biological and ecological functions of species are understood; particularly their spatiotemporal use of habitats and microhabitats, in order that appropriate conservation measures can be implemented (Nowakowski *et al.*, 2016; Meade *et al.*, 2018; Ozdemir *et al.*, 2018; Souza Terra *et al.*, 2018). This includes modelling species distributions and conservation translocation scenarios (Aubry *et al.*, 2017; Smeraldo *et al.*, 2017). Assessing microhabitat as opposed to wider habitat can show important mechanisms that may not be detected otherwise (Telve *et al.*, 2020).

Details around reptile monitoring in the UK, in terms of type, size and location of refugia, remain controversial, with research urgently required (Sewell *et al.*, 2013). Previous studies indicate that legged lizards, and particularly sand lizards, seldom use refugia (Reading, 1997; Sewell *et al.*, 2013). Where this study does not dismiss the importance of systematic transects to observe sand lizards in natural habitat, it suggests that methods could be adapted in order to optimise survey protocols. Detailed consideration of refugia – both type and location within habitat - can optimise detectability of sand lizards, and in a manner allowing for both intra-site and inter-site comparability.

Much herpetofauna monitoring in the UK is undertaken by volunteers, often under guidance from surveyors with expertise and experience, and using general protocol documents (Froglife, 1999; Langham, 2011; Langham, 2012; Sewell *et al.*, 2013). Reporting is encouraged using national recording schemes, such as Record Pool (Amphibian and Reptile Groups of the UK (ARG-UK) and ARC Trust). Volunteers are a vital resource in monitoring herpetofauna populations, and other species beyond this, for conservation purposes. A growing emphasis on citizen science has recognised this (Silvertown, 2009; Gardner *et al.*, 2019). As the capacity for information sharing and dissemination has increased, it is important that protocols for data collection are replicable (Frigerio *et al.*, 2018). Obtaining data that are comparable has never been more crucial, with climate change threatening many species and populations. Large scale citizen science projects have the capacity to offer much quantitative support in the observation of spatiotemporal trends (Dickinson *et al.*, 2010). Clear monitoring methods also allow reintroduction outcomes specifically to be more quantifiably assessed and project protocols evaluated accordingly (Armstrong and Seddon, 2007; IUCN, 2013; Taylor *et al.*, 2017). With robust baseline data sets, future predictive modelling of populations and ecosystems in the light of habitat and environmental changes will likewise be more robust and informative (Cruz *et al.*, 2015; Rytteri *et al.*, 2017; Préau *et al.*, 2019; Molloy *et al.*, 2020).

2.5.7 Further study

Sample size of adult lizards for this study was very small. While this allowed detailed examination of individual differences in spatiotemporal behaviour to be observed, it consequently limited the power of analyses and trends being seen across demographic groups. Only one clutch of juveniles was observed accounting for no inter-clutch variability in behaviour. Study of more individuals over multiple years would increase sample size and allow for more robust and detailed analyses. The study duration was limited to one part of the active reptile season; it would be beneficial to repeat the study at other points during the year to ascertain differences in visibility, microhabitat and refugium use in relation to changing environmental conditions and temporal differences in behaviour.

Spatial fidelity and dominance hierarchy affected refugium and microhabitat use by adult lizards, with some lizards' access to areas of the vivarium, and thus specific locations and refugia, effectively restricted by conspecifics. A future study could focus on use of space without social implications and hold individuals separately, to remove this confounding factor from microhabitat and refugium use determinants.

2.6 Conclusions

This *ex situ* study of small captive groups of male, female and juvenile sand lizards enabled visibility, use of microhabitat and use of artificial refugia to be assessed, whilst also examining sociality in terms of differential spatial fidelity, agonistic interactions and dominance hierarchies in adults and aggregatory behaviour in juveniles.

The study ascertained several key conclusions, that are explored further in a field setting for both sand lizards and the wider reptile assemblage in Chapters 3 and 4:

- Demographic variables of age and sex, microhabitat, weather, refugium type and individual differences all affected spatiotemporal behaviour of sand lizards. These are important considerations for conservation of this species in: management of suitable habitat, selecting sites for conservation translocation, and monitoring this species in the wild.

- Overall optimal surveying protocols for sand lizards across all demographics should include tile and slate refugia, placed in heterogeneous, partially vegetated, microhabitat. Surveys should not be conducted based on season and time of day alone; more detailed weather and microclimate variables should be considered. Undertaking surveys maintaining these conditions between years would also allow populations to be compared more accurately over longer timeframes.
- The presence of a dominance hierarchy in adult male sand lizards, and strong spatial fidelity (and potential hierarchy) in adult female sand lizards outside of breeding season, highlights individual variation that is likely to exist in a release population of sand lizards. There are potential implications of this on visibility, use of space and survivorship; these also have consequences for detectability and monitoring.
- Aggregatory behaviour appears to be primarily for thermoregulatory purposes in juvenile sand lizards and may have implications for collective post-release movement of this age group.
- Captive populations of cryptic species offer conservation biologists a unique opportunity to improve understanding of their spatiotemporal behaviour and inform best practice monitoring protocols. Understanding the ecophysiological and social behaviour of ectotherms is of high importance in the light of concern for amphibians and reptiles in the face of climate change and habitat loss on a global and local (UK) scale. Well-informed population monitoring has never been more crucial for these taxa.

Chapter 3 Environmental factors associated with detectability of a reintroduced population of sand lizards *Lacerta agilis* and implications for monitoring in dry heath habitat

3.1 Introduction

3.1.1 The importance of species monitoring

Conservation measures should be evidence-driven at all scales, from microhabitat to landscape-level, dictating pre-project modelling and decision-making, and post-project monitoring (Nichols and Williams, 2006; Bubac and Johnson, 2019). Species monitoring is increasingly important today for ecosystems that face significant threats from many directions; from habitat loss, fragmentation and degradation, to pollution, invasive species and climate change (Eigenbrod *et al.*, 2008; Gonzalez *et al.*, 2010; Wouters *et al.*, 2012; Kumar *et al.*, 2014; Horvath *et al.*, 2019; Powers and Jetz, 2019). Monitoring is essential in order to assess population changes as a result of these factors and prioritise conservation efforts through habitat preservation, management and restoration (Dunford and Berry, 2013; Radinger *et al.*, 2017; Menéndez-Guerrero *et al.*, 2020). This includes monitoring at sites both with an established population and that offer the potential for conservation translocation of a species (Nichols and Williams, 2006; Nichols and Armstrong, 2012; Barata *et al.*, 2017; West *et al.*, 2019). Advances in technology have enabled theoretical modelling to become a vital tool for predicting species distributions, shifts and extinctions in the light of changes to the environment (Umetsu *et al.*, 2008; Tanentzap *et al.*, 2012; Marcer *et al.*, 2013), and to assist in conservation translocation planning (Smeraldo *et al.*, 2017; Maes *et al.*, 2019). These models are in turn reliant on high quality, accurate, quantitative data at appropriate resolutions (Soberón, 2007; Aubry *et al.*, 2017); such data can be challenging to obtain (Jiménez-valverde *et al.*, 2008; Kamino *et al.*, 2012). A model will perform based on the adequacy of the data it includes. Inadequate data can lead to over-parameterisation and poor model performance, in turn leading to sub-optimal practical recommendations (Getz *et al.*, 2018).

In the light of the challenges species are facing, ecological understanding of spatiotemporal drivers of habitat and microhabitat use is crucial (Nowakowski *et al.*, 2016; Meade *et al.*, 2018; de Souza Terra *et al.*, 2018). By quantifying these details, models can be parameterised more accurately, offering improved predictions of species occupancy and distribution in different habitat and environmental scenarios (Aubry *et al.*, 2017; Meade *et al.*, 2018). Obtaining data at the microhabitat level can show small but important habitat selection mechanisms that may be otherwise missed (Telve *et al.*, 2020). In the field, conservation management decisions can then be made that best protect focal species of conservation concern (Ozdemir *et al.*, 2018; Liefferinge *et al.*, 2019). Survey protocols can also benefit from detailed spatiotemporal data that may inform best practice, particularly for cryptic species that may rely on attractant-based, or focal-location-based survey techniques (Cusack *et al.*, 2015; Stewart *et al.*, 2019) to ensure these are as efficient, objective and spatiotemporally comparable as possible. Techniques can be adapted with this evidence-base in the face of changing natural systems, to ensure presence/absence surveys remain reliable and any inferences around shifting temporal trends in populations and occupancy can be considered in the light of the monitoring methodology utilised.

3.1.2 Shifting species ranges and reptiles

There is a current drive to model expanding and shifting species ranges at landscape level, by incorporating various climate change scenarios (Cruz *et al.*, 2015; Préau *et al.*, 2019) to plan future conservation efforts accordingly (Rytteri *et al.*, 2017; Walls *et al.*, 2019). Reptiles are of particularly high susceptibility to changes in climate due to their biology being closely linked to temperature and moisture variables and their annual cycle defined by climatic conditions (Kumar *et al.*, 2014), such as annual brumation cycles in colder climes and the effect of temperature on sex ratio of offspring in many species (Janzen, 1994; Wapstra *et al.*, 2009). Many reptiles also have more limited dispersal abilities than other taxa, making them more vulnerable to rapid changes in climate and consequently habitat (Gibbons *et al.*, 2000). This leaves populations vulnerable to rapid decline, and along with anthropogenic factors such as habitat destruction, degradation and pollution (Gibbons *et al.*, 2000) decline is being observed for reptiles on a global scale (Saha *et al.*, 2018).

Changes in environmental conditions as a result of temperature rise may theoretically enable a species to occupy space previously unsuitable. Vegetation changes, for example, leading to improved foraging and hunting opportunities may offer ideal habitat for a species' range to shift into (Gonzalez *et al.*, 2010). However, suitable habitat corridors must be established enabling populations to translocate to new areas. For species with limited mobility and capacity to disperse, such as reptiles, this may still be insufficient. Reintroduction (or other conservation translocations) may be deemed the most suitable conservation measure, especially if the species to be translocated fulfils ecological function in the ecosystem (IUCN, 2013). However, details on reptile ecology are data deficient (Tingley *et al.*, 2016), therefore assessing suitability of introduction to new sites or reintroduction to sites within historic range, using theoretical modelling remains challenging (Getz *et al.*, 2018). The elusive nature of these species and the research and publication biases towards more charismatic taxa (Clark and May, 2002; Fazey *et al.*, 2005; Bajomi *et al.*, 2010) has left them comparably under-studied.

Habitat management is used to create and maintain high quality *foci* within wider reptile habitat and features in the UK (Moulton and Corbett, 1999; Edgar *et al.*, 2010). Ensuring this is optimal, based on microhabitat and microclimate needs is essential to concentrate efforts appropriately and optimise the success of conservation and reintroduction initiatives, as well as best survey practice. Targeted monitoring is essential to assess populations and wider UK metapopulations of a group of species broadly regarded as under threat (Sewell *et al.*, 2012; Mccoy *et al.*, 2014; Tingley *et al.*, 2016; Gardner *et al.*, 2019).

Along with the importance of monitoring reptiles to assess any shifts in population as a result of changing habitat and climate, it may be necessary to adapt surveying methodologies to ensure they are still optimal in changed environments. The close link between reptiles and environmental conditions for thermoregulatory purposes (Kumar *et al.*, 2014) (Froglife, 1999; Joint Nature Conservation Committee, 2004; Langham, 2012) means that as environmental conditions alter, monitoring techniques may require adapting accordingly.

3.1.3 Application of *ex situ* research with a captive population to a field scenario *in situ*

This chapter extends the monitoring methodology that was assessed in captivity (Chapter 2) and applies it to a reintroduced population of sand lizards *L. agilis* using a field experimental approach. The captive environment enabled detailed observation of a small number of sand lizards within a limited area over one part of the active season. It allowed a detailed study of microhabitat and refugium use temporally, in the context of ecophysiological, social and demographic factors, and when lizards were known to be within sight above ground – an element that is unquantifiable in a field setting. Undertaking this complimentary, field-based study allows assessment of the applicability of each setting in contrast to the other to provide optimal evidence-based recommendations to survey protocols for sand lizards.

3.1.4 Habitat selection and monitoring protocols for sand lizards in the UK

The sand lizard is predominantly restricted in the UK to dry heathland habitat, also occupying coastal dunes (Edgar *et al.*, 2010; Blanke and Fearnley, 2015). Sand lizard *foci* include south facing slopes and features and bare interfaces adjacent to the cover of vegetation (Corbett and Moulton, 1998; Blanke and Fearnley, 2015); in dry heath mature and degenerate heather and bryophytes and lichens appear favoured (Edgar *et al.*, 2010) (see also 1.3.3). Surveying techniques for sand lizards have suggested transect walks several times in spring and autumn along site-specific key features, such as edge habitat between heather and sandy traces (see 1.4) (Corbett and Moulton, 1998; Moulton and Corbett, 1999). However, detectability of this species is notoriously poor. Comparability between sites and inter-surveyor reliability is also challenging with this relatively arbitrary approach.

3.1.5 Aims

The overarching aim of this chapter is: **to assess survey methodologies and determine environmental factors affecting detectability of a reintroduced population of sand lizards in dry heath habitat.** Its objectives are:

1. To assess differences in sand lizard detectability between different survey methodologies: multi-material refugium arrays and walking transects.
2. To determine preferred refuge material by reintroduced sand lizards and microclimate and microhabitat factors associated with material use.
3. To compare microclimate and microhabitat conditions associated with observations of reintroduced sand lizards and wild common lizards on transect walks.

3.2 Methods

3.2.1 Sand lizard release protocols

Sand lizards were released in the dry heath habitat of Eelmoor Marsh SSSI on 15/09/2017 (26 yearlings, 54 hatchlings), and 13/09/2018 (10 yearlings, 51 hatchlings) and 18/09/2018 (10 2-year olds, 15 yearlings) (the third annual release, as per sand lizard reintroduction protocols discussed (1.4), was beyond the timeframe of this study and took place 12/09/2019 and 18/09/2019). The reintroduction was undertaken as part of the conservation efforts at the site, with consent from Natural England. Assistance was provided to obtain the data discussed below by P. Riordan, M. Wilkie, T. Gilbert, A. Thompson, B. Govier, C. Broom, E. Jordan and L. Furmidge. The release protocols followed current ARC Trust protocols as closely as possible, constituting the release of approximately 80 individuals a year for three consecutive years (the third release took place in 2019, not covered in this study) (Moulton and Corbett, 1999; Berglind *et al.*, 2015). Advice regarding the best timing each year was followed, through regular contact with ARC Trust in the lead up to releases. Releases usually take place in “early September… When possible… in warm and sunny conditions, which allow [lizards] to adapt to the site gradually” (ARC Trust, 2016), with limited rain forecast for the following few days (ARC Trust, pers. comm.); however “sunny and warm days during mid-April to early October” are considered suitable (Moulton and Corbett, 1999). Lizards were captured in their rearing vivaria at Marwell Zoo in the morning (and some the night prior and held in a smaller rearing vivarium overnight) and transported within their age groups to Eelmoor Marsh by car in plastic, "well-ventilated escape-proof" (ARC Trust, 2016) travel terrariums measuring approximately 45 x 30 x 20 cm.

Current sand lizard reintroduction protocols release small groups of juveniles in an area of optimal habitat within a wider site, with vegetation offering varied structure allowing both basking and sheltering opportunities (ARC Trust, pers. comm.). In this study, in order to best quantify individual movements, groups of ten lizards were released at regular 2 m intervals at eight release localities in the centre of a 60 m long, ~ 2 m wide, mown strip on the south-facing dry heath habitat; releases were at 1, 3, 5 and 7 m either side of the central point of this strip. Lizards were set down in groups of three or four by two people (one lizard in each hand) and were predominantly released within their age group, however a few mixed groups were released; in these cases it was ensured there was always more than one individual of each age group released together. At each of the eight release points a 50 X 50 cm 'release square' was placed, bounded by four pegs and a piece of string elevated by ~5 cm denoting the point of release for each group of lizards (Figure 16).

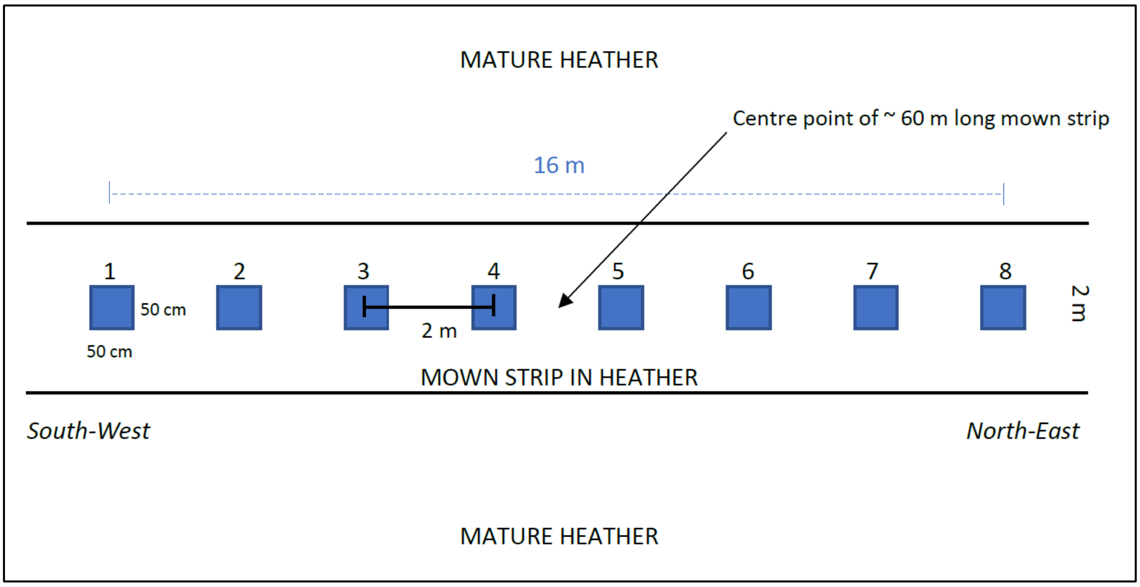


Figure 16 Sand lizard release squares along centre of ~60 m long mown strip in mature heather. Blue release squares were demarcated with pegs and elevated string.

Following the releases, ARC Trust protocols were followed to create a series of 'contingency burrows' by hammering a 45 cm steel spike into the ground along the top of the mown strip working westwards from the release squares (it is hypothesised that sand lizards have a tendency to migrate westwards immediately post-release (Moulton, N pers. comm.; Langham, S, pers. comm.)). It is thought that these may offer an essential sheltering opportunity (or starter burrow) for sand lizards post-release, especially in the event of early cold weather when they may not have established themselves sufficiently in their new habitat.

3.2.2 Post-release monitoring

Advice from practitioners has suggested sand lizards will disperse anywhere between 10 m and 100 m from the point of release (Moulton, N. pers. comm.). Thus, a grid of refugium arrays at intervals discussed below was deployed, consisting 500 x 250 mm size materials and initially covering 40,000 m^2 (Figure 17) (200 x 200 m). The survey grid centred on the middle of the mown release strip. (An additional ~ 2 m wide strip was mown and excavated in two lengths, up-slope of the release strip, over winter 2018-19 to allow the creation of two linear sand strips for oviposition by female sand lizards, as well as two small ~ 2 x 2 m sand patches just south of these. The sand was manually turned over to remove roots and then tamped flat with help from QinetiQ staff on 8th May 2019.)

The survey grid was oriented in line with the southeast aspect of Pyestock Hill (51.275N, 0.802W; Figure 17). An array was placed every 20 m wherever possible, measured using ranging poles and measuring tape (Figure 18). The limited accuracy of a GPS unit, of 'up to' 5 m, was deemed too poor precision when deploying arrays every 20 m. Some edges of the grid extended onto the surrounding metalled tracks and then completely off the SSSI, and several other arrays could not be placed due to inaccessibility as a result of dense thickets of gorse and brambles. The grid was extended, as indicated (Figure 17), by 40 m to the north-east and 40 m to the north-west following sand lizard sightings at its limits during post-release monitoring.

Immediately around the mown release strip a more intense grid of arrays were placed every 10 m, covering 2400 m^2 (60 m along the contour of the slope SW-NE, 40 m up-slope NW-SE; covering at least 20 m in each direction from the extremes of the release locations measured along the central 14 m of the mown strip). This grid allowed smaller movements around the release area to be assessed as it was suggested that the majority of lizards would not leave the release site for some time (Langham, S. pers. comm.; Moulton, N. pers. comm.). (The upper mown strip, converted to two linear sand strips as discussed above, was positioned at ~ 14-16 m north-west of the release mown strip, falling mid-way between the 10 and 20 m arrays to the north of the release locations; the two small sand patches were between the mown release strip and upper mown / sand strips.)

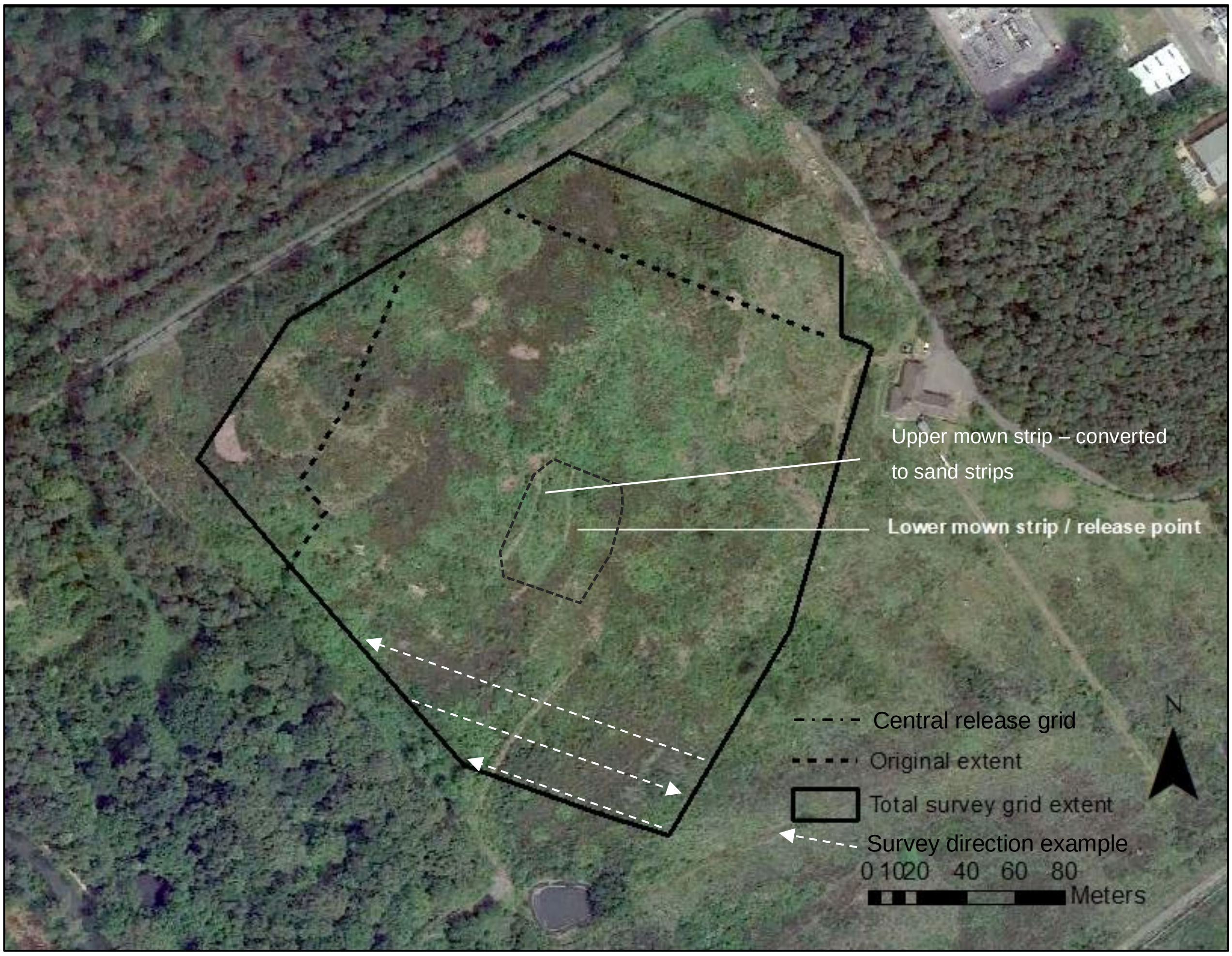


Figure 17 (Above) Dry heath habitat surveying grid, with original (200 x 200 m) and extended limits. The mown strip along which lizards were released is marked, as is the strip to its north-west which was later turned over to sand. Extent of grid limited by site boundary / dense vegetation in places. (Satellite imagery georeferenced and annotated from Google™ Earth image in ArcMap).

Figure 18 (Left) Ranging poles used for demarcating refugium grid across sand lizard release area.

The refugium arrays forming the grid consisted of four material types: Wildcare® thin sheet metal Reptile Profile Tin (< 1 mm thick, 3 cm high corrugations); Cembrit Ltd. natural Spanish roofing slate (4-6 mm thick); Marley® dark red plain clay roofing tile (25 mm thick); and IKO® bitumen underlay roofing felt (1 mm thick). Felt was included in place of the plywood in Chapter 2, due to wood being very seldom utilised here. Felt is already used as second preference to tin in current refugium-based reptile monitoring and its inclusion allowed both these materials to be assessed against slate and tile, which were used the most by sand lizards in the captive setting. Individual tins, slates and felts measured 500 x 250 mm; two approximately 250 mm x 250 mm tiles were used to cover the same ground area, leaving no gaps. (It was deemed important to provide comparable ground cover across refugium types rather than add an additional variable of area of coverage. It is acknowledged that thermal properties differ between a refugium made of one continuous sheet of material, as opposed to multiple. However, the purpose of this research was to assess practical options for reptile refugia, and commercially available tiles do not exist larger than those utilised. Alternatively, reducing all refugia to 250 x 250 mm would have restricted their use by the larger reptile species present, as also being studied (see Chapter 4).) The order of placement of material type in each array was random and a 250 mm gap was left between each material when initially distributed; subsequently there was some small natural movement of the materials as they settled within the vegetation. All arrays were aligned lengthways, following the grid centre's greatest angle of dip, to keep this uniform. Materials were deployed more than two weeks in advance of surveys commencing. Any damaged materials were replaced as soon as possible (damage through vegetation growth, livestock, etc.; it should be noted that no basking or refuging animals were ever found injured or killed as a result of this).

The refugium size used intended to strike a balance between a suitable survey intensity to recognise relatively short movements of lizards and not creating a semi-paved release area where materials had the potential to be used as 'stepping stones' to aid dispersal beyond that which would occur naturally. Consideration of other flora and fauna on site was also necessary. There is no quantitative evidence that reptiles require particularly large refugia and the size of the materials used was ample to cover the body size of all native UK reptile species if used as a refuge. The highly quoted historic reptile survey advice suggests 'tinning' using refugia of 0.5 m^2 (Froglife, 1999) - twice the size. Other recommendations suggest (tin) refugia of 1000 x 610 mm (Langham, 2011) or 3 x 2 feet (910 x 610 mm) (Langham, 2012) - four times the size, stating that generally larger refugia are better. The thermal properties of refugia will obviously differ based on size as well as material type, showing patchy temperature distribution depending on the placement and profile of the material (Hodges, 2018). However, using smaller refugia of 500 x 300 mm, has recently been recommended in survey guidelines for monitoring reptiles in peatlands due to the lack of evidence supporting the need for larger ones when this size “appear to be effective”, as well as the practical ease of transportation during deployment (Cathrine, 2018). The variety of thermoregulating opportunities provided by each refuge is of benefit to its users (Hodges, 2018).

Post release surveys were carried out between 19/09/2017 and 17/11/2017, 22/03/2018 and 14/11/2018, and 26/02/2019 and 29/08/2019. Surveys were conducted three times a week post-release (September) until the end of the season in November 2017 and 2018, and one-two times a week between mid-March and the year’s release in September. The exceptions to this were a reduction in surveys during a heatwave in 2018 when reptile observations plummeted making surveys unproductive, and an early survey in mild weather starting the 2019 season, on 26/02/2019. Surveys totalled 117, amounting to 614 hours and 50 minutes surveying time.

All surveys were undertaken in daylight hours, between 08:15 and 19:15. Arrays were visited in turn, walking in a south-east to north-west direction up one row before returning north-west to south-east down the next (see Figure 17). The central 10 x 10 m grid was surveyed in its entirety when reached, before returning to the 20 m arrays once complete, following the same route every time. Arrays were checked for both basking and refuging reptiles (on top or underneath). Transects between arrays were also actively surveyed and any sightings logged to the nearest ~1 m. Approximately one meter either side of the route taken, equivalent to a 2 m belt transect, could practicably be scanned by eye in this manner at a consistently slow walking pace (surveyor remained constant throughout all surveys); audio cues were also listened out for, such as the sound of a scurry or a slither as an animal moved in the vegetation. In addition to walking the wider grid, the length of the mown release area was walked to determine any individuals remaining in the immediate release vicinity; during 2019, the sand strips and patches were also checked for signs of sand lizard test burrows. When sand lizards were observed time was taken to obtain photographs of suitable quality to identify individuals. A ‘scurry’ (seen or heard), at an array or on a transect, was treated as a potential lizard and five minutes was allowed for the cause of the scurry to re-emerge; this five-minute window was refreshed if a further scurry or partial sighting occurred. If nothing emerged, the sighting was recorded as ‘unknown scurry’. This process and subsequent photograph-taking could take up to ~15 minutes per individual.

Microclimate data were collected on one/two of three weekly surveys in autumn following release, and every once-twice/week survey in spring-summer. Surveys including no microclimate data took ~3-4.5 hours; surveys including all data collection took up to 8 hours, but typically ~6-7 hours; variation as a result of number of observations, number of scurries and obtaining adequate photographs. For quicker non-microclimate surveys, the grid was either surveyed in the morning or the afternoon on rotation, always beginning from the southern corner; for microclimate surveys the grid was split into thirds and the start point systematically rotated across these thirds. This varied the time of day each array was visited. Microclimate data were obtained for the central 14400 m^2 of the grid (120 x 120 m), including all arrays forming the 60 x 40 m central area with refugia every 10 m. Following a pilot survey in 2017, pre-release, the central 14400 m^2 was concluded the maximum area possible to survey with microclimate readings in order to guarantee a completed survey within a day across all seasons (accounting for shifting daylight hours). Microclimate variables were recorded in part by a Kestrel 3000, measuring air temperature (°C), relative humidity (%) and average wind speed over 30 seconds at ground level (m /s). A light meter was used to measure light at refugium level (Lux), and a soil thermometer measured soil temperature at a depth of ~2-3 cm in the open by the array (°C). These instruments were given 30 seconds to settle prior to readings being taken. Finally, an infrared thermometer was used to measure surface temperature of each refuge material in its centre, and open vegetation temperature next to the array (°C). During the microclimate surveys, the same microclimate measurements were taken when sightings occurred on transects walks, as close to the lizard as possible (within ~2 m) but avoiding disturbance. (Where the sighting was in proximity of an array (~2 m), these array measurements were used for the same reason).

An Oregon Scientific WMR200 weather station situated on site was set to record data on local air temperature, humidity, wind speed, wind direction, rainfall and UV index at one-minute intervals to be downloaded to Weather Station Data Logger 5.3.6.1 (Sourceforge). Unfortunately, technical issues meant these data were inconsistently and non-continuously recorded; this was noted during the regular downloads and ultimately weather logged at nearby MET Office-registered stations was deemed both more reliable and continuous. These data were thus used instead in the analyses as follows: Farnborough South weather station (located at Farnborough airport, immediately adjacent to Eelmoor Marsh, 51.279N, 0.772W) was used by preference and contributed the vast majority of weather readings. U.K. Meteorological Office weather stations at Aldershot (51.249N, 0.753W) and Reading (51.442N, 0.937W) provided supplemental and rainfall / UV data respectively from July 2018. An SP Lite2 pyranometer (Kipp & Zonen) logged solar radiation on Eelmoor, data being downloaded regularly via LogChart II software (Novus).

Microhabitat variables were recorded at every array across the grid, including measures of vegetation structure, vertical cover, ground cover and canopy cover. Vegetation variables were recorded in July 2018 and again in March/April 2019, in the middle of the two-year survey period to obtain both minimal and maximum vegetation growth data. The application of the results of these data is to assist in refugium placement for reptile surveys to optimise refugium use and reptile detectability. Therefore, established methods were followed with small modifications to make the technique as straightforward for field surveyors as possible, whilst remaining objective, repeatable and reptile-specific. All vegetation height, structure and ground cover readings were made using a 50 x 50 cm quadrat with 100 x 5 cm^2 subdivisions. This acted both to quantify ground cover, and as a coverboard for vegetation height and vertical structure to be assessed. The quadrat was first placed flat to the immediate north, east, south and west of the array. Photographs were taken to later assess vegetation out of the field, using grouped broad categories adapted from Joint Nature Conservation Committee guidelines (Elkington *et al.*, 2001; Joint Nature Conservation Committee, 2004; Hall, Kirby and Whitbread, 2004; Rodwell, 2006). Structure was then measured using a modified version of the cover board method (Coulloudon *et al.*, 1999). The quadrat was placed vertically through the vegetation in each of these same four locations, at right angles to the tin/array, and held in place with a walking pole while the observer squatted until eye-level with the top of the quadrat, from which point a photograph was taken. The total distance from observer to quadrat was 1 m (including arm length and measured walking pole length). This provided vegetation cover, height and structure data for four locations surrounding each tin/array, at a scale suitable for examining reptile microhabitat and use of refugia. These microhabitat data were averaged across all four compass directions and then across the two seasons to provide three values for each refugium site: a measure based on Simpson's Diversity Index for diversity of ground cover taking into account broad vegetation categories (pioneer heather, building/mature heather, degenerate heather, gorse, bramble, broom, graminoid, forb/herb, scrub, bracken, bryophyte, fungi, bare ground, litter, dung) based on National Vegetation Classification documentation (Elkington *et al.*, 2001; Hall *et al.*, 2004; Rodwell, 2006); average vertical cover as a percentage; and a pooled standard deviation as a measure of relative vertical structural diversity/heterogeneity. The growth phases of heather as defined (JNCC, 2004a) were included to acknowledge the different structural phases and thus microhabitat provisions of the heather-dominated dry heath. A spherical convex densiometer was used to measure canopy cover at the central point of each tin/array, by facing all four compass directions and averaging the result to provide an overall canopy cover reading (%) (Lemon, 1956; Forestry Supplies Inc, 2008); this was averaged across the two seasons.

These same microhabitat variables were recorded for every walking transect reptile observation locality. The measurements were taken collectively in batches during the same part of the season in which the observation occurred (within ~1 month but accounting for rapid vegetation changes as required e.g. bracken growth). This was deemed both more practical and less disturbing than carrying vegetation-surveying equipment in addition to microclimate instruments on every survey. Disturbing an animal to take vegetation readings at the exact time of observation would also be counter intuitive as it may have discouraged it from using the same locality.

3.3 Analysis

All statistical analyses were undertaken in RStudio 1.1.463 (R version 3.5.3). All analyses assume significance at $P \leq 0.05$. Power analyses, as discussed in Results, were run in R package 'pwr'. (Spatial analyses in Appendix C.10 was performed in ArcGIS ArcMap 10.6.1.)

Data were assessed for zero-inflation and over-dispersion. Negative binomial GLMMs (accounting for over-dispersion) were used to assess the effect of refugium type and i) microclimate and ii) microhabitat variables on sand lizard counts at arrays. Refugium type and microclimate / microhabitat variables were fixed effects, array number was included as a random effect. Interactions between material and each microclimate / microhabitat variable were included to assess if any particular factor determined material type selected.

Differences in average microhabitat and microclimate conditions associated with use of an array material were examined. Each observation of presence was treated as an independent data point (with equal opportunity to select felt, slate, tile or tin) and analysed with either one-way ANOVA or non-parametric Kruskal-Wallis, depending on whether each data set met parametric assumptions or not. When the overall test result was significant, post-hoc testing was undertaken using Tukey's test, or the non-parametric Dunn test accordingly. This provided a summary of the most optimal environmental conditions in which sand lizards were found utilising different refugium types. The results of these analyses are displayed in box and whisker plots.

Common lizard and sand lizard microhabitat and microclimate conditions at observations on transect walks were compared using *t*-tests or non-parametric Mann-Whitney U to determine any differences between the conditions used by these lizards for (generally basking) visible behaviour. The results of these analyses are again displayed in box and whisker plots.

(Regression analyses for key weather variables recorded on site, and corresponding microclimate readings obtained during surveys in dry heath can be found in Appendix B.1. A point density analysis summarising sand lizard distribution and abundance post-release is shown in Appendix C.10 d), modelled using ArcGIS ArcMap 10.6.1.)

3.4 Results

3.4.1 Observation location and preferred refugium material of sand lizards

More sand lizards were observed on transect walks than using refugium arrays. Of the refugium material types, slate was used the most, followed by felt, then tin, being in the open near an array, and finally tile (Figure 19a). There was a weak positive relationship between number of sand lizard observations at arrays and on walking transects during the same survey (R^2 = 0.161) (Figure 19b).

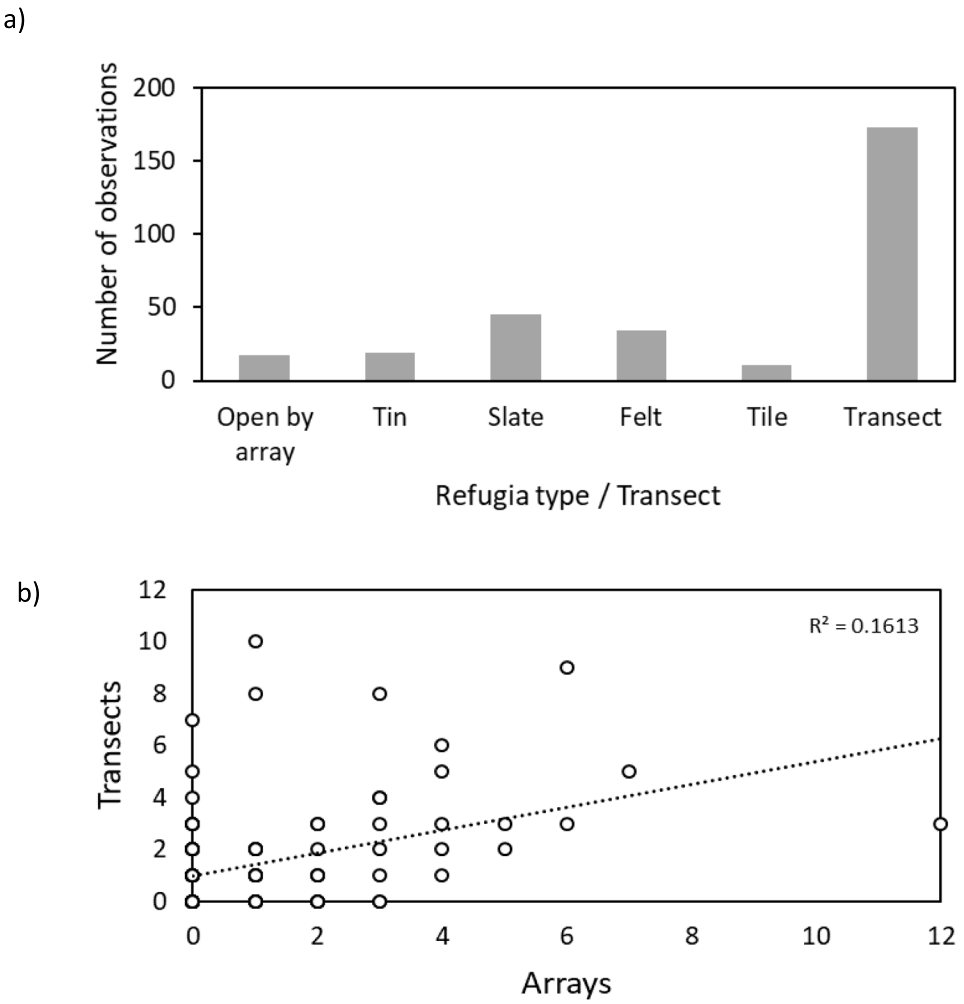


Figure 19 a) Locations of sand lizard observations on dry heath of Eelmoor Marsh and b) frequency of sand lizard observations on transect walks and at refugium arrays per survey.

3.4.2 The effect of environmental conditions on refugium selection by sand lizards in dry heath

Increased humidity and decreased light levels (lux) significantly predicted use of refugium arrays. There was a negative interaction of open habitat use at arrays with humidity compared to tin, indicating the importance of lower humidity levels in use of open space and greater in use of refugia. Increase in structural diversity of vegetation had a significantly positive effect on use of felt by sand lizards (Table 9).

Table 9 Minimum adequate negative binomial GLMMs considering microclimate (left) and microhabitat (right) factors and material type selected by sand lizard. ‘Tin’ was the reference level for material type.

Microclimate					**Microhabitat**				
Coefficients	Estimate	Std. Err.	z value	*P*	Coefficients	Estimate	Std. Err.	z value	*P*
Intercept	-13.960	2.088	-6.687	**<0.001**	**Intercept**	-12.304	1.584	-7.770	**<0.001**
Open: humidity	-0.053	0.024	-2.191	**0.029**	**Felt: structural diversity**	0.127	0.047	2.724	**0.006**
Humidity	0.045	0.018	2.477	**0.013**	**Felt**	-1.774	0.868	-2.044	**0.041**
Light	-0.025	0.011	-2.252	**0.024**	**Slate**	1.949	0.831	2.344	**0.019**
Open	3.515	1.721	2.043	**0.041**	**Structural diversity**	-0.022	0.078	-0.285	0.776
Air temp	0.086	0.059	1.451	0.147	**Open**	-0.348	0.950	-0.366	0.714
Soil temp	-0.065	0.064	-1.016	0.310	**Tile**	-1.525	1.065	-1.432	0.152
Mat temp	-0.012	0.028	-0.419	0.675	**Open: structural diversity**	0.016	0.055	0.281	0.779
Felt	2.376	1.615	1.471	0.141	**Slate: structural diversity**	-0.074	0.052	-1.409	0.159
Slate	1.948	1.562	1.248	0.212	**Tile: structural diversity**	0.058	0.058	1.002	0.316
Tile	1.802	1.847	0.976	0.329	**AIC**	1457.60			
Felt: humidity	-0.025	0.021	-1.156	0.248					
Slate: humidity	-0.013	0.020	-0.618	0.537					
Tile: humidity	-0.028	0.025	-1.123	0.261					
AIC	949.4								

Material use did not vary as a result of material temperature, air temperature, soil temperature, wind speed or relative humidity, although humidity was very nearly significant ($p = 0.052$), (Table 10, Figure 20). There was a difference between light and material selected; light was greater when open vegetation was selected compared to felt or slate ($p = 0.018$ and $p = 0.027$ respectively).

There was no difference between vertical vegetation cover or canopy cover and material use (Table 10, Figure 20). There was a difference between material selected and ground cover diversity; felt was utilised in instances of lower ground cover diversity compared to slate ($p = 0.019$). There was a difference between structural diversity and material type use; structural diversity was significantly greater in instances of use of felt compared to open vegetation, slate or tin ($p = 0.036$, $p < 0.0001$ and $p = 0.023$ respectively).

There were strong positive relationships between microclimate variables on the ground and wider weather variables within dry heath, highlighting the predictability of field conditions for monitoring when the weather is known (Appendix B.1).

Table 10 Environmental factors associated with sand lizard material type use in dry heath habitat, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are bold italicised and any significantly different material pairs following post-hoc testing are highlighted.

	Felt					Open					Slate					Tile					Tin					Main model (ANOVA/Kruskal-Wallis)					
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	**F value**	**Kruskal-Wallis χ^2**	**df**	**P value**	**Significant pairs**	**Transformation**
MICROCLIMATE																															
Mat.temp °C	20.75	21.30	16.65	24.65	23	21.38	24.20	15.80	26.30	13	18.32	18.10	15.30	22.70	33	21.89	21.70	20.00	23.40	9	20.06	19.75	18.50	22.10	14	1.080		4,87	0.372	NA	NA
Air temp °C	19.92	19.90	17.60	22.40	24	19.22	20.40	15.70	21.50	13	17.72	17.80	15.30	21.85	35	20.94	20.40	20.00	21.70	9	18.35	18.65	17.70	20.00	14	1.542		4,90	0.197	NA	NA
Soil temp °C	15.62	15.00	14.00	18.00	21	15.45	16.00	12.00	17.50	11	14.85	15.00	13.00	17.00	33	17.44	18.00	16.00	18.00	9	15.42	16.00	15.00	17.00	12	0.917		4,81	0.458	NA	NA
Rel hum %	67.83	68.00	58.20	74.35	24	57.96	58.20	45.20	62.10	13	71.21	71.80	62.65	78.90	35	67.08	73.80	53.60	81.90	9	73.69	75.30	67.60	79.10	14	2.445		4,90	0.052	NA	NA
Light Lux	16.54	12.29	9.25	20.97	24	30.98	34.86	16.37	46.10	13	17.98	14.99	7.71	22.89	35	17.48	15.22	11.48	18.28	9	16.56	13.44	8.70	21.56	14	2.880		4,90	**0.027**	Op/Fe, Op/Sl	log
Wind sp m/s	0.11	0.00	0.00	0.15	24	0.12	0.00	0.00	0.30	13	0.19	0.00	0.00	0.30	35	0.16	0.00	0.00	0.30	9	0.16	0.00	0.00	0.30	14		2.239	4	0.692	NA	NA
MICROHABITAT																															
Cover div SI	0.51	0.61	0.24	0.78	34	0.63	0.72	0.61	0.76	17	0.72	0.76	0.73	0.78	45	0.53	0.70	0.27	0.74	11	0.67	0.73	0.70	0.76	19		12.792	4	**0.012**	Fe/Sl	NA
Struct st.dev	21.76	20.56	14.09	29.62	34	16.40	14.47	12.83	20.74	17	13.89	13.67	12.28	14.47	45	18.16	15.80	13.34	23.00	11	15.83	14.47	13.02	15.80	19	8.076		4, 121	**<0.001**	Fe/Op, Fe/Sl, Fe/Tn	log
Height cover %	34.55	35.88	19.47	44.34	34	33.37	31.16	27.25	42.88	17	28.04	27.25	18.38	37.28	45	34.10	35.88	27.64	42.88	11	29.56	34.41	18.38	37.28	19	2.100		4, 121	0.085	NA	NA
Can cov %	0.85	0.00	0.00	0.13	34	1.25	0.00	0.00	0.39	17	1.23	0.00	0.00	0.39	45	0.56	0.00	0.00	0.26	11	1.66	0.00	0.00	0.39	19		3.091	4	0.543	NA	NA

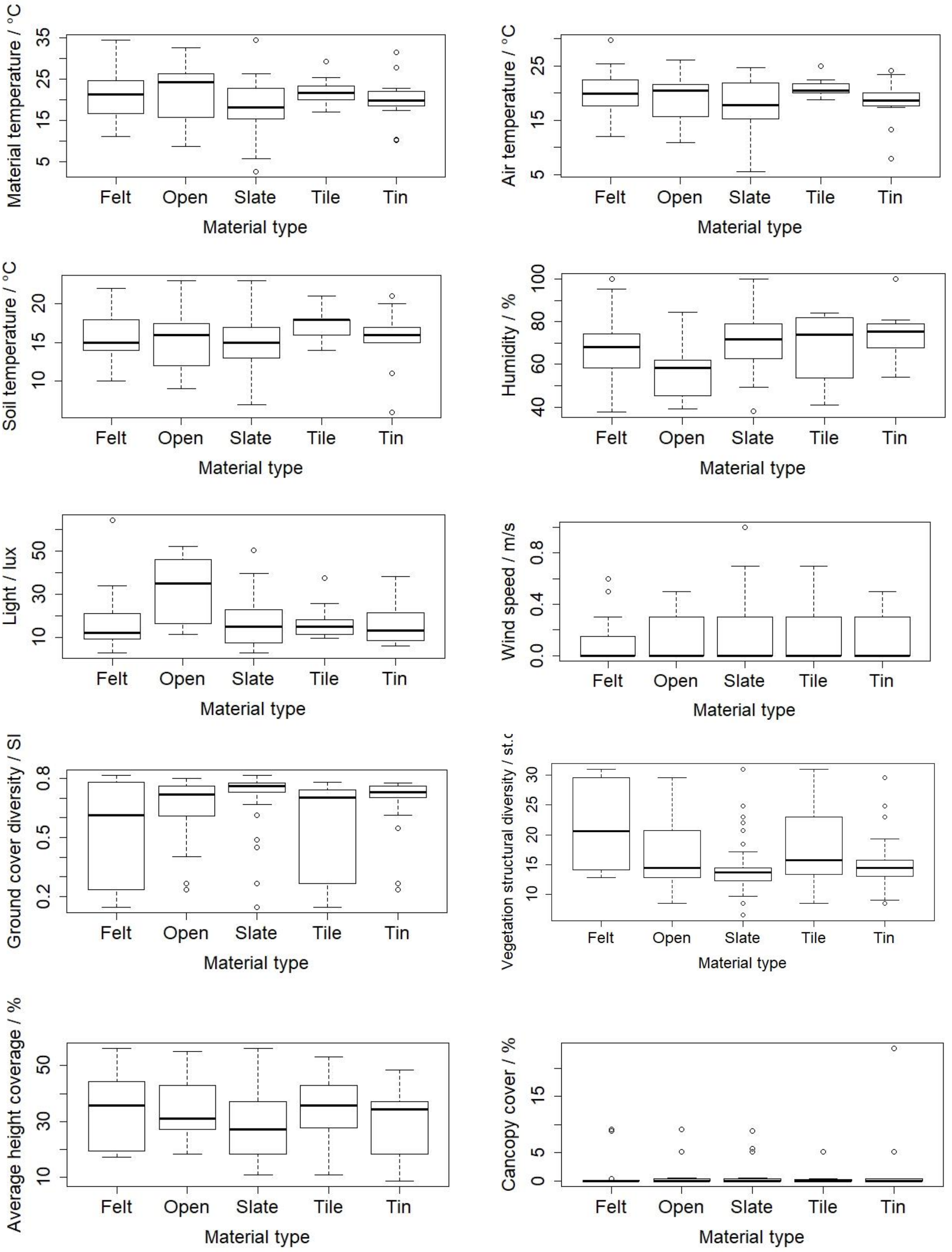


Figure 20 Microclimate and microhabitat conditions associated with material use by sand lizards in dry heath. Plots highlight the median, interquartile range, extreme values and outliers.

3.4.3 Differences in sand lizard and common lizard use of natural habitats (transect walks)

There were significant differences between microclimate and microhabitat factors recorded at locations where common lizards and sand lizards were observed during walking transects in dry heath (Table 11, Figure 21). Microclimate factors of vegetation temperature, air temperature, soil temperature and light were significantly lower for sand lizards than common lizards, and humidity and wind speed significantly higher. This held true for all factors except soil temperature (which became non-significantly different) when surveys during November were removed (when only persistent post-release sand lizards were recorded). Ground cover diversity was significantly higher for sand lizards than common lizards, and structural diversity and vertical/height cover significantly lower for sand lizards; canopy cover did not differ significantly. These results also held true when surveys from November were excluded.

Table 11 (overleaf) Environmental factors associated with observations of common lizards and sand lizards on transect walks in dry heath habitat, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are bold italicised and any significantly different material pairs following post-hoc testing are highlighted.

	Common lizard					Sand lizard					Main model (*t*-test / Mann-Whitney U)				
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	***t*-test / t value**	**Mann-Whitney U / U value**	**df**	**P value**	**Trans-formation**
MICROCLIMATE															
Veg temp °C	22.83	22.45	19.55	25.75	132	20.05	18.80	14.80	23.65	107	3.544		132,107	**<0.001**	NA
Air temp °C	23.94	23.60	21.00	27.40	133	20.79	20.80	16.80	23.65	107	4.811		133,107	**<0.001**	NA
Soil temp °C	18.26	18.00	16.00	21.00	130	17.00	17.00	13.00	20.00	105	2.068		130, 105	**0.040**	NA
Rel hum %	49.45	48.90	40.50	57.90	133	57.10	55.80	47.60	67.60	107	-4.022		133,107	**<0.001**	NA
Light Lux	35.03	34.67	20.93	46.50	133	27.79	27.48	14.01	38.03	107	3.268		133,107	**0.001**	Sq.rt.
Wind sp m/s	0.13	0.00	0.00	0.30	133	0.19	0.00	0.00	0.35	107		5965.50	133,107	**0.015**	NA
MICROHABITAT															
Cover div SI	0.51	0.56	0.38	0.68	158	0.68	0.72	0.62	0.76	175		6420.50	158,175	**<0.001**	NA
Struct st.dev	22.04	22.71	16.76	28.02	158	19.58	20.22	15.16	24.87	175	3.109		158,175	**0.002**	NA
Height cover %	38.48	36.94	25.69	50.13	158	30.61	31.44	21.28	39.41	175	4.882		158,175	**<0.001**	NA
Can cov %	2.66	0.00	0.00	1.30	158	2.52	0.00	0.00	0.78	175		14586.00	158,175	0.268	NA
	Surveys excluding November														
MICROCLIMATE															
Veg.temp °C	22.83	22.45	19.55	25.75	132	21.06	20.30	17.50	24.60	94	2.205		132, 94	0.029	NA
Air temp °C	23.94	23.60	21.00	27.40	133	21.67	21.20	18.90	24.00	94	3.440		133,94	<0.001	NA
Soil temp °C	18.26	18.00	16.00	21.00	130	17.83	18.00	15.00	20.00	92	0.704		130,92	0.482	NA
Rel hum %	49.45	48.90	40.50	57.90	133	55.43	55.25	45.50	62.50	94	-3.063		133,94	**0.003**	NA
Light Lux	35.03	34.67	20.93	46.50	133	29.06	30.55	14.66	38.78	94	2.563		133,94	**0.011**	Sq.rt.
Wind sp m/s	0.13	0.00	0.00	0.30	133	0.19	0.00	0.00	0.40	94		5271.00	133,94	**0.023**	NA
MICROHABITAT															
Cover div SI	0.51	0.56	0.38	0.68	158	0.67	0.72	0.61	0.76	149		5919.50	158,149	**<0.001**	NA
Struct st.dev	22.04	22.71	16.76	28.02	158	19.88	21.13	15.67	25.12	149	2.603		158,149	**0.010**	NA
Height cover %	38.48	36.94	25.69	50.13	158	31.85	33.44	22.06	40.00	149	3.981		158,149	**<0.001**	NA
Can cov %	2.66	0.00	0.00	1.30	158	2.89	0.00	0.00	1.17	149		12176.00	158,149	0.475	NA

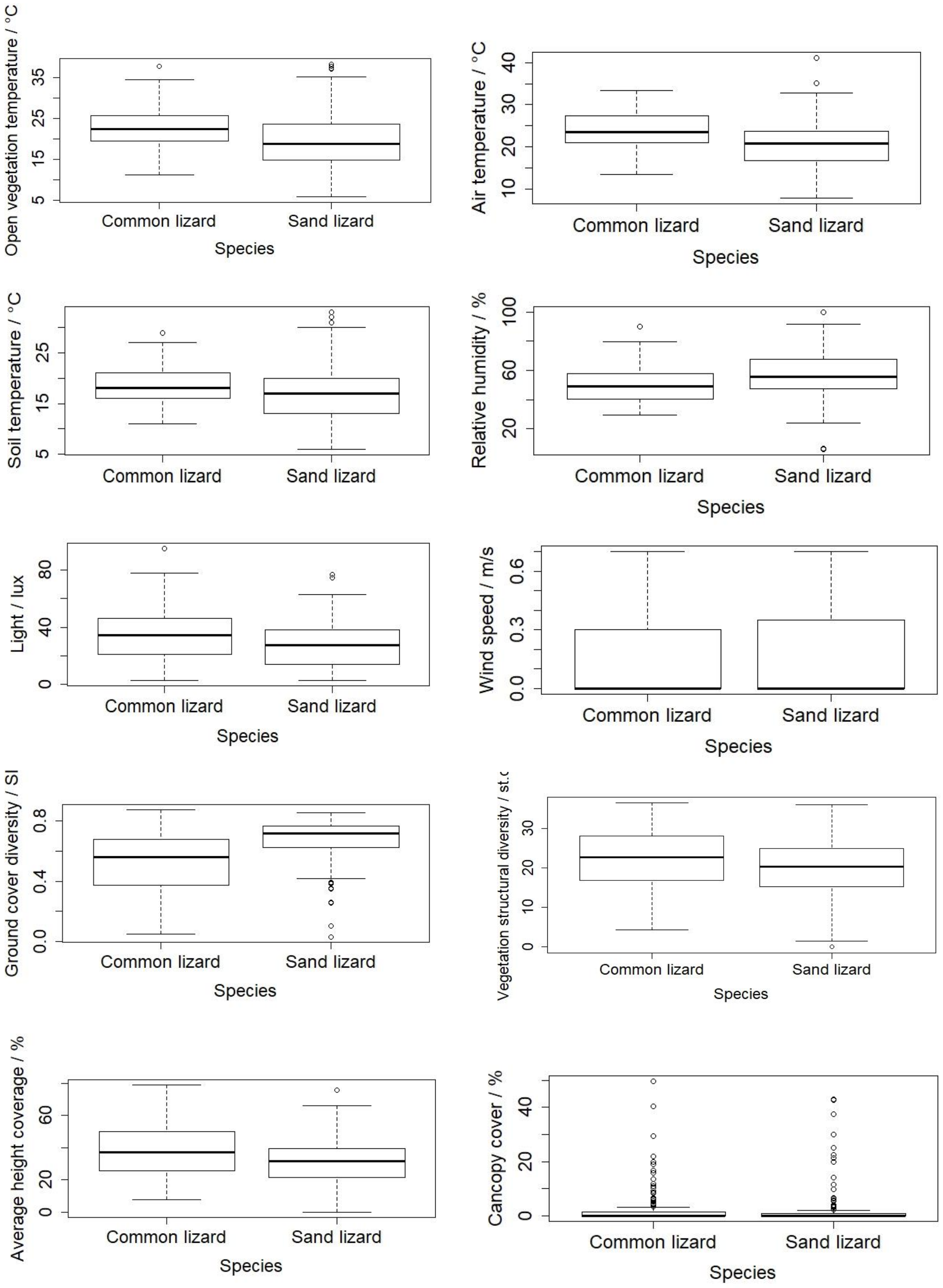


Figure 21 Microclimate and microhabitat conditions associated with common lizard and sand lizard observations on transect walks in dry heath. Plots highlight the median, interquartile range, extreme values and outliers.

3.5 Discussion

3.5.1 The effect of survey methodology

Sand lizards were observed on transect walks almost twice as frequently as using materials in refugium arrays collectively. This should be considered in the light of the majority of sand lizard observations occurring around the reintroduction release area, a mown strip in the heather offering ideal edge habitat for basking and sheltering. Where refugia act as *foci* themselves in wider mature heath, the release area was also effectively a large *focus* (Edgar *et al.*, 2010). Where this perhaps reduced refugium use here, a few sand lizards were still observed using materials on the strip itself. Many of the observations of sand lizards beyond the immediate release area and particularly at the extremities of the survey grid (discussed in Chapters 5 and 6) were utilising refugia. Had these refugia not been present, whether these wider-ranging lizards would have been detected is questionable. It can therefore be inferred that refugia do aid surveys for sand lizards, although their use may not be as great as by some reptile species.

The highest count for sand lizards at arrays were not always associated with the highest counts on transects, highlighting the benefit of surveying for this species using refugia as well as walking transects. These data indicate days where refugia may offer a greater comparable benefit (a higher temperature gradient) for thermoregulation, such as in cooler conditions (Blanke and Fearnley, 2015).

3.5.2 Microclimate and microhabitat conditions associated with sand lizard observations

Vegetation heterogeneity was a factor in the final sand lizard microhabitat model, with high heterogeneity being consistent with use of felt, the second most utilised refugium type. The interaction between felt and more structurally diverse or covered microhabitat indicates material selection may be beyond thermoregulatory benefits. The use of felt, which is comparably lightweight in relation to the other materials and may be viewed by reptiles as less protective, is particularly associated with increased natural cover opportunities. The importance of structurally diverse vegetation is highlighted for UK legged lizard species overall, providing optimal basking and sheltering options; this is also discussed in the nature of focusing walking survey effort on edge habitat (Spellerberg and House, 1980; House and Spellerberg, 1983; Dent, 1986; Moulton and Corbett, 1999; Edgar and Bird, 2006; Blanke and Fearnley, 2015).

Sand lizard use of refugia was predicted by overall higher humidity (although significantly lower when in the open) and lower light (lux) at the microhabitat level. The latter was shown to be positively correlated with solar radiation (see B.1), which agrees with previous studies that this has a key effect on sand lizard detection probability, particularly for male sand lizards (Fearnley, 2009). Here, in conditions of lower light, refugia may offer the maximum thermoregulation potential over surrounding natural habitat. Humidity is also known to influence lizard behaviour (Nicholson *et al.*, 2005; Nemes *et al.*, 2006), and is inversely correlated with temperature (as seen in Chapter 2); both of these variables define the annual cycle of reptile species and highlight the reason they are so at risk with climatic changes (Kumar *et al.*, 2014).

3.5.3 Environmental variables affecting sand lizard and common lizard observations on transect walks

Considering observations of sand lizards and common lizards on walking transects, vertical cover and structural heterogeneity were significantly lower for sand lizards than common lizards. Both species favour microhabitats with high structural heterogeneity (House and Spellerberg, 1983; Moulton and Corbett, 1999; Inns, 2009; Edgar *et al.*, 2010; Blanke and Fearnley, 2015). However, sand lizards sightings are not found to differ between lower, more uniform, ungrazed and grazed dry heath habitat in the UK, whereas common lizards were observed significantly more frequently in ungrazed areas (Reading and Jofré, 2016), indicating the comparable reliance on this height and diversity may be greater for common lizard. Less diverse and short vegetation leaves sand lizards more exposed to solar radiation, which is essential for basking, however also more exposed to the elements in colder conditions. More homogeneous vegetation structure and at lower height also leaves the sand lizard more exposed to threats of predation as escape mechanisms through structurally diverse habitat would be compromised. In the context of this post-release scenario, low less diverse vegetation is likely to reflect the majority of sand lizard observations away from refugia being on and around the mown strip release area, with a very high number of incidences pre-winter. Here, vegetation transitioned from very low to mature heather which would have resulted in low values for both variables; this is also likely to have impacted the ground cover diversity observed as being higher for sand lizards. It is difficult to determine in this instance what is cause and what is effect; whether sand lizards are remaining in the release vicinity due to its microhabitat, or for site fidelity reasons in spite of the microhabitat. The study by Reading and Jofré (2016) indicates the former may be the case. Undertaking this research at a site with wild sand lizards and common lizards would confirm or counter this.

The significant differences between microclimatic conditions for common lizards and sand lizards on transect walks show sand lizards were found in colder, more humid and lower lighting conditions than common lizards. This was driven by the continued high observations of sand lizards into late October and November post-release, outside of typical seasonal activity patterns (Inns, 2009). Even when removing November, all differences apart from soil temperature were still significant. This indicates that sand lizard activity was abnormal even within the typical active cycle. This is most obviously attributable to the fact these lizards had just been reintroduced to the wild. The intention of release timing is to enable body mass gain in captivity prior to release and then encourage lizards to overwinter in the immediate vicinity of the release site, where habitat is optimal and where contingency burrows have been created (Moulton, N., pers comm.). The tendency of lizards at release, however, is to disperse. Hatchlings, which are predominantly the age group released under current protocols, will likely still be showing some postnatal dispersal tendencies, that may be influenced further by social factors (Aragon *et al.*, 2006; Cote and Clobert, 2007). Subadults (as included in releases in this research) will be influenced by other social cues, such as developing dominance hierarchies and territoriality (Govier, 2017; Michelangeli *et al.*, 2017). Individuals are also likely to display particular dispersal syndromes (Sih *et al.*, 2012; Legrand *et al.*, 2016). Ultimate post-release habitat selection is likely to differ from natal habitat selection, for example social effects may differ depending on age released, as observed in two releases of hihi *Notiomystis cincta* (Richardson and Ewen, 2016).

Timing of reintroduction has been shown as crucial for a variety of species. Survival of brown hares *Lepus europaeus* following release is found to be highly season-dependent, with releases in summer months significantly increasing survival duration (Cukor *et al.*, 2018). Particularly high and fast mortality is observed, with 82 % of individuals dying within 6 months and 41 % within 10 days of release. Most individuals that die are predated by red foxes, however during this season resources including food and crucially, shelter, are most abundant. Guidance for water vole *Arvicola amphibius* reintroduction in the UK has suggested individuals should not be released in autumn due to high risk of overwintering mortality. However, a recent study finds that overwinter mortality is no greater for released voles than resident voles, suggesting that autumn translocations are a viable option alongside spring and given the animal health and welfare and financial costs of housing them overwinter in captivity (Baker *et al.*, 2018). These studies show the diverse effects of season on survival and how important it is to quantify such inferences in a real scenario, even if assumptions can be made considering species biology.

It is suggested that many sand lizards may be neither dispersing, nor properly establishing themselves near the release site in good time for overwintering. By remaining active into the start of winter, lizards are likely to lose condition as prey numbers decline and they face colder temperatures, and then become less likely to survive brumation. Any sand lizards observed active late in the year or very early in the spring in the captive population at Marwell Zoo had poor body condition and often did not survive (R. Gardner, personal observation). This same pattern is observed in other sand lizard studies, with both adults and juveniles showing poor survivorship in such instances (Blanke, 2006; Blanke and Fearnley, 2015). The release methods employed to try to optimise survival may in fact be having the opposite effect.

3.5.4 Conservation practice and monitoring implications

The results from this study highlight the importance of transect walks when surveying for sand lizards. However, refugia are also an important monitoring tool, utilised at times when open basking in vegetation may be less preferable. Refugia as focus points of a survey enable easier detectability as well as optimising repeat routes walked over time; this is particularly useful for less experienced surveyors. The study shows key refugium types and environmental conditions that are preferable and can be accounted for in survey design and refugium placement to optimise refugium use and thus detectability. Regardless of refugium type being used, the results indicate the most optimal microhabitat conditions to place them in and the best weather and microclimate conditions to survey during, as well as how this may differ between materials.

Through comparisons with common lizards, it is suggested that the timing of sand lizard release in early-mid September may not allow the lizards enough time to settle into their new habitat and locate suitable overwintering locations. Timing of release could therefore benefit from reconsideration and more research.

3.5.5 Further study

Collecting data on a wild, or established reintroduced, population of sand lizards would allow assessment of the assumptions around seasonally late observations being a result of mid-autumn release. It would also remove the effect of the release area on microhabitat use observations.

Continued monitoring is essential in order to ensure appropriate site management in accordance with the distribution of sand lizards as they become established (Moulton and Corbett, 1999; Edgar *et al.*, 2010).

3.6 Conclusions

This chapter applied the approach of Chapter 2, within a captive experimental setup, to a wild scenario following reintroduction to Eelmoor Marsh SSSI. It intended to further determine factors contributing to the detection of sand lizards in the wild. The monitoring protocols employed in turn allowed the reintroduced sand lizard cohorts to be studied in detail within their receptor site, as is the subject of Chapters 5 and 6.

The key conclusions of this chapter were as follows:

- Sand lizards are more likely to be detected on walking transects during a survey combining this method with a variety of refugium materials.

- When utilising refugia, sand lizards were found to use slate and felt above tin and tile in dry heath. Increased vegetation structural heterogeneity was an important factor in their selection of felt refugia. Given the more limited use of traditional refugium-based reptile survey methods by legged lizards, this is an important finding to help inform refugium deployment protocols, especially for this threatened native species.

- Sand lizards were observed in significantly less favourable microclimatic conditions than common lizards. This was partially indicative of their being active later in the season, but also held true through observations excluding November. It could be the result of release in mid-autumn, with lizards trying to maximise resources available in their novel environment before brumation. However, such behaviour will likely have a negative affect and may impact survivorship of those individuals entering brumation late.

- By reconsidering and optimising monitoring methods, reintroduced sand lizards can be monitored more quantitatively and effectively. This allows evidence-led habitat management and future reintroduction and conservation decisions to be made.

Chapter 4 Monitoring techniques and environmental factors associated with detectability of widespread native UK reptile species in multiple habitats

4.1 Introduction

4.1.1 Conservation translocations and effects on the wider ecosystem

In a conservation translocation, there will be effects on both the species being released and the species assemblage already present (Ciechanowski *et al.*, 2011; Burke *et al.*, 2020; Robinson *et al.*, 2020). Prolonged absence of a species followed by reintroduction may have predictable or unexpected consequences on the species at a release site (IUCN, 2013) as well as on wider ecological processes (Genes *et al.*, 2018). The IUCN Guidelines for reintroductions highlight the importance of ecological monitoring pre- and post-release in conservation translocations. Pre-release, baseline data on species “most likely to be affected by the translocation” should be obtained, and this should be continued post-release as it is “most unlikely that any translocated organism can attain its intended demographic targets without evident ecological impacts” (IUCN, 2013). Despite this, community-level or ecosystem-level studies are rare in relation to the number of reintroductions undertaken (Taylor *et al.*, 2017). At ecosystem level, a review of reintroduction of keystone species, that were specifically reintroduced to restore the key ecological processes missing in their natural range, found that only 11 of 30 projects actually assessed these ecosystem-level effects following release (Hale and Koprowski, 2018). This broader ecological approach has predominantly been examined with reference to non-native, invasive species introductions (Cerasoli *et al.*, 2019; Hill *et al.*, 2019).

Dry heath habitat favoured by the sand lizard *Lacerta agilis* is also ideal habitat for the five other native reptile species in the UK (Edgar *et al.*, 2010). Understanding the effect of translocations of this species on the wider reptile community is important. Previous studies have specifically compared the sand lizard and common lizard *Zootoca vivipara* and their ecological niche overlap (Nicholson, 1980; Dent, 1986). Adding one species to an area of known occupation by the other may affect the population already present if resources are competed for or they both act as prey for the same predatory species. However, it is not just common lizard populations that may show a response to the reintroduction of sand lizards. Adders *Vipera berus* and grass snakes *Natrix helvetica* are known predators of the sand lizard (Edgar and Bird, 2006) and this addition of prey to their established home and seasonal ranges could impact their activity and movement patterns. It is therefore vital that not only the sand lizard population being reintroduced is sufficiently monitored, but also the wider reptile assemblage, and particularly in the long-term as impacts may not be evident for years (IUCN, 2013). In order to undertake such monitoring appropriately, techniques must acknowledge the differing behavioural ecology and microhabitat selection of the species assemblage.

4.1.2 Habitat selection and monitoring protocols for widespread reptiles in the UK

Understanding the biological and ecological drivers of habitat use by species is vital in the light of current biodiversity loss (Dirzo *et al.*, 2014; Ceballos *et al.*, 2017), where habitat destruction, fragmentation and degradation have occurred at unprecedented levels and climate change is further altering species distributions (Gonzalez *et al.*, 2010; Horvath *et al.*, 2019; Powers and Jetz, 2019). Cryptic species are particularly at threat, where they have not received due research focus (Clark and May, 2002; Fazey, Fischer and Lindenmayer, 2005), and in this category there is high concern for herpetofauna due to their often low mobility and biological cycles being so closely linked to temperature and moisture (Beebee, 2013; Kumar *et al.*, 2014; Nowakowski *et al.*, 2016; Préau *et al.*, 2019). Better understanding the habitat drivers, and particularly microhabitat drivers, of spatiotemporal behaviour is vital. For the sand lizard in the UK, reintroduction has offered a lifeline to return populations to former areas they would have occupied, once these have been restored and adequately protected (Corbett and Moulton, 1998; Moulton and Corbett, 1999; Woodfine *et al.*, 2017). Chapter 3 focused on furthering understanding of how this cryptic species utilises microhabitat in the dry heath habitat in which it is restricted, along with coastal dunes. A comparable study of sympatric native reptile species in dry heath would greatly aid conservation and monitoring of the wider reptile assemblage. Furthermore, evidence-based recommendations across multiple habitat types for these species would enable application of monitoring techniques in the light of ecological spatiotemporal drivers to be optimised as far as possible.

The less specialist, more common UK reptile species occupy a variety of habitats. Adders are found mostly in woodland, heathland, moorland and in coastal areas; they favour dry, sunny areas with close dense ground cover and will utilise wetter areas such as mires in the summer provided dry tussocks exist for basking (Edgar *et al.*, 2010; Gardner *et al.*, 2019). Grass snakes have a strong preference for habitat interfaces offering both basking site availability and nearby dense vegetation providing cover, however where they are more mobile than other native reptiles, they do not rely on one site for their entire annual cycle and thus cover and structural diversity of vegetation in one locality is less essential (Reading and Jofré, 2009; Edgar *et al.*, 2010). They avoid woodland and grazed areas (Reading and Jofré, 2009), favouring wetlands, with which they are most associated (Edgar *et al.*, 2010) and have been observed to use wet heath more than dry heath (Reading and

Jofré, 2016). Slow worms *Anguis fragilis* are mostly fossorial and occupy a wider variety of habitats than sand lizards or common lizards; their operative body temperature covers a wide range (Smith, 1990). They require dense vegetation, particularly grasses, with sunny areas and preferably a loose substrate to burrow, although this is not essential (Edgar *et al.*, 2010). Common lizards do not have the same habitat requirement of sandy soil as sand lizards, given that they are viviparous, and thus occupy a range of habitats as well as dry heath. Common lizards occupy sites with greater structural diversity of vegetation than slow worms (also wide-ranging); they are also present in humid and dry microhabitats (Edgar *et al.*, 2010). (See also 1.3.6.)

The fossorial behaviour of slow worms means they are common users of reptile refugia and rarely seen in the open. Snake species are commonly found using both refugia and natural habitat for thermoregulation. Legged lizards are poorer users of refugia and found more commonly on walking transects (Reading, 1997; Moulton and Corbett, 1999; NARRS, 2011).

Most reptile survey guidance in the UK is in the form of reports and advice sheets produced over the last several decades (Inns, 1996; Froglife, 1999; NARRS, 2011; Langham, 2012; Sewell *et al.*, 2013). These consist of refugium and walking transect surveys as discussed in Chapter 1 (1.3.7), as well as first-hand advice and expertise from organisations such as ARC Trust and ARG-UK. Further recommendations can be gleaned from broader reptile literature extending beyond UK species, regarding survey design and additional considerations, such as microhabitat variables (McDiarmid *et al.*, 2012; Dodd, 2016). Suggestions have been put forward with evidence both quantitative and qualitative to improve techniques for specific habitat types (Reading, 1997; Cathrine, 2018). There has been assessment and recommendations for survey effort required to detect species declines of differing rates for common reptile species using current refugium (tin or felt) and transect survey methodologies, and the mixture of volunteer and expert surveyors typically present in herpetological surveys (Sewell *et al.*, 2012). The need to grow this vital body of protocols and guidance, to further inform methodologies and optimise survey effort, is acknowledged within the herpetofauna conservation and research communities (NARRS, 2011; Sewell *et al.*, 2013; ARG UK, pers.comm.).

Due to the cryptic nature of reptiles, monitoring is heavily reliant on the physical presence of people in the field; remote sensing is a challenge for small ectotherms. Databases for native UK reptiles, and amphibians, have been populated predominantly by the volunteer workforces, headed by charitable organisations such as ARC Trust and ARG UK, as well as independent ecological surveyors. Such data have allowed, for example, concerning trends in adder populations in the UK to be observed (Gardner *et al.*, 2019). Despite concerns around some aspects of citizen science, this network offers a resource with enormous capacity for biological and ecological monitoring that could never be replicated by scientists and conservation biologists alone (Silvertown, 2009; Dickinson *et al.*, 2010; Frigerio *et al.*, 2018). In order to maximise potential of such human resource networks, appropriate training and guidance is essential to ensure the recording of high quality data, that are ideally spatiotemporally comparable (McKinley *et al.*, 2017; Stockwell and Gallo, 2017).

This research offers a contribution to optimising reptile survey protocols. It addresses the need for scientific rigour, systematic, repeated methodology, and high survey effort across multiple seasons. It offers evidence-based recommendations for monitoring techniques that intend to optimise species detectability. With an increasingly holistic approach to site management and conservation at community, rather than species, level it is imperative that resources are maximised to better understand how a whole species assemblage is using a site, and feed this into planning accordingly. The approaches apply to many UK reptile monitoring scenarios, vital in in order to make evidence-based and quantitative spatiotemporal predictions of reptile presence and abundance, and to detect population changes in this taxonomic group that is so threatened (Dunford and Berry, 2013; Kumar *et al.*, 2014; Gardner *et al.*, 2019).

4.1.3 History of reptile monitoring at Eelmoor Marsh

Long term monitoring of the reptile assemblage inhabiting Eelmoor Marsh has been investigated using a series of tin refugia across the site since 2004, when 68 refugia were initially deployed to assess the reptile species present and indicate their distribution (Hutchins, 2004). *Ad hoc* refugium and walking surveys have since been undertaken in 2008, 2010 and 2015 (Langham, 2015), as well as three Masters projects exploring the impact of grazing on the reptile assemblage (Marum, 2010; Rose, 2010; Broom, 2018).

4.1.4 Aims

The overarching aim of this chapter was: **to assess survey methodologies and determine environmental factors affecting widespread reptile species detectability in multiple habitat types, with a focus on dry heath where they are sympatric with the sand lizard.** Its objectives were:

1. To determine relative reptile species abundance across habitat types.
2. To assess differences in surveying methodologies (tin refugia; mixed-material arrays; and walking transects) on species observations.
3. To determine preferred refuge materials by each species and microclimate and microhabitat factors associated with material use.

The study's approach was two-fold. It initially explored the variety of habitats across Eelmoor Marsh SSSI. It then focused on the dry heath habitat in which the sand lizard reintroduction took place, with increased survey effort.

4.2 Methods

4.2.1 Surveying methods

In the initial phase of this research across Eelmoor Marsh's wider habitat, maps of the site were assessed to determine the location of reptile tins already present, as per surveys until 2015. (Some tins had been removed, replaced or re-located over the years.) Forty-eight tins were located and logged using a GPS.

A map of the site showing the general vegetation communities present was then used to determine the location of an equal number of additional four-material refugium arrays (Figure 22) (Sanderson, 2003). The general vegetation communities were combined into five broad habitat types: dry heath (24.8 Ha), wet heath (5.6 Ha), grassland (7.0 Ha), mire (11.5 Ha) and woodland (16.7 Ha). The same number of new refugium arrays as tins were deployed and positioned across the site in a systematic manner as follows. After georeferencing the habitat map in ArcGIS (version 10.2.2), a grid was overlain splitting the site into 1.4 Ha units in order to allocate the 48 additional refugium arrays proportional to the area of each habitat type present. This ensured comparable density of arrays between habitat types. The allocated number of arrays per habitat type *x* were then deployed to the *x* 1.4 Ha units showing the most coverage of that habitat type. Within each unit, placement of the array was then randomly selected by creating a further grid, each subdivision measuring 2 x 2 m (the coverage area of an array to the nearest metre) and a random number generator provided the coordinates of the 2 x 2 grid cell in which the array should be placed. (This process was independent of location of historical tins as no direct pairing of tins and arrays was being made.) The locations were uploaded to a GPS unit for deployment of refugia in the field.

Arrays consisted of four material types, as in Chapter 3: Wildcare® thin sheet metal Reptile Profile Tin (< 1 mm, 3 cm high corrugations); Cembrit Ltd. natural Spanish roofing slate (4-6 mm thick); Marley® dark red plain clay roofing tile (25 mm thick); and IKO® bitumen underlay roofing felt (1 mm thick). Area coverage by each refugium material was double that of Chapter 3; this was still not as large as traditional survey refugia, however the concerns with using larger refugia in such a dense manner, as highlighted in Chapter 3, were not relevant here. Tins and felts thus measured 500 x 500 mm, where two 250 x 500 mm slates and four 250 mm x 250 mm tiles were used to cover the same ground area, leaving no gaps. (As in Chapter 3, it was deemed important to provide comparable ground cover across refugium types rather than add an additional variable of area of coverage.) The order of placement of each material type in each array was random and a 500 mm gap was left between each material when initially distributed; subsequently there was some small natural movement of the materials as they settled within the vegetation. All arrays were aligned on north-south, east-west compass bearings for consistency (Figure 23). Materials were deployed at least two weeks in advance of surveys commencing and were not shifted for the duration of the study. Any damaged materials were replaced as soon as possible (damage through vegetation growth, livestock, etc.; no basking or refuging animals were ever found injured or killed as a result of this).

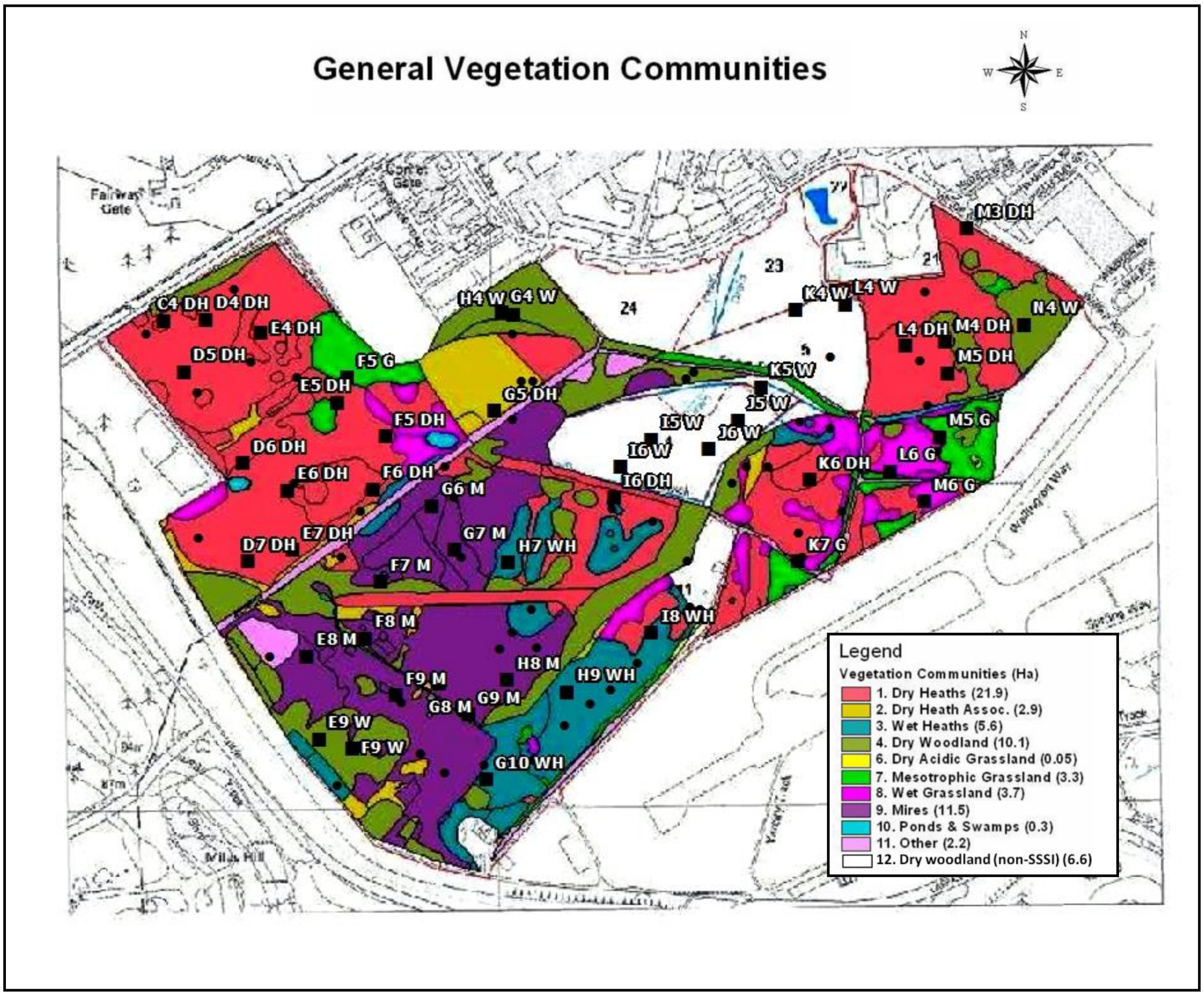


Figure 22 Eelmoor Marsh general vegetation communities, amended from Sanderson (2003) and Langham (2015). Black squares represent additional refugium array deployment (letter(s) following grid reference (e.g. F9 M) represent broader habitat type. DH = dry heathland (consisting defined communities 1 and 2), G = grassland (communities 6, 7 and 8), M = mire (community 9), W = woodland (communities 4 and 12), WH = wet heathland (community 3). (Communities 10 and 11 were not included in this broader categorisation.) Black circles represent longstanding historic tins.

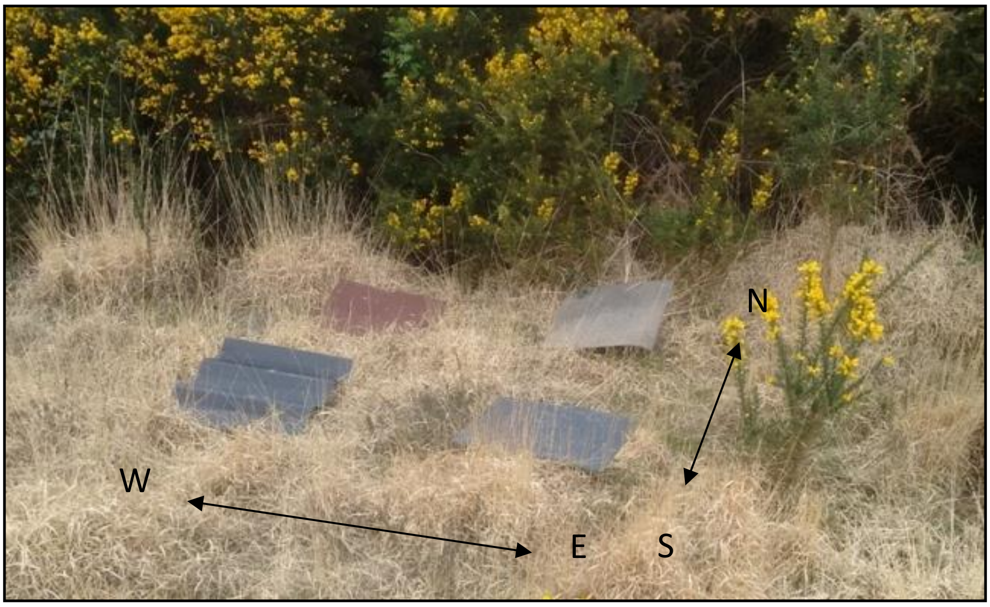


Figure 23 A refugium array *in situ* showing clockwise from top left: tile, felt, slate and tin, oriented along north-south and east-west bearings

Refugium checks and walking transect surveys were conducted between May and November 2017 and March and November 2018. Twenty-one full surveys, each completed over three days, were undertaken across the wider site across these two seasons, with each habitat being surveyed no more frequently than once a week. During each survey, every refuge (longstanding tins and new arrays) located within each habitat type was visited in a systematic order, with visual – and audio – surveys being conducted on the walking route within that habitat between each refugium. C. Broom kindly contributed two surveys' datasets for species found utilising old tins for her MRes thesis (Broom, 2018); the corresponding array and walking transect data for these two surveys were collected as standard, ensuring the normal transect route was followed in full. Approximately one meter either side of the walked transects between refugia, equivalent to a 2 m belt transect, could practicably be scanned by eye in this manner at a consistently slow walking pace. Where scurries were heard (again these fell within an approximately 2 m belt transect), the observer waited for five minutes before continuing the survey in order to allow for re-emergence of the source of the scurry (often common lizards, occasionally small mammals, frogs or birds); crouching as required to minimise the visual disturbance caused by observer or their shadow. If the source of the sound did not emerge in this time or the scurry was not repeated (which would re-start the wait time to five minutes), the survey continued with this logged as 'unknown scurry'. Surveying all five habitats took the equivalent of three days in total, with surveying undertaken in daylight between the hours of 08:00 and 18:00. There were four time-blocks for surveying; early morning, late morning/noon, early afternoon and late afternoon/early evening. The grassland, wet heath, mire and woodland routes were split in half and the longer dry heath route in thirds, allowing each to be systematically rotated between these windows to ensure variation in the time each refuge was visited.

Presence of reptiles, be this on, under or around a refugium and along walking transects was noted (and GPS recorded if on a transect walk). Information on species, age group (juvenile, sub-adult, adult), sex (if distinguishable), and any notable features (such as scarring, unusual colouration or tail autotomy) was recorded. Animals were not handled and were photographed *in situ* where possible. Additional note was made of use of refugia by other animals, e.g. significant use by ants (categorised into 'half' or 'full' sub-refugium coverage) or the presence of amphibians or rodents. Reptile sloughs were also recorded and species noted if identifiable.

At every refugium, and when a reptile was observed on a transect walk, a number of microclimate variables were recorded. A Kestrel 3000 was used to take a measure of air temperature (°C), relative humidity (%) and average wind speed over 30 seconds at ground level (m /s). A light meter was also used to measure light at refugium level (Lux), and soil temperature at a depth of ~2-3 cm was obtained in the open (°C). Instruments were given 30 seconds to settle prior to readings being taken. An infrared thermometer was used to measure surface temperature of each refugium at its centre and open vegetation temperature next to the refugium/array (°C).

Weather data was collected for the site as discussed in 3.2.2. A series of small temperature probes (iButton® Thermochrons) were deployed at one array 'type site' within each habitat for the duration of the field season – one underneath each material type and one in the open. Those underneath materials were placed ~1-2 cm below the material when in situ and thus recorded air temperature underneath the refugia rather than the underside temperature of the material itself. These were set to log temperature (°C) every ten minutes and logged data were downloaded to software Thermodata® Viewer 3.2.8 during of each survey.

Microhabitat variables were recorded as per 3.2.2 at each multi-refugium array, once during the spring and once during the summer of 2018 to cover both extremes of vegetation growth during months of reptile activity.

4.3 Analysis

All statistical analyses were undertaken in RStudio 1.1.463 (R version 3.5.3). All analyses assume significance at $P \leq 0.05$. Power analyses, as discussed in Results, were run in R package 'pwr'. (Spatial analyses in Appendix C.10 was performed in ArcGIS ArcMap 10.6.1.)

Relative abundance of species by habitat type and surveying method (new arrays, historic tins, walking transects on wider site; arrays and walking transects on dry heath) were calculated:

$$\text{Relative abundance} = \frac{\text{number of individuals of species } X}{\text{total number of individuals of all species}}$$

Encounter rate on walking transects was calculated for each species in each habitat type, by dividing the number of sightings by the transect length (m). A negative binomial GLM took each observation as independent to perform a basic analysis of the effects of, and interactions between, variables of species and survey method (historic tins, new arrays, transect walks) on observations.

A Poisson and negative binomial GLMM were used to assess the effects on common lizard and slow worm observations respectively, of fixed effects of habitat type and refugium type in new arrays including interactions; array location was a random effect. The minimum adequate model was selected using AIC values and Likelihood Ratio Tests. See Appendices C.3 and C.4.

Refugium selection and associated environmental (microclimate and microhabitat) variables across habitat types at Eelmoor Marsh were assessed using a series of generalised linear mixed models (GLMM); observation totals meant this was only possible for common lizard and slow worm. A Poisson GLMM using count data was selected for common lizard data from three possible mixed models as being best fit using AIC scores (also tested were a binomial GLMM treating the response as binary, and a zero-inflated Poisson (ZIP) mixed model in package ‘glmmTMB’); data included many zeros, but were not over-dispersed. For slow worm, where count data were both zero-inflated and over-dispersed, a negative binomial GLMM was determined as the best model fit (with a zero-inflated negative binomial (ZIMB) in package ‘glmmTMB’ also being tested) using AIC scores and Likelihood Ratio. ‘Array location’ was set as a random effect (n = 48), and categorical independent variables of refuge material type and the microclimate / microhabitat factors discussed were fixed effects; an interaction term between material type and each environmental variable was included. The models were reduced from maximal, to minimum adequate by removing successive non-significant terms and comparing AIC values.

Following these analyses, any differences in average microhabitat and microclimate conditions associated with use of an array material were examined. Each observation of presence was treated as an independent data point (with equal opportunity to select felt, slate, tile or tin) and analysed with either one-way ANOVA or non-parametric Kruskal-Wallis, depending on whether each data set met parametric assumptions or not. When the overall test result was significant, post-hoc testing was undertaken using Tukey’s test, or the non-parametric Dunn test accordingly.

For the more focused dry heath surveys, the same approach to assessing refugium material preference in relation to microclimate and microhabitat was undertaken for all widespread species with negative binomial or Poisson GLMM models being fitted for zero-inflated and over-dispersed count data for adder, common lizard, grass snake and slow worm. Microclimate analyses covered only the central 60 x 60 m grid where this was measured (as discussed); data were re-scaled prior to model fit as required. An additional material category of 'Open' was included given the large number of observations of species using natural habitat at an array. ANOVA or Kruskal-Wallis tests were again run for each variable following this broader modelling. Results regarding environmental variables presented in the main chapter summarise key findings. Full model outputs for each species can be found in Appendices C.5, C.6, C.11, C.12, C.13 and C.14.

Regression analyses for key weather variables recorded on site, and corresponding microclimate readings obtained during surveys in across the SSSI can be found in Appendix C.7.. Appendix C.8. shows example data readings for the thermal dataloggers. Seasonal variation in observations of species was summarised using box and whisker plots in Appendix C.9. A visual summary of distribution and abundance of each species in dry heath habitat were modelled using ArcGIS ArcMap 10.6.1.

4.4 Results

4.4.1 Multiple habitat types

4.4.1.1 Relative abundance and differing survey methodologies

Survey time for the wider site surveys totalled 423 hours and 55 minutes including data collection of all microclimate variables. A total of 611 successful observations were made for all reptile species across all habitat types including all survey methods; this included 21 adders, 110 common lizards, nine grass snakes and 471 slow worms (see Appendix C.1).

The majority of observations at old tins and new arrays for all habitat types were slow worms. Common lizards constituted a greater proportion of observations at arrays than historic tins and made up the majority proportion of observations during transect walks. The highest relative abundance values for grass snake and adder were utilising historic tins (Figure 24). Slow worms were found significantly less on transect walks; common lizards were found significantly less on old tins. There were significantly more observations of slow worm and common lizard overall (Table 12).

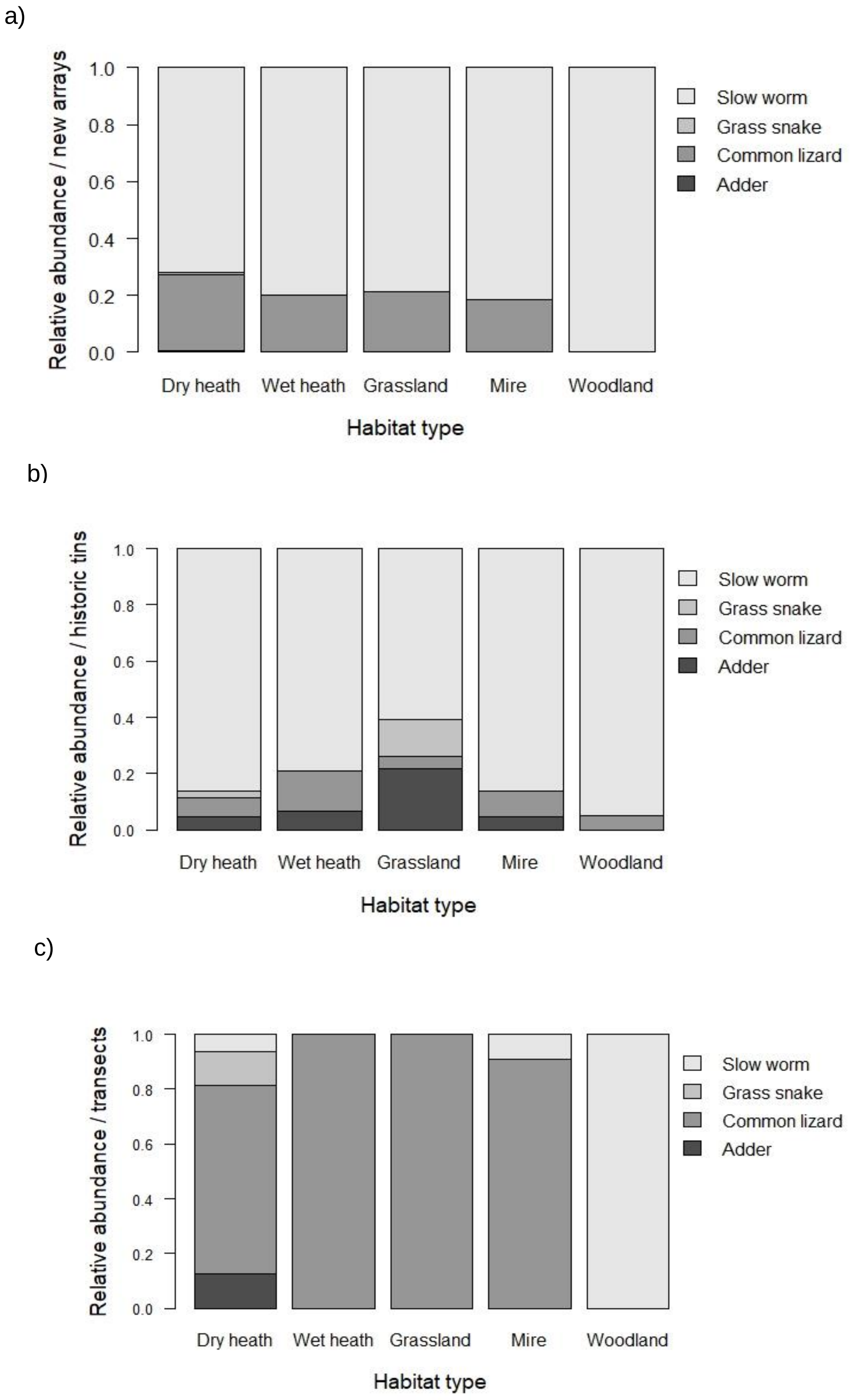


Figure 24 Relative abundance of four common reptile species across five broad habitat types at Eelmoor Marsh, using data from a) new arrays; b) historical tins; and c) walking transects.

Table 12 Species observations and survey method, fitted by a negative binomial GLM, with ‘Adder’ as reference level for species, and ‘New arrays’ as reference level for survey method.

	Estimate	**Std. Err.**	**z value**	***P***
Intercept	-3.045	1.020	-2.986	**0.003**
Slow worm : Transects	-4.942	1.413	-3.498	**<0.001**
Common lizard : Historic tins	-2.594	1.175	-2.208	**0.027**
Slow worm	5.347	1.041	5.136	**<0.001**
Common lizard	4.060	1.047	3.878	**<0.001**
Grass snake : Historic tins	1.099	1.543	0.712	0.476
Slow worm : Historic tins	-1.586	1.155	-1.373	0.170
Common lizard : Transects	-1.981	1.318	-1.503	0.133
Grass snake : Transects	0.406	1.730	0.234	0.815
Grass snake	0.000	1.442	0.000	1.000
Historic tins	1.792	1.116	1.605	0.108
Transects	0.693	1.257	0.552	0.581

4.4.1.2 Refugium selection and associated environmental conditions across multiple habitats

Sample sizes of grass snake and adder across wider site surveys were not large enough to take these species forward for subsequent, species-specific analyses.

Common lizards and slow worms showed different preferences for refugium types (Figure 25, Appendix C.2). Common lizards were found using felt the most, followed by tin, tile and slate. Slow worms utilised tin and slate an almost comparable number of times (69 and 64 respectively), then tile and felt the least.

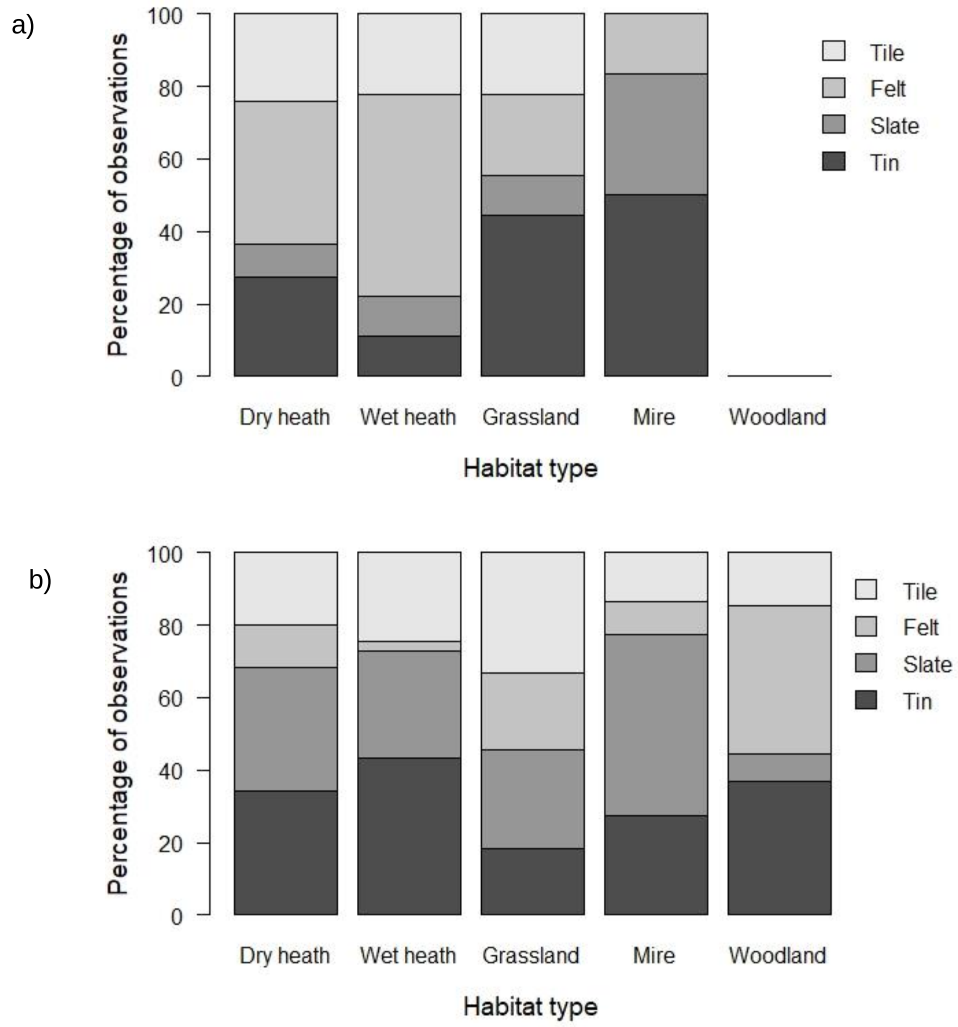


Figure 25 Percentage of observations of use of each refugium type by a) common lizards and b) slow worms within each habitat type.

There were no significant interactions between habitat type and material selection for common lizard observations. Slate was used significantly infrequently considering all habitat types (*p* = 0.048) (see Appendix C.3). Interactions between habitat type and material selection had to be retained in the model for slow worm, although none were significant. Felt was used significantly infrequently overall (*p* = 0.005) (see Appendix C.4).

Common lizards utilised felt the most and slate the least. There was no significant effect of, or difference between, the type of refugium material selected by common lizards and any microclimate variables measured (Table 13, Appendix C.5). There was a significant positive relationship between increased structural diversity of vegetation and use of felt and slate, compared to tin and tile. There was a significant negative relationship between increased canopy cover and greater vertical vegetation cover and use of refugia collectively. Vertical vegetation cover (%) was significantly greater in uses of felt compared to tin, and also greater in uses of slate than tin (*p* = 0.014 and *p* = 0.004 respectively). There was also a difference between vegetation structural diversity and material selection; vegetation had a greater structural diversity in uses of felt than tin and in uses of slate than tin (p = 0.002 and p = 0.006 respectively).

Slow worms utilised tin the most and felt the least. Felt was used significantly less than other materials by slow worm in conditions of lower light and humidity (Table 13, Appendix C.6). Increased soil temperature and humidity had an overall positive effect on use of refugia. Felt was utilised less than any other material types by slow worms. There was a difference in light (lux) between material type selected. Light varied significantly between slate and felt, and tile and felt (*p* = 0.007 and *p* = 0.004 respectively); felt required lower light levels to be utilised, compared to thicker, heavier materials of slate and tile. Increased structural diversity had a positive impact on refugium use in general; although, felt was utilised in conditions of lower structural diversity than slate, tile and tin (*p* < 0.0001, p = 0.032 and *p* <0.001 respectively). Increased canopy cover had a positive effect on use of felt and negative on use of slate; felt was utilised in conditions of greater canopy cover than slate, tile or tin (*p* = <0.0001, *p* = 0.002, and *p* = 0.015 respectively). Felt, slate and tile were all used more than tin in instances of greater ground cover diversity.

Table 13 (overleaf) Environmental factors associated with use of refugia by common lizards and slow worms across multiple habitat types. Key microclimate and microhabitat considerations highlight those factors of significance in GLMM models for refugium use generally, and for specific material type use for each species. Mean conditions are single-boxed to match key factors for consideration and double-boxed where significance existed for both refugium use generally and that material type.

MICROCLIMATE

		Ranked refugia	Key microclimate considerations in relation to refugium type			Material temp. (°C)	Air temp. (°C)	Soil temp. (°C)	Rel. humidity (%)	Light (Lux)	Wind speed (m/s)
						Mean microclimate conditions in relation to detectability for use of each refugium type					
Common lizard *Zootoca vivipara*	1	Felt				29.62	21.31	15.19	63.73	30.43	0.25
	2	Tin				29.96	23.85	18.00	63.01	31.35	0.15
	3	Tile				23.28	21.84	16.33	64.22	23.06	0.34
	4	Slate				24.24	21.49	15.29	66.93	15.53	0.10
Slow worm *Anguis fragilis*	1	Tin	↑ soil temp.	↑humidity		23.81	21.6	16.09	68.47	19.59	0.12
	2	Slate				21.68	21.3	16.36	67.18	22.98	0.14
	3	Tile				24.72	22.28	17.11	64.55	24.52	0.16
	4	Felt			↑light ↑ humidity	23.67	22.28	16.63	62.62	16.93	0.14

MICROHABITAT

		Ranked refugia	Key microhabitat considerations in relation to refugium type			Veg. ground cover div. (SI)	Veg. structural div. (st. dev)	Vertical veg. cover (%)	Canopy cover (%)
						Mean microhabitat conditions in relation to detectability for use of each refugium type			
Common lizard *Zootoca vivipara*	1	Felt	↓ canopy cover	↓ vertical veg. cover	↑veg. structural div.	0.35	23.82	33.32	2.77
	2	Tin			↑ veg. structural div.	0.38	17.11	21.44	2.63
	3	Tile				0.32	20.51	34.47	3.15
	4	Slate				0.24	25.22	44.72	6.80
Slow worm *Anguis fragilis*	1	Tin	↑ veg. structural div.			0.31	20.72	37.66	16.21
	2	Slate		↓ canopy cover	↑ veg. ground cover div.	0.41	21.97	42.36	7.74
	3	Tile			↑ veg. ground cover div.	0.41	19.68	44.18	11.4
	4	Felt		↑ canopy cover	↑ veg. ground cover div.	0.4	16.19	36.48	33.95

There are typically positive correlations between wider weather and microclimate variables on the ground, as already seen in Appendix B.1 for dry heath specifically (Appendix C.7). The strength of these relationships varies by habitat type, becoming less predictable in more covered habitats. This extends to sub-refugium temperatures (Appendix C.8). Consideration of these relationships allows field conditions to be predicted when weather conditions are known (with habitat-specific variation of accuracy); this can help inform monitoring.

4.4.2 Dry heath surveys

4.4.2.1 Relative abundance and differing survey methodologies

There were 2,638 live widespread reptile species observations during surveys following the sand lizard release in the dry heath habitat of Eelmoor Marsh (excluding indirect evidence such as sloughed skins) (Table 14). A total of 217 surveys took place between September 2017 and August 2019. An additional 93 signs (visual/audio) were suspected reptiles. (These figures include the original and subsequently expanded survey extents (Figure 17)).

Table 14 Total counts of reptile species during surveys in dry heath habitat, Eelmoor Marsh.

	Adder	**Common lizard**	**Grass snake**	**Slow worm**	***Scurry (unknown)***	***Slither (unknown)***
Array	60	444	43	1864	*NA*	*NA*
Transect	38	160	16	13	*85*	*8*
Transect encounter rate	*0.141×10^{-3}*	*0.557×10^{-3}*	*0.056×10^{-3}*	*0.048×10^{-3}*		
TOTALS	**98**	**604**	**59**	**1877**	***93***	

As shown for the surveys across multiple habitat types, survey method resulted in different relative species abundances in dry heath habitat also (Figure 26). Slow worm constituted 77.3 % of observations at refugium arrays, whereas common lizards made up 70.5 % of observations on walking transects. Like common lizards, adder and grass snake were more commonly found on transects (16.7 % and 7.0 % of observations, compared to 2.5 % and 1.8 % at arrays respectively).

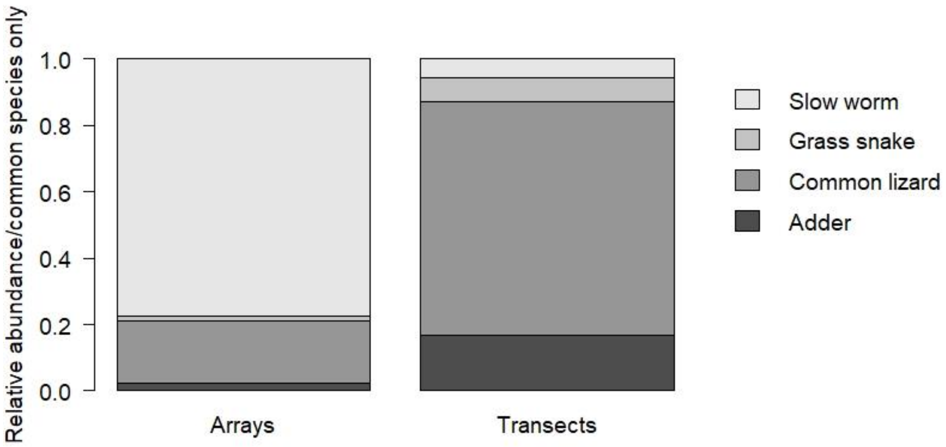


Figure 26 Relative abundance of common reptile species in dry heath habitat at Eelmoor Marsh.

Seasonal peaks and troughs in observations broadly corresponded between arrays and transect walks for each species (Appendix C.9). However, peaks for observations were not uniform between all species; common lizard counts were highest in the spring and early autumn whereas adder, grass snake and slow worm counts reached their highest levels over the summer months.

4.4.2.2 Observation location, refugium selection and associated environmental conditions

Observations of each widespread reptile species are detailed in Figure 27. The spatial distribution of observations can be seen in Appendix C.10. This highlights the disparity in surveying methodologies, both between arrays and walking transects, and also between material types. Adders and common lizards were found using felt most, then slate (with a higher number of common lizard observations on felt than on transect walks). Grass snakes used tin the most, followed by slate. Slow worm were found predominantly using slate, followed by tile.

Key microclimate and microhabitat factors associated with each species and use of materials (including mean values) are summarised in Table 15 and Table 16. For full analyses results, see Appendices C.11 (adder), C.12 (common lizard), C.13 (grass snake) and C.14 (slow worm).

For adder, there was a significant positive correlation between refugium use and higher air temperature; however, air temperature when tile was used was significantly lower. Temperature of open vegetation was significantly lower than felt and tin when selected (p = 0.005 and p = 0.009), and tile was also significantly lower than tin (p = 0.040). Air temperature was significantly higher when tin was selected compared to open vegetation and tile (*p* = 0.032 and *p* = 0.006 respectively). Tin was utilised in significantly higher soil temperatures than open vegetation (*p* = 0.0313). There were no specific microhabitat factors significantly affecting adder use of material type in dry heath.

Air temperature had an overall positive relationship with common lizard observations and soil temperature a negative one. Open vegetation had to be significantly warmer than refugia to be used by common lizards. Air temperature was significantly higher when common lizards were utilising open vegetation than any of felt, slate, tile or tin (*p* < 0.001, *p* < 0.001, *p* = 0.033 and *p* = 0.001 respectively). Soil temperatures were greater for open vegetation use than use of felt, slate or tin (*p* < 0.001, *p* < 0.001 and *p* = 0.040 respectively). Relative humidity was significantly higher in instances of use of felt over open vegetation (*p* = 0.001). Light (lux) also varied between material types, however post-hoc analyses showed no individual significant pairs. The greatest difference was seen between slate and open vegetation (p = 0.067), with open vegetation being used in instances of greater light. Structural diversity had a significantly positive relationship with refugium use overall. Within this, use of felt had a significantly negative relationship with structural diversity; felt use and vertical cover had a positive relationship. Canopy cover was significantly greater for open vegetation observations compared to refugium use. There was a significant relationship of vegetation structural diversity however no pairs proved significantly different; the greatest difference was seen between tin and felt (*p* = 0.065), with tin use in greater structural diversity than felt use. Canopy cover also differed significantly between material types overall. However, again no pair of materials had a significantly differing level of canopy cover; felt and open vegetation differed the most (*p* = 0.053), with open vegetation being used in instances of greater canopy cover than felt.

No microclimate factors significantly affected use of arrays by grass snakes. Greater structural diversity was particularly important for the use of slate and tile. Ground cover diversity was significantly lower when slate was used compared to tin or open vegetation (*p* = 0.002 and *p* = 0.026 respectively). There was significantly less structural diversity in instances of tin use over slate and tile (*p* = 0.002 and *p* = 0.038 respectively). Tin was used significantly more than slate where canopy cover was greater (*p* = 0.014).

Increased wind speed and air temperature had a positive effect on refugium use by slow worm and increased light had a negative effect. Use of tile and felt occurred in significantly lower humidity than other materials. Increased canopy cover, increased vertical vegetation cover and increased structural diversity all significantly positively impacted refugium use overall. There was significantly greater ground cover diversity for observations of slow worms in the open (although *n* = 2) and significantly lower for use of tile refugia. There was significantly higher structural diversity for use of tiles than other refugia. There was a significant difference between material temperature and material type, however, no significantly different pairs of materials; the greatest difference was seen between slate and felt (p = 0.051). Air temperature was significantly greater when felt was used over slate and tin (p = <0.001 and p = 0.018 respectively). Soil temperature was greater in instances of use of felt over slate and tile ($p < 0.0001$ and p = 0.009 respectively). Relative humidity was greater when slate was used than when felt or tile were used (p <0.0001 and $p < 0.0001$) and also when tin was used over felt or tile (p = 0.013 and p = 0.010 respectively). Wind speed was greater in uses of felt than slate (p = 0.025). Slow worms used felt in instances of greater ground cover diversity than slate (p = 0.001) and used tile in instances of lower ground cover diversity than felt, slate or tin (all values of $p < 0.001$). Vertical structural diversity was significantly lower for felt than slate (p = 0.002) and significantly greater for tile compared to felt, slate or tin (all values of $p <$ 0.001). Felt was used in instances of greater canopy cover than slate or tin (p < 0.001 and p = 0.002 respectively) and tile was used in significantly decreased canopy cover compared to felt, slate and tin (all values p < 0.0001). (N.B. There were only two records of slow worms found in the open at arrays, compared to multiple hundreds for each refugium material type.)

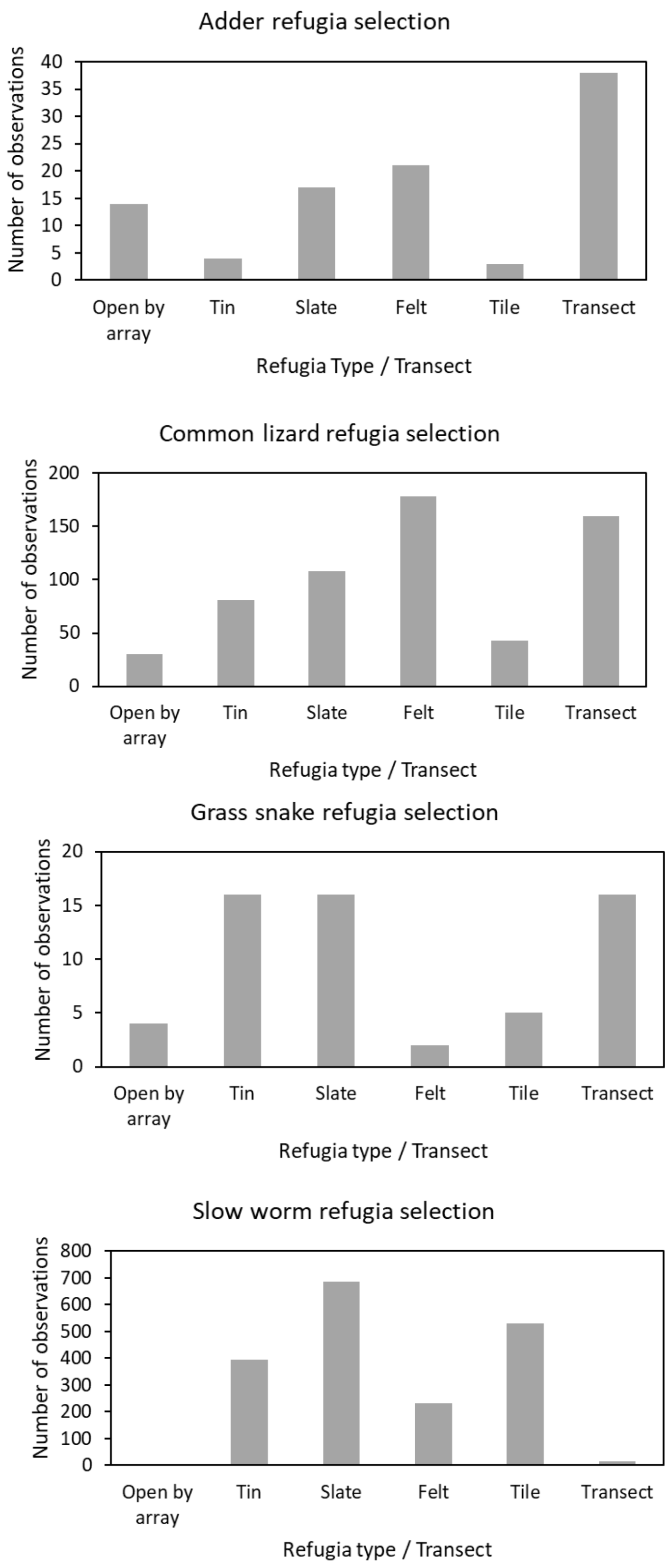


Figure 27 Locations of widespread reptile species observations in dry heath habitat of Eelmoor Marsh.

Table 15 Microclimate factors associated with use of refugia by common reptile species in dry heath. Key microclimate considerations highlight those factors of significance in GLMM models for refugium use generally, and for specific material type use for each species. Mean conditions are single-boxed to match key factors for consideration and double-boxed where significance existed for both refugium use generally and that material type.

MICROCLIMATE			**Key microclimate considerations in relation to refugium type**	**Mean microclimate conditions in relation to detectability for use of each refugium type**					
		Ranked refugia		**Material temp. (°C)**	**Air temp. (°C)**	**Soil temp. (°C)**	**Rel. humidity (%)**	**Light (Lux)**	**Wind speed (m/s)**
Adder *Vipera berus*	1	Felt		30.77	23.66	16.82	54.59	37.74	0.09
	2	Slate		29.03	23.36	15.33	53.54	36.94	0.15
	3	Open	↑ air temp.	20.28	21.80	15.00	57.25	39.71	0.14
	4	Tin		35.23	29.65	22.00	45.08	35.51	0.08
	5	Tile	↓ air temp.	18.60	17.03	13.33	51.80	27.55	0.20
Common lizard *Zootoca vivipara*	1	Felt		21.29	19.03	14.22	62.62	24.57	0.17
	2	Slate		22.20	19.22	14.09	57.53	20.49	0.14
	3	Tin	↑ air temp. ↓ soil temp.	24.71	21.33	16.10	55.69	22.65	0.12
	4	Tile		24.35	21.57	16.60	56.36	32.68	0.16
	5	Open	↑ soil temp.	25.45	27.03	19.56	45.95	35.70	0.13
Grass snake *Natrix helvetica*	1	Tin (1)		30.33	24.69	19.63	59.98	22.46	0.11
	2	Slate (1)		28.03	23.12	17.00	53.83	29.64	0.12
	3	Tile		27.00	25.80	22.00	61.50	11.95	0.00
	4	Open		13.00	21.45	12.00	63.75	15.51	0.00
	5	Felt		27.50	29.10	23.00	53.60	47.90	0.00
Slow worm *Anguis fragilis*	1	Slate		22.59	21.17	8.70	62.72	22.20	0.12
	2	Tile	↓ humidity	24.03	22.27	9.17	54.52	24.61	0.14
	3	Tin	↑ air temp. ↓ light levels ↑ wind speed	24.14	21.59	9.60	59.51	23.11	0.15
	4	Felt	↓ humidity	24.44	23.14	10.72	54.11	24.56	0.18
	5	Open		NA	NA	NA	NA	NA	NA

Table 16 Microhabitat factors associated with use of refugia by common reptile species in dry heath. Key microclimate and microhabitat considerations highlight those factors of significance in GLMM models for refugium use generally, and for specific material type use for each species. Mean conditions are single-boxed to match key factors for consideration and double-boxed where significance existed for both refugium use generally and that material type.

MICROHABITAT			Key microhabitat considerations in relation to refugium type					Mean microhabitat conditions in relation to detectability for use of each refugium type			
		Ranked refugia						Veg. ground cover div. (SI)	Veg. structural div. (st. dev)	Vertical veg. cover (%)	Canopy cover (%)
Adder *Vipera berus*	1	Felt						0.59	17.49	48.16	
	2	Slate						0.50	18.29	45.51	0.28
	3	Open						0.48	16.97	44.55	0.00
	4	Tin						0.53	19.91	47.88	0.00
	5	Tile						0.57	18.61	48.40	0.00
Common lizard *Zootoca vivpara*	1	Felt	↑ veg. structural div.	↓ veg. structural div.	↑ vertical veg. cover			0.52	18.27	42.64	1.99
	2	Slate						0.51	18.86	42.19	2.66
	3	Tin						0.51	20.37	37.99	1.58
	4	Tile						0.52	19.79	39.28	2.46
	5	Open		↑ canopy cover				0.53	17.07	36.45	6.38
Grass snake *Natrix helvetica*	1	Tin (1)						0.47	16.47	48.78	6.54
	2	Slate (1)	↑ veg. structural div.					0.22	25.91	47.79	1.15
	3	Tile	↑ veg. structural div.					0.31	26.54	46.55	0.00
	4	Open						0.52	21.08	48.64	0.46
	5	Felt						0.58	16.95	36.14	17.75
Slow worm *Anguis fragilis*	1	Slate	↑ veg. structural div.	↑ vertical veg. cover	↑ canopy cover			0.50	19.23	42.69	5.62
	2	Tile				↓ veg. ground cover div.	↑ veg. structural div.	0.43	21.20	43.42	3.28
	3	Tin						0.53	18.85	42.05	5.58
	4	Felt						0.57	17.44	40.54	7.22
	5	Open				↑ veg. ground cover div.		0.74	14.41	35.84	0.39

4.5 Discussion

4.5.1 Relative species abundance and the effect of surveying methodology and seasonality

For both wider site surveys and dry heath habitat, relative species abundances differed depending on survey methodology. This indicates that assumptions about reptile presence and distribution at a site should not be made according to one method alone, highlighted in guidance to date in terms of refugium deployment and walking transects (Reading, 1996, 1997; Froglife, 1999; Moulton and Corbett, 1999; Guenioui, 2012; Sewell *et al.*, 2013). Common lizards were encountered more frequently than other species on transect walks, as highlighted previously (Reading, 1997; Sewell *et al.*, 2013). New refugium arrays of multiple material types improved the detectability of common lizards in comparison to old tin and transect surveys in wider habitat. In dry heath, proportionally more common lizards were observed using arrays in comparison to walking transects, than adder and grass snake using arrays compared to walking transects. This suggests that detectability of common lizards was much improved by providing refugium arrays of different materials.

Current survey guidelines highlight that reptiles are less detectable over summer months and that surveying is better undertaken in spring and autumn, as reptiles may spend only a short time basking in hotter conditions before retreating to shade (Moulton and Corbett, 1999; Cathrine, 2018). Surveys in this study were undertaken throughout the day across the seasons and order of refugia was rotated to ensure habitat types and refugia were not checked at the same times in consecutive surveys. Reptiles were observed as frequently per survey, if not more so, in summer months (June, July, August, September) than in spring and autumn, although common lizard sightings notably dropped in August. This broadly agrees with findings by Reading (1997), that show peaks in May, June and September. Results herein still lack the lower values Reading (1997) observes in July and August. Surveys should therefore not be discounted in the summer months, but instead specific conditions assessed to determine optimal surveying days and times. These data also cover a period of nationwide heatwave in July/August 2018 where the hottest days resulted in few observations, highlighting the point that reptiles can be observed by selecting surveying days based on conditions within such periods accordingly.

4.5.2 Microhabitat and microclimatic conditions associated with reptile observations

Reptile use of refugium arrays and specific material types in surveys were predicted by various microclimate and/or microhabitat variables. These, and the specific variable metrics associated with them, can be seen in detail in the Results. They allow recommendations to be made for common lizard and slow worm surveying techniques and refugium type and location, across habitats widely utilised by these species. They also offer recommendations for adder, common lizard, grass snake, sand lizard and slow worm in dry heath habitat specifically, in reference to this vegetation community and corresponding microclimates; as mentioned, dry heath is the only habitat type in the UK in which all six native reptile species are found. The differing significant variables by species are reflective of their differing habitat and microhabitat selection within the wider environment, and their particular thermoregulatory requirements (Edgar *et al.*, 2010) as summarised earlier in this Chapter (4.1.2) and discussed in 1.3.6. All variables considered in this study were hypothesised to influence use of refugia for the reptile assemblage collectively, given their reliance on overall structurally diverse habitat offering both thermoregulatory and sheltering opportunities. For slow worm and common lizard, refugium preference differed between wider site surveys, across a variety of habitats, and dry heath surveys. This highlights the importance of considering context for refugium type in relation to placement and survey timing. For example, the overall effects of increased canopy cover and vertical vegetation cover on common lizard observations in a variety of habitats were significantly negative, however in dry heath habitat, structural diversity became significant, with individual effects of vegetation variables on specific material types. This shift in factors of importance reflects the shift from surveys showing great variability in sightings between highly vertically heterogeneous habitats, to dry heath where the variability is more limited and in which this species is widely prevalent.

Specific material types were favoured by different species, for example felt and slate by both common lizards and sand lizards in dry heath; tile and slate for slow worm. Depending on the purpose of the monitoring being undertaken, refugium type could be selected to improve the detectability probability of a particular species. The experimental nature of the surveys undertaken highlighted that in order to optimise survey effort when monitoring the UK reptile species assemblage (smooth snakes not considered), a multi-method approach to refugium deployment where feasible and practical would best optimise return as material preference not only differed by species, but by microclimate and microhabitat conditions as well; this builds further on the concept of employing multiple surveying techniques (Moulton and Corbett, 1999; Langham, 2012). Offering a variety of refugia, in addition to walking transects, would account for this and increase observation potential. The number of observations of legged lizards, adder and grass snake in the open at refugia indicates that refugia not only offer thermoregulatory benefit in their own right, but create areas of more open habitat by their deployment, depressing the vegetation in the immediate vicinity and creating a *focus* with its own microclimate. Such *foci* are highlighted as areas of high importance for reptiles within the wider habitat (Edgar *et al.*, 2010). The number of observations for open habitat at refugia is comparable to use of some material types for all four species.

The focus on dry heath habitat in this study was in response to the sand lizard reintroductions into that habitat type, and the need to understand the interactions and shared habitat use between the released sand lizards and sympatric reptile species such as the common lizard, a potential competitor, and predators such as the grass snake and adder. Dry heath habitat is the only habitat type in the UK in which all reptile species co-exist, highlighting the additional importance of this focus in the light of concerns for native reptile populations more holistically (Edgar *et al.*, 2010; Dunford and Berry, 2013; Gardner *et al.*, 2019).

Improving understanding of common lizard spatiotemporal use of microhabitat in dry heath alongside the sand lizard, in particular, is important to inform monitoring recommendations and habitat management specific to these legged lizard species which are rarely found using traditional refugia (Sewell *et al.*, 2013). Along with sand lizards (Chapter 3), common lizards were found to favour slate and felt in dry heath. Common lizards favoured felt with surroundings of higher vegetation cover with lower heterogeneity, but overall refugium use was in areas of high vegetation heterogeneity. When observed utilising open space at arrays, canopy cover was preferred. Microclimate air temperature was indicative of common lizard observation, and previously shown to be highly positively correlated with wider air temperature, enabling prediction of optimal survey timing in relation to this. Air temperature is directly linked to thermoregulatory capacity of reptiles (Edgar *et al.*, 2010). The interaction between felt and more covered microhabitat for the common lizard, in addition to the interaction between felt and vegetation structural heterogeneity for the sand lizard (Chapter 3), builds further on the concept that refugia are considered not only in the light of thermoregulatory benefits. Their other properties, such as weight and thus sheltering security, may encourage use in more specific microhabitat conditions.

4.5.3 Conservation monitoring implications

This chapter has drawn attention to the current gaps in monitoring protocols for widespread native reptile species in the UK (Sewell *et al.*, 2013). It has highlighted disparities in monitoring methods employed and suggested how the results included can help improve surveying techniques. Significant microhabitat variables, and measures of variables, can be quickly extracted to optimise type and best deployment of refugia within the habitat. Weather conditions, with corresponding measures of microclimate conditions, can then optimise detection probability for common lizards and slow worms across a variety of habitats, and for all common species in dry heath habitat.

There is much debate about the use of ‘attractants’ to accurately infer species occupancy and distribution and the very nature of their deterring species to show typical spatiotemporal behaviour. This has been widely discussed in the capacity of baiting traps and camera traps (Balme *et al.*, 2014; Preez *et al.*, 2014; Stewart *et al.*, 2019). However, a systematic method that optimises detectability of cryptic species (or assemblages) of conservation concern allows spatiotemporal comparability within and between sites. Through this, optimal survey effort can be employed in a meaningful and robust manner to make inferences about populations (Sewell *et al.*, 2012; Preez *et al.*, 2014; Stewart *et al.*, 2019).

The rigorous approach to data collection undertaken herein has provided evidence-based recommendations. These can be employed by field surveyors to ensure future data collection is an optimal use of resources, and in turn provide robust data. With the increasing use of citizen science (Silvertown, 2009; Dickinson *et al.*, 2010) and a strong contingent of volunteers within the herpetofauna community in the UK (Gardner *et al.*, 2019), further guidance on best practice reptile surveying methodology would be greatly beneficial. This is particularly true for legged lizards, one of which is of particular conservation concern in the UK. For reptile species at a global scale in the face of a changing (Gibbons *et al.*, 2000; Kumar *et al.*, 2014), it is a race against time to improve understanding of basic biology and ecology (Tingley *et al.*, 2016), largely driven by thermoregulatory requirements (Kumar *et al.*, 2014), in order to focus conservation efforts on this taxonomic group (Clark and May, 2002; Bajomi *et al.*, 2010; Gilbert *et al.*, 2017).

4.5.4 Further study

It would be beneficial to repeat a multi-refugium monitoring study across multiple sites in order to eliminate any site effects on the dataset. It would also be ideal to include sites occupied by smooth snake in order to complete recommendations for the whole UK reptile assemblage. Further surveys of broader habitat types would increase grass snake and adder counts to enable preferences of these species to be assessed. Increasing sample size, particularly for these same species, in dry heath would improve predictive power.

Mark-recapture methodologies, identifying individuals of all species, would offer insights into individual preferences in microhabitat use and surveying conditions to be assessed in the light of wider trends seen.

Size of refugia were discussed and varied between methodologies in Chapters 3 and 4, however they were not directly compared. Both larger 500 x 500 mm and smaller 500 x 250 mm materials were found to be utilised by all widespread species studied. Given the recent recommendations that smaller refugia appear as suitable as larger sheets, a quantitative analysis of this would be beneficial (Langham, 2011; Cathrine, 2018).

This study offered baseline data on which further monitoring of the reptile community following sand lizard release can build. As the reintroduced population enters the persistence phase, continued monitoring of the wider ecosystem is essential in order to observe any shifts in species distributions potentially attributable to the releases, which may not be observed for some years to come (IUCN, 2013).

4.6 Conclusions

This chapter applied the multi-method monitoring techniques of Chapter 3 for the sand lizard to the widespread UK reptile community. Recommendations are made for reptile monitoring methods in the UK going forward.

The key conclusions of this chapter were as follows:

- Relative abundance of reptile species was heavily dependent on surveying methodology. Methodology should target the species in question accordingly or incorporate a variety of techniques to monitor the whole community, depending on monitoring purpose.
- Refugium type and location, and optimal surveying conditions varied by species. Data showed the key factors and measures associated with reptile observations and suggest the utility of applying these to optimise detecting each species.
- Like sand lizards (see Chapter 3), common lizards were found to utilise felt and slate above other refugium types in dry heath and be influenced in location particularly by vertical vegetation (height or structural heterogeneity). Current UK reptile monitoring guidelines are most lacking for legged lizards and the detailed results herein could provide direct field survey recommendations.
- By improving monitoring methods and consequently understanding the species community at a reintroduction receptor site holistically, continued monitoring and habitat management can be targeted most efficiently. This is particularly important for UK reptiles in the light of threatened habitats and climate change.

Chapter 5 Demographic trends in sand lizard *Lacerta agilis* response to reintroduction at a lowland dry heath site

5.1 Introduction

The importance of a rigorous, evidence-based approach to species reintroduction has been highlighted for decades (Seddon, 1999; Fischer and Lindenmayer, 2000). However, it has been repeatedly re-visited due to studies indicating a broadly opportunistic approach is still being taken (Taylor *et al.*, 2017; Bubac and Johnson, 2019). Post-release monitoring is one of the seven reintroduction project stages outlined in the 'IUCN Guidelines for reintroduction and other conservation translocations' and requires no less rigour than the preparatory and release phases (Nichols and Williams, 2006; IUCN, 2013; Barata *et al.*, 2017), although this is often not the case in practise (Fischer and Lindenmayer, 2000). There is also a need for research to be conducted on not only the establishment of a population, but also its persistence, and more widely species recovery at the metapopulation and ecosystem level (Armstrong and Seddon, 2008; Taylor *et al.*, 2017). The importance of establishing *a priori* questions has been raised, in concert with advance planning to ensure that monitoring evaluates the aims of a project, avoiding more *ad hoc* methods (Armstrong and Seddon, 2007; Taylor *et al.*, 2017). These approaches allow a project to measure success against its own targets, which might vary substantially. The temporally dynamic nature of any population has also been raised as a point of consideration (Seddon, 1999; Bernardo *et al.*, 2011; Parker *et al.*, 2013).

The most informative requirement to ensure a reintroduction project is approached appropriately is a detailed understanding of the ecology of the species being released. Attempts to make critical reintroduction protocol decisions with insufficient comprehension of a species' biology should be avoided (West *et al.*, 2019). A recent review of the Global Re-introduction Perspective Series finds that a third of difficulties encountered by practitioners during animal translocations are due to an aspect of the target species' ecology or biology, such as quality and suitability of the release area and animal behaviour (Berger-Tal *et al.*, 2019). The three most reported problems by projects are monitoring difficulties (32.8 % case studies reviewed), a lack of funding (32.4 %) and animal behaviour issues (27.6 %). The most common behavioural issue encountered (almost half of what is reported) is around movement or dispersal. Animal behavioural issues are particularly prevalent in reptiles, birds and mammals (Berger-Tal *et al.*, 2019). Understanding a species' biology enables meaningful aims for a conservation translocation to be established, as well as species-appropriate post-release monitoring. Biological understanding also allows appropriate management of the population and wider ecosystem to optimise population establishment and persistence (Fischer and Lindenmayer, 2000; Seddon *et al.*, 2007; Armstrong and Seddon, 2008). Monitoring feeds directly back into species recovery, highlighting areas for improvement of reintroduction protocols.

5.1.1 Post-release monitoring to inform reintroduction protocols

Post-release monitoring allows the variability in post-release movement and survival to be observed within a release cohort and to feed back into protocols. Elk *Cervus elaphus* movement post-release is found to be influenced by a soft or hard release, with longer acclimation periods causing elk to disperse shorter distances, which is preferred in this instance (Ryckman *et al.*, 2010). Calves stay in closer proximity to the release location than adults, indicating less exploratory behaviour with decreased age. A tammar wallaby *Macropus eugenii* reintroduction is not deemed successful due to survival of fewer than 1/3 of animals, however vital lessons are learned regarding vegetative habitat requirements for future efforts, and the importance of reporting results regardless of success is highlighted (Watkins *et al.*, 2018). An 18-month radio telemetry study of Chinese giant salamander *Andrias davidianus* finds survival rate of released captive-bred individuals is not impacted by body mass; however, it is affected by recovery time allowed from transmitter implantation pre-release (Zhang *et al.*, 2016). It therefore highlights the importance of releasing older juveniles to account for the latter in future radio telemetry monitoring of the species.

For small, cryptic and otherwise challenging species to monitor, obtaining detailed data on this post-release stage often unearths unanticipated results. The critically endangered thrush *Myadestes palmeri* is monitored using radio telemetry for 8-10 weeks post-release (Foster *et al.*, 2003). Two of these captive bred individuals disperse to more than 3 km from the release site within a single day of release, and there is an overall 57 % dispersal rate out of the target area suggesting that multiple releases may be necessary to repopulate the intended habitat. The wide-ranging dispersal and consequent gene flow also suggests that a small captive stock is likely enough for reintroduction purposes. Population augmentation of the lacertid lizard *Psammodromus algirus* compares the release of captive-bred with native lizards at a site, finding introduced juveniles are ~ 25% larger than native ones, however survival rates of each are comparable (Santos *et al.*, 2009). The study also highlights that captive breeding this species is both cost-effective and practicable and a tool that would likely suit other small species with fragmented natural populations. A study assessing differences between translocation of a wild population and a captive-reared cohort of water snakes *Nerodia sipedon sipedon* shows different negative behaviours post-release (Roe *et al.*, 2010). The former has restricted movement and abnormal habitat use, and the latter frequently move off site; both result in high overwinter mortality. Reintroduction protocols can be improved by better matching natural habitats condition and enriching environmental conditions in captivity. A similar response was observed in reintroduced grey partridge *Perdix perdix* in southern England, with release captive-bred animals suffering an ‘ecological trap’ that was not experienced by their wild counterparts (Rantanen *et al.*, 2010).

5.1.2 Demographic differences in a translocated population

Immediate response to a novel release environment may be indicative of subsequent behaviour. Such behavioural characteristics may be underlain by demographic as well as individual differences (the latter are considered in Chapter 6). There is reflection of behavioural traits in long-term post-release survival and movement, however they are not consistent across species, highlighting the importance of studying this on a species-specific level (Bremner-Harrison *et al.*, 2004; Conrad *et al.*, 2011; Haage *et al.*, 2017). Responses to a new environment have been examined historically to ascertain behavioural measures such as boldness, exploration and activity using Open Field Tests (Hall and Ballachey, 1932; Perals *et al.*, 2017). Testing consists of a measured spatial study, or behavioural assay, sometimes involving novel objects and often set within a bounded ‘arena’ environment. Understanding the questions being asked and ensuring they are reflected in the experimental design that has been set up is important. It is recommended that it is conducted in conjunction with additional behavioural assays and repeatability determined through time where possible (Carter *et al.*, 2013; Perals *et al.*, 2017).

Following release, there may be demographic trends in long-term post-release movement and survival of a reintroduced population. Sex-related differences in post-release behaviour have been observed. Female river otters *Lontra canadensis* are found to disperse on average 8.7 km further than males, likely a result of intra-sexual territoriality observed in females and not males in this species, and seeking out higher quality habitats in order to successfully rear young (Spinola *et al.*, 2008). The opposite is observed in Persian fallow deer *Dama mesopotamica* where males move long distances to avoid other males, potentially incurring a survival cost. Conspecific size also plays a role where smaller males are tolerated by larger males to some extent as they pose less threat (Dolev *et al.*, 2002). Post-release movement of water voles *Arvicola amphibius* shows mean weekly distances travelled do not alter over the 10-week study period. However, there is evidence that some males have located an area in which to settle, where this is not shown for any females (Baker *et al.*, 2018). In a study of mountain gazelle *Gazella gazella*, no difference was found between dispersal distances of females and territorial males (Dunham, 2000).

Dispersal and survival trends also differ by age between species (Letty *et al.*, 2007; Le Gouar *et al.*, 2012). Although caution is raised around transferring knowledge of natal dispersal tendencies of a species (Clobert *et al.*, 2001) to a population post-release, there are tendencies of younger individuals to disperse further in some species. For example, younger females in the reintroduced group of mountain gazelles *G. gazella* are found to travel further than older females (Dunham, 2000). The majority of gazelles remain in the immediate release vicinity (within 3.5 km of the release site) which has a positive effect on breeding success and indicates site selection and release numbers are appropriate. However, the maximum distance dispersed, of 12.1 km, shows variability is present. Reintroduction of captive-bred kaki *Himantopus novaezelandiae* show juveniles make longer moves than subadults, taking them further from the release site within the months post-release (Heezik *et al.*, 2009). This might be considered problematic as it takes these individuals away from the breeding population, however the importance of long-term monitoring is highlighted here as it shows that comparable proportions of both juveniles and subadults returned to their release location two years later for breeding. Conversely to observations of increased dispersal in younger individuals, the research into elk reintroduction *C. elaphus* (Ryckman *et al.*, 2010) finds calves, and particularly male calves, remain closer to the release locality than adults. In the case of Alpine ibex *Capra ibex ibex* reintroduction no effect is found of age on post-release spatial behaviour (Scillitani *et al.*, 2012). However, this study releases sub-adults and adults, suggesting that a younger age grouping may have shown a tendency to disperse less. In snowshoe hare *Lepus americanus*, juveniles show poor survival rate due to greater predation (Wirsing *et al.*, 2002). Survival of translocated red squirrels *Sciurus vulgaris* also finds survival to be poorer in subadult than adult males (Wauters *et al.*, 1997).

The variable nature of demographic differences post-release, as discussed, highlights the importance of studying this on a species-specific basis. Many biological factors may underly the differences seen. For reptiles in the UK, a key element related to age is being of suitable body mass to survive brumation (Gregory, 1982; Blanke and Fearnley, 2015). It is therefore important to understand growth trends across age demographics in conjunction with survival rates. For sand lizards *L. agilis*, growth and body condition are also key drivers of early breeding success (Bischoff, 1984), highlighting further the importance of understanding growth patterns. Demographics of the release population must be appropriate for the species being reintroduced in order to optimise survival and reproductive success.

5.1.3 Aims

This research has so far addressed the need to suitably monitor a cryptic species, the sand lizard, of which our understanding is limited in terms of its post-release behaviour, due to its poor detectability (Fearnley, 2009; Blanke and Fearnley, 2015). Improving its monitoring has been considered in an experimental, captive study (Chapter 2), and then in a wild setting (Chapter 3).

Evidence-based recommendations have also been made to improve surveying protocols across the wider species assemblage (Chapter 4). This intends to offer adaptable approaches that are species- and habitat-specific, and thus also resilient to environmental changes.

This chapter considers our current understanding of sand lizard response to release and survival following fifty years of reintroductions to deemed suitable sites (Woodfine *et al.*, 2017). It applies, for the first time, a detailed and quantitative approach to assessing and analysing sand lizard response to reintroduction, across the release cohort demographic, during the immediate population establishment phase. Quantifying the heterogeneity of release populations is important to ensure that protocol amendment recommendations are made objectively, from pre-release decision-making, through the release itself, and into population monitoring. The results of this research may offer insights for comparable reintroduction initiatives for cryptic species.

The overarching aim of this chapter is: **to assess post-release observation trends and determine demographic factors affecting post-release movement and post-winter survival of reintroduced sand lizards, and implications for reintroduction protocols.** Its objectives are:

1. To summarise temporal trends in numbers of post-release observations of sand lizards following reintroduction, in consideration of corresponding weather conditions.
2. To determine the influence of age and sex on
    i. response to release,
    ii. overwinter survival,
    iii. distance travelled pre-winter.
3. To determine key growth periods for captive juvenile sand lizards during their first year.

5.2 Methods

5.2.1 Immediate response of sand lizards to release into a novel environment

The full sand lizard release protocols are detailed in Chapter 3 (3.2.1).

At each marked release square, a video camera (Sony Handycam) was installed on a tripod directly overhead. Lizards were removed from their plastic terrarium and were photographed; any missing toes/tail or scarring was noted. They were then set down in their groups of three or four in the centre of the square, and the time was noted (Figure 28). Each lizard was audibly numbered in order to correspond video footage with photographs and thus individually identify them. The video camera was left running following each release for ten minutes, or until every lizard had moved out of the square (whichever occurred sooner). Video footage was subsequently analysed using VLC Media Player (Figure 29). Using methodology similar to an open field test (Hall and Ballachey, 1932; Perals *et al.*, 2017) to assess behavioural differences in terms of activity and exploration of the immediate release environment, the release square had 25 smaller squares of equal size manually super-imposed over the top (each representing 10 x 10 cm). The footage was replayed at a frame rate of 10 frames per second (FPS) and times of moving between squares recorded (entry of a new square equating to a lizard's nose crossing the boundary). If a lizard disappeared under vegetation this was noted as Out of Sight. Data were summarised by: time taken to completely leave the 50 X 50 cm release square (tail tip crossing line), the total number of transitions between smaller squares (as a measure of activity) and the number of smaller squares entered, excluding the central square (as a measure of exploration).

Figure 28 a) Placement of video camera directly overhead release square on mown heather strip (*image R. Gardner*); b) releasing three sand lizards in the centre of the square (*image P. Riordan*).

Figure 29 Paused video footage of Release Square 8 containing three subadult sand lizards (circled).

On the rare occasions an individual went out of sight within the release square and did not emerge (i.e. under vegetation) or remained within the release square for > 10 minutes post-release, they were not included in analyses as their response to release could not be quantified in a comparable way to the other individuals. (Dense vegetation within the release squares was intentionally minimal, as they were within the mown heather strip.)

5.2.2 Post-release monitoring

Post-release survey methodologies are detailed in Chapter 3 (3.2.2).

Age and sex, where known, of sand lizards observed post-release were recorded, along with their location. Photographs were obtained wherever possible to allow individual identification. Surveys were kept as regular and comparable between the two years of monitoring as possible, as described in 3.2.2. The 29 surveys in spring/summer 2018, as opposed to 38 in spring/summer 2019, reflect the heatwave in summer 2018 when conditions were so hot that surveys were returning few reptiles and no sand lizards, as can be seen in the results.

Average monthly weather data were obtained from the MET Office historic station data for Heathrow Airport (~ 30 km away): mean daily temperatures (℃, maximum and minimum), days of frost, total rainfall (mm) and total sunshine duration (hours). These data offered an overview of weather conditions experienced by the two release cohorts in their first year/s post-release (Sept 2017- August 2018, Sept 2018-August 2019).

5.2.3 Pre-release morphometrics

Within 48 hours of each clutch laid in captivity hatching in its incubator at Marwell Zoo, sand lizards were photographed allowing individual identification (detailed in 6.2.1). Conventional lizard morphometric parameters were recorded, prior to their being moved to outdoor rearing vivaria, including body mass (0.01 g precision); snout-vent length (SVL); tail length; left rear tibial length; head length; and head width (0.01 mm precision). Further photographs and morphometric measurements were taken pre-release (of all individuals, whether released or not), post-overwintering (in early summer) of individuals retained in captivity, and pre-release once more for these retained animals. The first date of morphometrics being taken was considered Day 1 post-hatching. Lizards that arrived from another private breeding facility and any that hatched in the adult vivarium missed having the first post-hatching data recorded. These lizards were included in subsequent data collection of morphometrics and photographs and therefore had pre-release data recorded (and further data, if retained). Where every effort was made to catch all lizards to obtain the repeat morphometric data, several were typically missed on each occasion. Some loss was expected throughout the rearing process therefore knowing exactly how many lizards should be present to be searched for and caught at each stage was not possible.

5.3 Analysis

All statistical analyses were undertaken in RStudio 1.1.463 (R version 3.5.3)

Time-series analyses were used to summarise total post-release counts, demographic counts by age group and percentage of each demographic group observed; this information was further summarised into pre- and post-winter observations by age. Differences in number of observations of sand lizards per survey were crudely analysed pre- and post-winter and between release years using the non-parametric Mann Whitney U (Wilcoxon Rank Sum) test.

The effects of age (hatchlings, yearlings, two-year olds), and sex when known in subadult lizards, on apparent overwinter survival (binary, counted as an observation of the lizard at any point following their first winter) were analysed with Fisher's Exact Test.

Differences between response to release by age group were analysed using Kruskal-Wallis for exploration and activity, and one-way ANOVA (with data log-transformed) and post-hoc Tukey's

Test for time taken to leave the release square. Response differences between sexes were analysed using Mann-Whitney U and student's *t*-test. Results were displayed as a series of boxplots.

Differences between individual maximum furthest distances observed from point of release by different age groups were analysed using Kruskal-Wallis and the post-hoc Dunn Test, as distance data were not parametric and could not be successfully transformed. Differences between maximum furthest distances travelled and sex (when known) in subadult lizards were analysed using the Mann-Whitney U test. Lizards were only included when they were observed at least once post-release. Results were displayed as a series of boxplots.

Weather data for the year following each release were summarised in line graphs and a written overview given.

Trends in inter-annual growth (body mass) were summarised and displayed as a boxplot.

5.4 Results

5.4.1 Summary of post-release observations

Sand lizards were observed on 307 occasions following the first release in September 2017, until the end of August 2019; this included two indirect observations of sloughed skins. Five of these observations were made opportunistically outside of formal surveys, which were excluded from some, but not all, analyses depending on applicability. Of 307 observations, 284 were photographed with sufficient clarity for lizards to be accurately identified. Of the total of 166 lizards released in 2017 and 2018, 85 (51.2%) were observed at least once during formal post-release surveys; one of the five opportunistic sightings was a sand lizard not observed in formal surveys (see Figure 30 and Table 17 for summaries; see Appendix D.1 for full observation details).

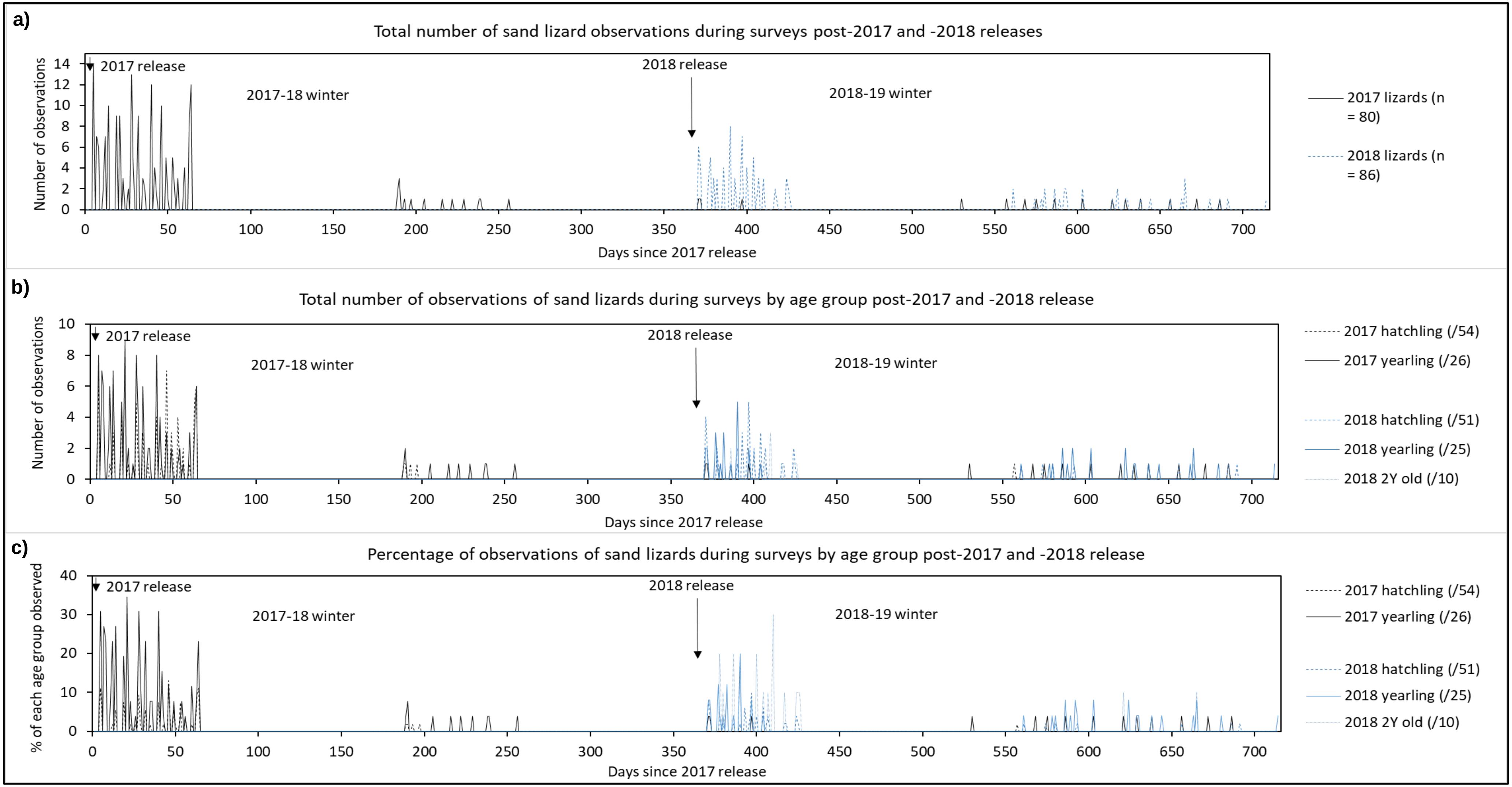


Figure 30 Summary of number of observations (total n = 307) of a) total sand lizards, and sand lizards by b) age group and c) percent of age group released in 2017 and 2018 (including two sub-adult sloughed skins, 11/05/2018 and 23/10/2018).

5.4.2 Survival considering demographic differences

There were more observations per survey (with consistent survey effort across comparable area) pre-winter September-November than post-winter March-August for both the 2017 ($U_{27,27}$ = 15.5, p <0.001) and 2018 ($U_{24,38}$ = 152.0, p <0.001) release cohorts (Table 17). There were a greater number of observations per survey pre-winter of the 2017 release cohort than the 2018 release cohort ($U_{27,24}$ = 480.5, p = 0.003), but there was no difference in the number of observations per survey post-winter between the 2017 and 2018 release cohorts ($U_{27,38}$ = 406.0, p = 0.121). There was no difference between the number of observations per survey of the first, 2017 release cohort between 2018 surveys March-August and 2019 surveys March-August ($U_{27,38}$ = 572.0, p = 0.351).

Table 17 Summary of live individual sand lizard observations during post-release surveys 2017-2019.

	2017 release					**2018 release**		
		Pre-winter	**Post-winter**	***Pre-winter***	***Post-winter***		**Pre-winter**	**Post-winter**
	Survey n	***27***	***29***	***24***	***38***	***Survey n***	***24***	***38***
Number of individuals observed at least once post-release	Hatchling (/54)	23	1	*0*	*1*	Hatchling (/51)	12	5
	Yearling (/26)	22	7	*1*	*2*	Yearling (/25)	11	10
						2-year old (/10)	7	2
% of individuals observed at least once post-release	Hatchling (/54)	42.59	1.85	*0.00*	*1.85*	Hatchling (/51)	23.53	9.80
	Yearling (/26)	84.62	26.92	*3.85*	*7.69*	Yearling (/25)	44.00	40.00
						2-year old (/10)	70	20.00
Maximum number of observations of one individual	Hatchling (/54)	7	2	*0*	*1*	Hatchling (/51)	11	2
	Yearling (/26)	16	4	*3*	*9*	Yearling (/25)	4	10
						2-year old (/10)	9	1
Mean number of observations per individual	Hatchling (/54)	2.0	0.15	*0.00*	*0.02*	Hatchling (/51)	1.2	0.14
	Yearling (/26)	3.9	0.35	*0.12*	*0.42*	Yearling (/25)	0.7	0.88
						2-year old (/10)	1.8	0.20
Median number of observations per individual	Hatchling (/54)	0.0	0.00	*0.00*	*0.00*	Hatchling (/51)	0.0	0.00
	Yearling (/26)	3.5	0.00	*0.00*	*0.00*	Yearling (/25)	0.0	0.00
						2-year old (/10)	1.0	0.00
Additional observations						Definite test burrow		1
						Possible test burrow		2
						Oviposition		1

The numbers of individuals observed during surveys declined following winter (Figure 30, Table 17). An estimated minimum of 10.0 % and 19.8 % of released lizards survived their first winter in 2017-18 and 2018-19 respectively. Further observations of the 2017 release cohort in 2019 allowed revision of the estimated survival from the minimum of 10.0% to at least 13.8 %.

The percentage of each age group of individuals observed was consistently greater for yearlings than hatchlings (Table 17). Just one of 54 (2017) and five of 51 (2018) individual hatchlings were observed following the first winter post-release; the 2017 hatchling later died, however a 2017 hatchling was then observed in spring 2019, having survived two winters and not been seen at all in 2018 – an individual that had travelled 67 m from the release site by survey number 6 on 28/09/2017. Comparably, seven of 26 (2017) and ten of 25 (2018) yearlings respectively, were observed following their first winter. Only two of ten 2-year olds were observed post-winter (Table 17, Appendix D.1).

In spring-summer 2019, 19/39 observations (48.7%) were of just two individuals. One of these was a 2017 cohort yearling that had not been seen once in 2018 (observations in 2019, n = 9). The other was a 2018 yearling that had been seen only once post-release on 26/09/2018 (observations in 2019, n = 10). Between March and August 2018, and February/March and August 2019, there were 12 and 39 observations during these periods representing 8 and 17 lizards respectively (Table 17, Appendix D.1).

One lizard observation in 2019 was a 2018-release yearling female (at this stage two-year old) digging a burrow on the sand strip on 06/06/2019 (Figure 31); the burrow was backfilled on checking post-survey, suggesting eggs were laid. This female was the 2018-release lizard observed 10 times during surveys in 2019 (Appendix D.1). One other definite and two possible test burrows were also observed in June 2019 (Table 17). This showed post-release breeding had occurred within a year of release of a yearling.

Figure 31 Female sand lizard digging test burrow in prepared sand strip at Eelmoor Marsh; this was backfilled by the end of the day suggesting oviposition had occurred.

The number of individuals observed following the first winter post-release was significantly different between the three age groups (Fisher's Exact Test: $p < 0.001$). Yearlings were observed significantly more than hatchlings (Fisher's Exact Test: $p < 0.001$) (Table 17). There was no significant difference between male and female encounter rates (over-winter survival) (Fisher's Exact Test: $p = 0.764$) where sex was known in subadults (Table 18).

Table 18 Number of known male and female subadults (yearlings and two-year olds) observed post-winter.

	Males	**Females**
Observed post-winter	11	10
Not observed post-winter	10	12

5.4.3 Response to release and post-release movement considering demographic differences

There was no difference in exploratory behaviour, measured as the number of small 'squares' entered in the release square, between age groups (Kruskal-Wallis $\chi^2 = 3.373$, d.f. = 2; $p = 0.185$) or in active behaviour, measured as the number of transitions between 'squares', between age groups (Kruskal-Wallis $\chi^2 = 5.049$, $p = 0.080$, 2 d.f.). There was a significant difference in the amount of time taken to leave the release square by different age groups ($F_{2,137} = 15.75$, $p < 0.001$). Yearlings took significantly longer than two-year olds (Tukey's Test: $p = 0.009$) and hatchlings (Tukey's Test: $p < 0.001$) to leave the square, but there was no difference between hatchlings and two-year olds (Tukey's Test: $p = 0.985$) (Figure 32a).

There was no difference in exploratory behaviour, measured as number of small squares entered, between sexes ($U_{19,22} = 168.5$, $p = 0.273$). Neither was there a difference between males and females in active behaviour, measured as number of transitions between squares ($U_{19,22} = 164.0$, $p = 0.230$), nor the amount of time taken by each sex to leave the release square ($t_{39} = -0.133$, $p = 0.895$) (Figure 32b).

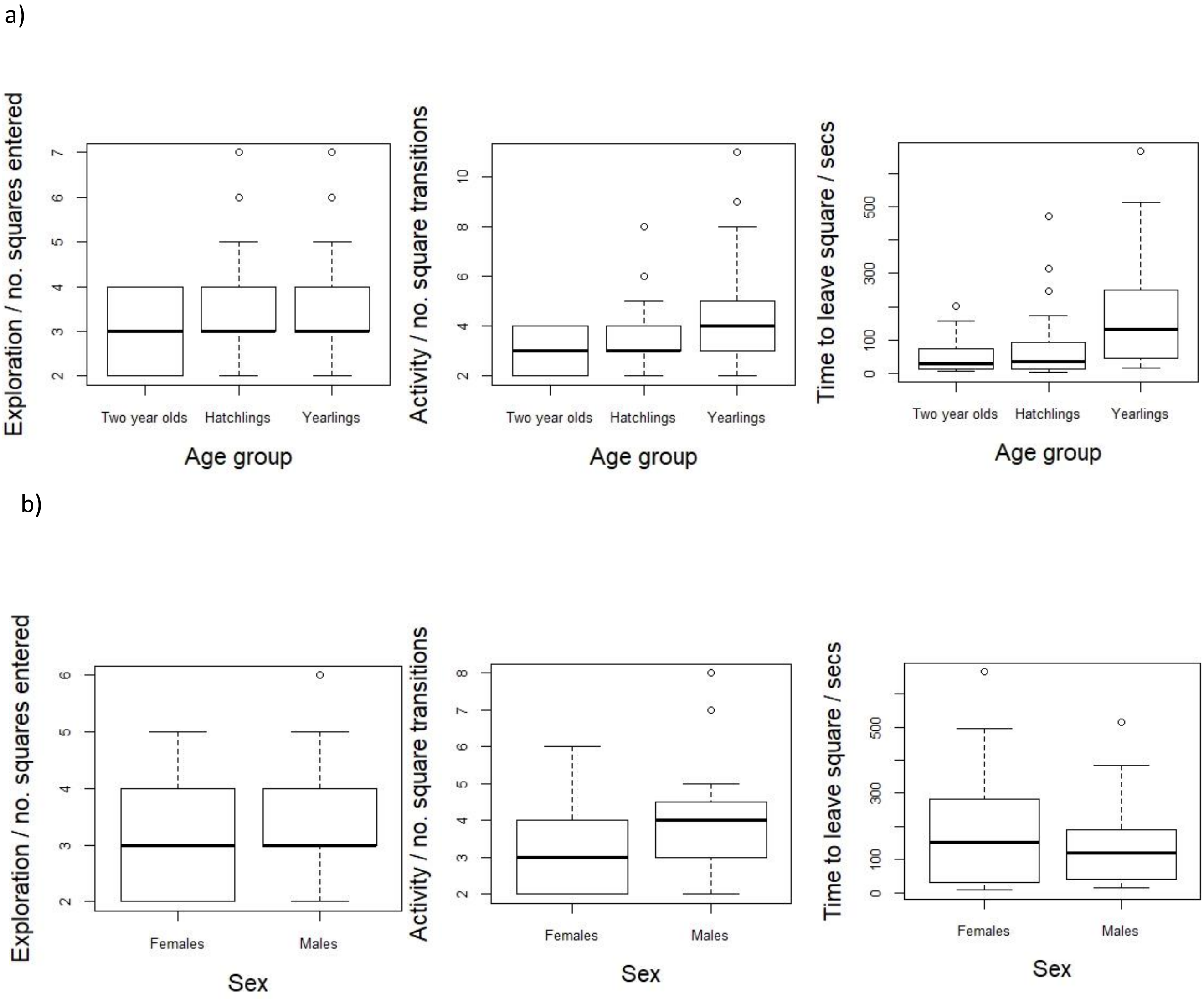


Figure 32 Response to release by a) different age groups, by measures of exploration, activity and time taken to leave the release square; two-year olds (n = 10), hatchlings (n = 81), yearlings (n = 49); and b) by males and females, by measures of exploration, activity and time taken to leave the release square; females (n = 22), males (n = 19). Plots highlight the median, interquartile range, extreme values and outliers.

Different age groups were found to differ in the pre-winter distances they travelled following release (Kruskal-Wallis χ^2 = 8.410, 2d.f., p = 0.015). Hatchlings travelled significantly further than two-year olds pre-winter (Dunn Test: p = 0.041). Hatchlings also travelled further than yearlings, approaching significance (Dunn Test: p = 0.056), but there was no difference between two-year olds and yearlings (Dunn Test: p = 0.347) (Figure 33a). There was no significant difference between the furthest distance travelled by male and female sand lizards before overwintering ($U_{20,18}$ = 169.0, p = 0.758), although the distance travelled by males appeared to be more variable, with a greater interquartile range than females and the largest outlier (Figure 33b).

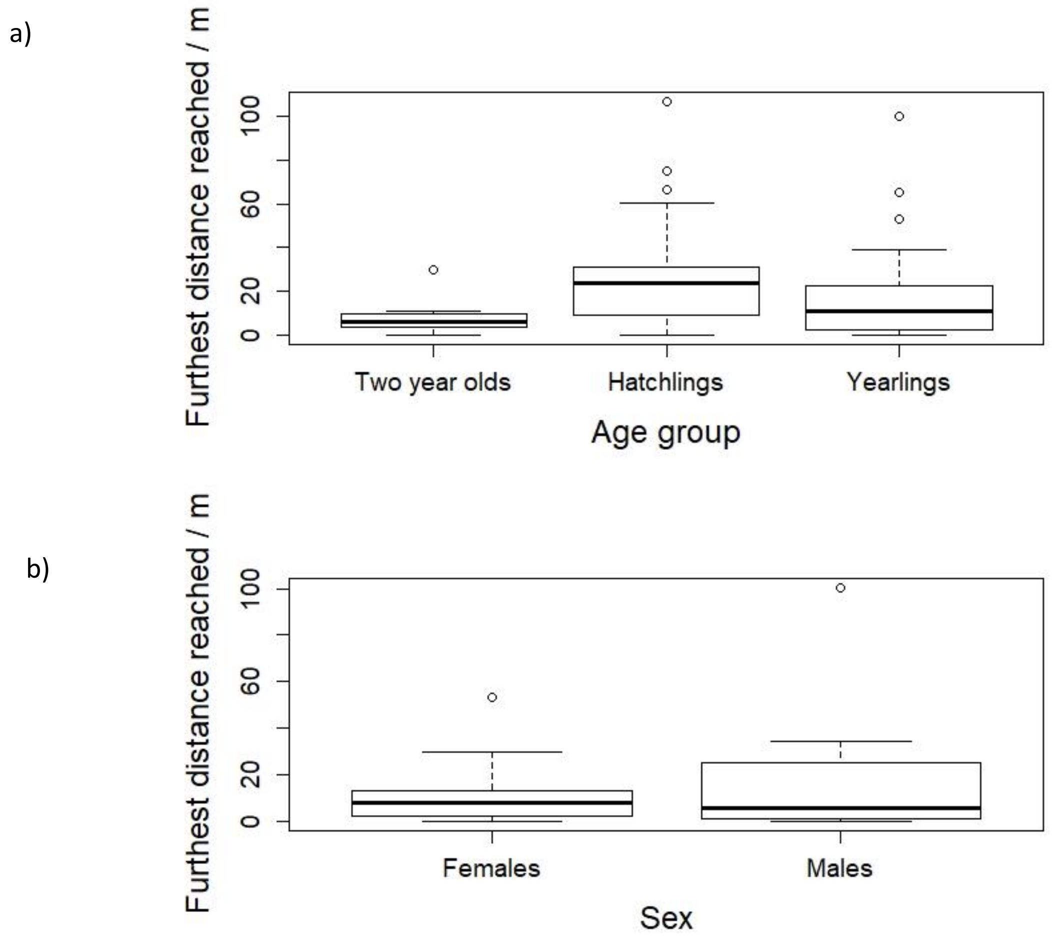


Figure 33 Furthest distance reached by a) different age groups of sand lizard from point of release pre-overwintering (including only lizards observed at least once post-release in release-year or following years); two-year olds (n = 8), hatchlings (n = 38), yearlings (n = 39); b) male and female sand lizards from point of release pre-overwintering (including only lizards observed at least once post-release in release-year or following years and lizards of known sex); females (n = 20), males (n = 18). Plots highlight the median, interquartile range, extreme values and outliers.

5.4.4 Overview of weather conditions experienced by sand lizards post-release

There were differences in the weather experienced by the two release cohorts during their first year/s post-release (Figure 34). Notably, the 2017 lizards experienced very little rainfall in October following release (<10 mm) compared to >60 mm in October 2018, and just under half the amount in November 2017 compared to 2018. The peaks in air-frost days in December 2017 (7 days) and then almost double this number in February 2018 (12 days) and a continuation of this cold spell into March (6 days) show the extremity of this late cold spell (colloquially termed the ‘Beast from the East’). By comparison, the 2018-19 winter showed a more typical decline to minimum temperatures and maximum air-frost days in January before temperatures rose again. Maximum temperatures in February in 2019 were however atypical, averaging 12.4 ℃, which was unseasonably warm. Minimum and maximum daily temperatures for May – July were higher in 2018 than 2019, by typically > 2 ℃. Rainfall during June-July 2018 was low (less than 20 mm in total), particularly in comparison to a very wet June in 2019 (more than 80 mm); heatwave conditions were declared nationally in 2018.

In summary, the 2017 release cohort experienced milder, dryer weather conditions immediately post-release, however then faced a late, cold winter followed by a wet spring and dry, hot summer. The 2018 release cohort experienced cooler, wetter conditions pre-overwintering, an early warm spell in February, and a warm and wet summer.

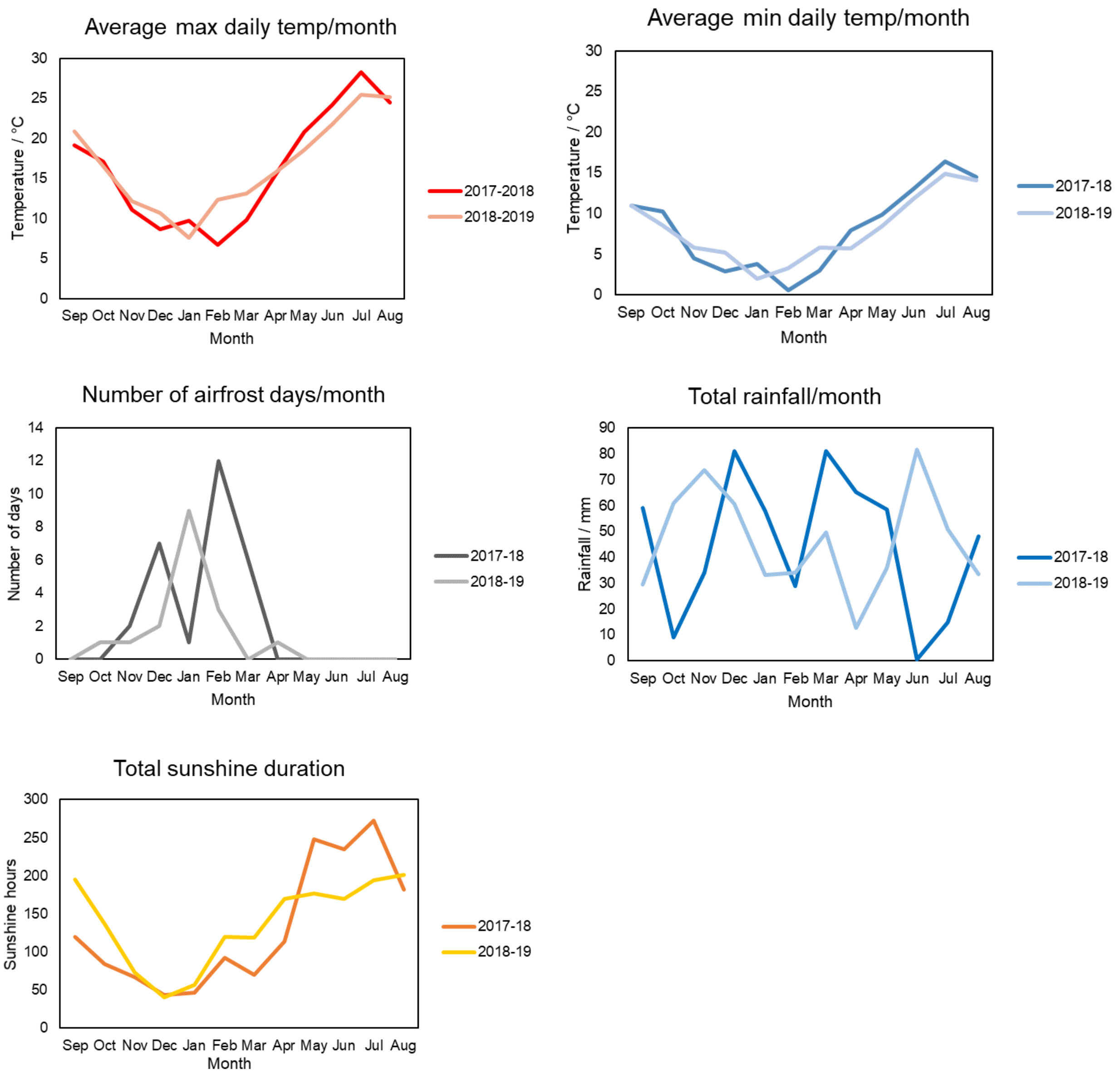


Figure 34 Average monthly weather conditions for the 2017-18 and 2018-19 years following sand lizard releases. (Raw data extracted from Heathrow Airport MET Office historic records (MET Office, 2020).)

5.4.5 Trends in juvenile sand lizard growth

Between September/October 2016 and mid-June 2017, 2016 hatchling lizard body mass had increased from an average of 1.28 g +/- 0.25 SD to 2.15 g +/- 0.56 SD by 0.87 g; a rate of approximately 0.10 g / month (Figure 35). By early September 2017, 2.5 months later, average body mass had increased by 1.95 g to 4.10 g +/-1.12 SD; a rate of approximately 0.78 g / month. A similar trend was seen for the 2017 hatchlings, with an increases from an average of 0.90 g +/- 0.13 SD in early September and .07 g +/- 0.29 SD in September October, to an average of 1.60 g +/-0.30 SD in mid-May 2018 (0.70 g and 0.53 g increase respectively); rates of approximately 0.09 g / month and 0.07 g /month respectively. This was followed by an increase to an average of 5.61 g +/- 1.60 SD by 4.01 g early in September 2018, just under four months later; a rate of approximately 1.15 g /month. Hatchling sand lizards were able to more than double their weight over ~2-3 months between hatching and first autumn release (the first September data shown in Figure 35) (Appendix D.2.)

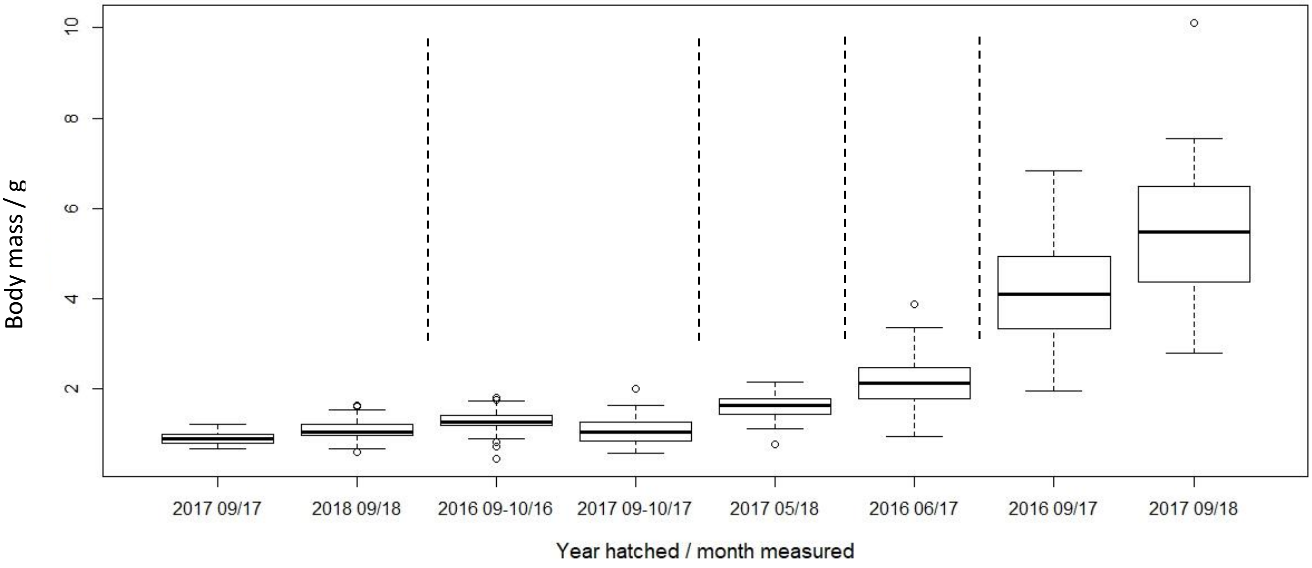


Figure 35 Body mass of lizards across years, measured September / September-October; May / June; and September. Grouped by comparable measurement months where they existed. X-axis categories listed by year hatched and date of measurement, month/year. 2017 09/17 = Marwell's 2017 hatchlings measured 06-11/09/2017, n = 76; 2018 09/18 = Marwell's hatchlings measured 06-07/09/2017, n = 63; 2016 09-10/16 = Marwell's and additional hatchlings measured 23/09-03/10/2016, n = 66; 2017 09-10/17 = additional hatchlings measured 27/09-02/10/2017, n = 49; 2017 05/18 = all 2017 hatchlings at ~ 1 year measured 14/05/2018, n = 42; 2016 06/17 = all 2017 hatchlings at ~ 1 year measured 14/06/2017, n = 56; 2016 09/17 = all 2016 hatchlings measured 02/09/2017, n = 51; 2017 09/18 = all 2017 hatchlings measured 05/09/2018, n = 24. Plot highlights the median, interquartile range, extreme values and outliers.

5.5 Discussion

5.5.1 Post-release survival of sand lizards

In the establishment stage post-release, over-winter survivorship is greater for yearling sand lizards than hatchlings and perhaps 2-year-old sand lizards (although overall sample size for this age group was very low). There were more yearling individuals seen, and a higher percentage of the yearling cohort seen, than there were hatchlings in both years, and 2-year-olds in the second release post-winter. Hatchlings may be less detectable due to their size. However, higher activity levels of juveniles and high densities post-hatching have also shown them to be the best way to determine presence of a small sand lizard population (Blanke and Fearnley, 2015). Either way, ongoing monitoring at the site will help confirm age group survival trends.

There is low apparent survival overall (although likely to be underestimated). This is not a unique observation in species reintroductions, and it may be representative of survival of wild-hatched lizards, which has not been studied. It is also not necessarily indicative of survival potential going forward. In a translocation of osprey *Pandion haliaetus* in Italy, low apparent survival is observed in juveniles over the first winter post-release, of 0.20, compared to 0.50 seen in wild-hatched juveniles elsewhere (Monti *et al.*, 2014). However, this then increases to 0.69 over subsequent winters, more comparable with the 0.87 observed in subadults at another site. A lack of parental teaching is considered the likely cause of high initial mortality, suggesting further consideration of release population demographics is necessary. During a wild-wild translocation of Texas horned lizards *Phrynosoma cornutum*, estimated survivorship is comparable to wild populations over the first two winters at 13.4 – 47.2 % and 8.9 – 54% (Miller *et al.*, 2020). The lower ends of these values are comparable to the figures observed following winter for sand lizard release for each cohort. In the instance of the horned lizards, they are actively radio tracked and located, enabling definitive monitoring of the whole population. Declines seen after two winters are attributed to the release of only 57 individuals over three years and natural lifespan being only five years. Larger release cohorts are likely therefore necessary for this species.

Number of sand lizard observations post-release suggest that either survival during the first few months post-release was greater in the 2017 cohort than the 2018 cohort, or that they were more detectable during this period. The 2017 cohort arguably experienced better conditions of milder and dryer weather during this period than the 2018 cohort, which would likely have resulted in the latter both seeking overwintering sites earlier, as well as being less active and therefore less detectable during surveys. Although the number of observations per survey did not differ between the 2017 and 2018 release cohorts, observations in 2019 continued throughout the summer, whereas these dropped off in 2018. The 2018 heatwave is likely to have again influenced detectability during this period, and as discussed survey effort was also reduced due to this. The study reiterates the importance of not only sufficient post-release monitoring, but also its longevity and consistency (Parker *et al.*, 2013), when individuals may miss detection for extended periods due to factors out of survey control. It highlights the apparent temporally shifting nature of the population's state and thus dynamic success of the reintroduction as a whole (Seddon, 1999).

5.5.2 Effects of demographic differences on survival and movement post-release

Yearling sand lizards showed higher levels of activity within the release square than both hatchling and two-year old lizards (although this was not significant) and took significantly longer to leave this space. This suggests a more tentative approach to the novel environment than the juvenile lizards, who are perhaps displaying natal-type dispersal at this stage (Meylan *et al.*, 2002), and older animals that have developed bolder traits through sexual maturity (Olsson *et al.*, 2010). There was no difference between sex (where known) in terms of response to the release environment although exploration and activity was overall higher for males. There was also greater range in furthest distance reached and the greatest maximum distance outlier was for male lizards, although again this was not significant. This difference may increase to show significance were it examined during breeding season, or with larger sample size, due to the increased sexual selection pressure on males (Blanke and Fearnley, 2015). Aggression exhibited in competing male lizards is stated to drive optimal distribution of individuals across a site (Verbeek, 1972).

The furthest distances reached by hatchling lizards over and above the other age groups pre-winter indicate that the diversity within juvenile sand lizards in terms of their natal dispersal is retained within individuals reared in captivity for 1-2 months. Depending on the release site in question, dispersing widely may be problematic due to habitat suitability beyond the immediate release area. Movement out of intended habitats immediately following release is observed in other species as has been discussed in 5.1.1, such as over half of thrush *M. palmeri* individuals released ultimately leaving their target area (Foster *et al.*, 2003). Timing of current protocols intends for movement away from a release location by sand lizards to be limited in the immediate post-release phase by seasonal shifts towards winter. It is hoped that lizards will more likely successfully brumate in afore-determined optimal habitat near the point of release to optimise survival (perhaps utilising the artificial starter burrows created) (ARC Trust, pers. comm.). This study suggests that for hatchlings at least, the current primary release demographic, this may not be occurring.

5.5.3 Trends in inter-annual growth

Sand lizards were able to more than double their body weight in the first 2-3 month period following hatching. This rate of growth then slowed over their first winter and the following spring, as indicated by both the 2016 and 2017 hatchlings, prior to a rapid body mass gain over their summer as a yearling lizard. The growth patterns highlight the importance of the spring and summer following a successful overwintering period for juveniles to regain lost condition and then undergo a phase of rapid growth into subadults prior to the following brumation period, in order to optimise their survival to the following spring. The growth rates correspond with studies suggesting sand lizards have the capacity to reach adult body size in their first summer following brumation (Nicholson, 1980).

Where growth and body condition is sufficient, breeding in sand lizards' second year can be seen (Bischoff, 1984; Blanke and Fearnley, 2015), as it has been for this release cohort through the observation of several test burrows and a female lizard in the action of digging and backfilling a burrow. Confirmation of breeding at this early stage shows individuals have survived the winter and regained body condition sufficiently to reach sexual maturity and develop eggs, implying they have established themselves well in the wild setting. Early breeding by the released individuals allows potential for hatching of wild-bred individuals within just three-four years post-release, a marker coined as one measure of project success (Sarrazin and Barbault, 1996a).

5.5.4 Conservation implications of research and future directions

Current protocols suggest release of sand lizard cohorts as hatchlings. This age group has comparatively low body mass (even if optimal for their age), appearing to result in lower survival through the winter of this age group compared with older animals. Sand lizards are able to breed after two winters if conditions have been favourable to their development (Bischoff, 1984), as was observed inconsistently across a captive population of two-year olds in captivity (R. Gardner, personal observation) and evidenced post-release at Eelmoor Marsh.

If sand lizards were retained in captivity for their first year, they would more likely brumate successfully due to ample food and good burrowing opportunities, as shown in survival of 79.7 % of hatchling juveniles over winter in captivity 2016-17 (author's own obs). They would then gain more optimal condition before being released as subadults in time to establish themselves at the release site and prepare for their second winter – which they would also be more likely to survive due to their greater body mass than hatchling lizards post-release, as shown in this study. More lizards released as yearlings would be likely able to breed in their second year due to improved body condition (Bischoff, 1984) gained from captive conditions, than had they been released as hatchlings.

In summary, retaining hatchlings (or at least a proportion of them) for a year would result in a more optimal body mass on release, leading to greater survivorship and earlier breeding. This would also discourage a post-release genetic bottleneck, potentially narrower than that already existing in the captive population from which the lizards have come. Reintroduction-induced bottlenecks have been shown to cause loss of great genetic diversity from the release generation (Bristol *et al.*, 2013). Minimising this as far as possible is therefore advantageous to the population going forward.

By releasing subadults there is also potentially scope to decrease the number of lizards released at a site, or the number of annual releases, due to the increased survivorship and faster rate of recruitment. This would offer potential for lizards to instead be divided across a greater number of release sites each year. Establishing the limitations for this would need careful consideration. Extrapolating apparent post-winter survival summarised herein (taking the overall more positive 2018 release data) suggest that out of a prospective 80 hatchlings released, 8 (7.8) may survive the winter; however if 80 yearlings were released, this survival could be 32 individuals, four times the number. Ongoing surveys at Eelmoor Marsh will help ascertain whether hatchling survival has been underestimated due to poor detectability of younger lizards. However, the results to date infer a clear advantage in releasing yearlings to maximise this animal resource.

If release cohort numbers were dropped due to greater release generation survival, the rate at which this species is being returned to its former range could be increased, in line with increasing habitat restoration. By doing this, ultimately the gaps and corridors between fragmented populations of optimal sand lizard habitat that have not been traversed due to low mobility of the species, may be re-populated more quickly. This would allow faster reconnection of isolated populations, increased gene flow and the southern UK heaths sand lizard metapopulation to faster reach positive status (Edgar and Bird, 2006).

The implications of retaining individuals in captivity for longer must however be considered, not in the least in terms of their heightening or relaxing behavioural traits due to effects of the captive environment (e.g. perceived threats never materialising, unnaturally high densities of conspecifics). The apparently poorer survival of two-year old lizards suggests that retention in captivity to this age may be too long, although sample size was small. A detailed examination into the effects of captive retention on behaviour, age-related thresholds for this, and methods that could minimise it would be a helpful future research direction.

Realistically, retaining and releasing solely yearling sand lizards is likely impractical for most breeders involved in the reintroduction initiative, due to breeding facility capacity and additional husbandry considerations. Attempting to release some yearlings as part of the release cohort would be more optimal than not doing this at all.

5.6 Conclusions

- Walking transects and refugia allowed meaningful data to be obtained on two release cohorts of sand lizards, confirming the validity of this method for post-release monitoring, when time resources allow and in the absence of reliable remote monitoring methods for this species at present.
- Sand lizards that had overwintered once in captivity and were released at a full year old showed higher survival than those released as hatchlings (current reintroduction guidance).
    - The spring-summer period following juvenile sand lizards' first winter is a key time of growth, which can be optimised in a captive setting with ample food resource. Captivity also offers optimal overwintering opportunities for hatchlings leading to good survival rates.
- Where sex was known, pre-winter distances travelled were more variable for males than females.
- Hatchling lizards were significantly quicker at leaving the immediate release area than yearlings and went on to travel almost significantly further from their release location than yearlings pre-winter. In doing this, hatchlings may have left the optimal habitat at a release location and also moved away from conspecifics and thus future breeding potential.
- Lizards released as yearlings have the capacity to breed in the wild within a year of release (at two years of age). Breeding by the first wild-hatched generation is therefore possible within three-four years of release.
- Apparent improved survival and greater release area fidelity in yearlings, as well as more rapid population recruitment, suggests this age demographic may be preferable for release.

Chapter 6 The effect of individual variation in sand lizard *Lacerta agilis* response to reintroduction at a dry lowland heath site

6.1 Introduction

Variation in individuals will likely affect survivorship and dispersal following reintroduction (Meylan *et al.*, 2002; Dingemanse *et al.*, 2003). It is therefore beneficial to follow a population at individual level through the process. Assessment of such factors as morphometrics, locomotive fitness and behaviour, allows a gauge of the common characteristics of those individuals that persist, and their variability in dispersal distance and rate, to inform future translocation efforts.

For reintroductions of the sand lizard *L. agilis* in the UK, which have taken place for fifty years (Corbett and Moulton, 1998; Moulton and Corbett, 1999; Woodfine *et al.*, 2017), it would be of great benefit to conservation practitioners to understand in more detail how lizards move and establish themselves within their new habitat. Examining this alongside individual variation would contribute to an assessment of whether current reintroduction protocols are appropriate, in terms of numbers of animals released, habitat management at the release site and the selection of animals; the latter is currently based primarily on availability within captive breeding populations.

Specific movement behaviour is important in terms of dispersal and it is found that metapopulation model dynamics can be sensitive to small differences in assumed post-release movement (Hawkes, 2009). There is therefore a need to understand the movement patterns shown by individuals post-release for such data to be incorporated into wider, landscape-scale modelling. It is increasingly important for this to be accurate, with pockets of suitable habitat adjacent to land that is not, and corridors and landscape permeability at the national scale through an increasingly human-dominated landscape (Crick *et al.*, 2020).

6.1.1 Individual differences of morphometrics, body condition, locomotive performance and behavioural response to release

Morphometric factors have been found to show differing trends in terms of post-release survivorship and movement for different species. There has been focus on this topic for some years likely given the relative ease of obtaining such data pre-release and the common hypothesis that larger animals are more likely to survive longer. Some studies agree with this (Janzen *et al.*, 2000; Nagy *et al.*, 2015). Nagy *et al.* (2015) find survivorship of juvenile Agassiz's desert tortoises *Gopherus* *agassizii* head-started in captivity increases with size and age. However, others show no link, with survivorship of neonatal Western rattlesnakes *Crotalus viridis* being independent of both weight and condition at birth (Charland, 1989) and a long-term release and recapture experiment of snapping turtles *Chelydra serpentine* showing no suggestion of the "bigger is better" concept. Body mass is seen to positively correlate with survival for hatchling common lizards *Zootoca vivipara* between birth and hibernation (Sorci and Clobert, 1999). High early winter masses also improve both overwinter and annual survival rate of Canvasback *Aythya valisineri* males (Haramis *et al.*, 1986). It is suggested that larger sand lizard hatchlings have greater chances of survival (Blanke and Fearnley, 2015). Reptiles can lose up to 10 % of body mass during overwintering (Gregory, 1982) therefore optimising body mass prior to this period is advantageous, particularly for younger individuals.

Trends have also been observed between body size and post-release spatial behaviour following reintroduction. Body size-dependent strategies are observed in natal dispersal (Einum *et al.*, 2012), however direct application of this to post-release movement of a species following reintroduction should again be cautionary, due to variation in other factors that may be influencing habitat selection between natal and post-release dispersers, such as varying effects of social factors (Richardson and Ewen, 2016). A positive correlation between dispersal and body size, closely linked to fitness, is observed in green frogs *Rana clamitans* released to experimental ponds (Searcy *et al.*, 2018). Reintroduction of Hermann's tortoise *Testudo hermanni hermanni* observed a positive correlation between body size and home range size established post-release (Dreschler *et al.*, 2016). This highlights the importance of species-specific assessments to allow predictions for post-release movement and survivorship to maximise chances of success.

Linked to morphometrics is the body condition of individuals being released. Some lizards, including the sand lizard, are capable of anti-predatory caudal autotomy, enabling them to drop their tails to deter a predator while they escape. This may have associated fitness costs to the individual in terms of locomotion, behavioural changes and metabolism; studies into this have been thoroughly reviewed (Arnold, 1984; Cooper *et al.*, 2004; Bateman and Fleming, 2009). Tail loss is shown to affect locomotion speed, and thus capacity to escape predation, in some studies but not all (McElroy and Bergmann, 2013; Gillis and Higham, 2016). The capacity for compensatory shifts in hind limb movement, such as changes in stride length and frequency may result in no effect being seen in some cases (Gillis and Higham, 2016). Additional impacts, such as on manoeuvrability and climbing may also occur depending on the species (Gillis and Higham, 2016). The tail is also an important store of lipids within many lizards, and therefore a key energy source (Doughty *et al.*, 2003). Juvenile sand lizards appear less likely to suffer tail break than older lizards (Blanke and Fearnley, 2015) and it is found that larger male lizards suffer greater associated stress than smaller males and females; following tail loss they have also been observed to shift to a more cryptic lifestyle (Olsson *et al.*, 2010).

Another variable component of body condition in lizards is toe loss which, unlike tail loss where some degree of regeneration occurs, is permanent. Toe loss is a common occurrence in sand lizards and is noted during health screening of captive lizards at Marwell Zoo. Toe clipping has long been used as a technique to identify individual legged reptiles and amphibians (McDiarmid *et al.*, 2012; Dodd, 2016). However, where some studies find this has no effect on stress levels (Langkilde and Shine, 2004), or despite some concern still advocate it instead of other potential marking mechanisms such as PIT tags (Guimarães *et al.*, 2014), others view it as a particularly poor method with regards to animal welfare (Parris *et al.*, 2010). Typical measures of the effect of toes loss are of survival based on recapture rate (Parris *et al.*, 2010; Guimarães *et al.*, 2014) or stress represented by corticosterone levels (Langkilde and Shine, 2004). The former is difficult to confirm as a causal relationship when it is possible that toe loss has instead caused a shift in behaviour that may result in lower recapture likelihood due to increased crypsis, as observed with male sand lizards and tail autotomy (Olsson, Pauliny, *et al.*, 2010). A measure of individual ability to respond to threat would better determine the effects of toe loss on an individual (be this through clipping or natural loss).

The effects of morphometrics, tail loss and toe loss on the ability of an animal to respond to a perceived threat are vital parameters to consider when reintroducing a population. If locomotion for example, is affected by loss of appendages or tail (Guimarães *et al.*, 2014) or this causes a shift in behaviour to acting more cryptic (Olsson *et al.*, 2010), there will be knock-on and, to a degree, predictable effect on the release population if this data is gained about individuals in advance. Flight from a predator occurs as the relative cost of remaining and escaping shift, as a response to predator proximity (Ydenberg and Dill, 1986). Flight initiation also varies in relation to individual differences such as level of experience, with greater experience of predators increasing perceived risk (Stankowich and Blumstein, 2005). In the Balearic lizard *Podarcis lilfordi* flight initiation distance is greater for lizards in better body condition, however also greater for those that have undergone tail autotomy (Hawlena *et al.*, 2009). Both morphologically fit individuals protecting their condition and those that no longer have tail autotomy as a survival advantage opt to flee when the threat is comparatively low. The type of relationship between such variables varies between species in different habitats and with different social behaviour (Stankowich and Blumstein, 2005; Cabido *et al.*, 2008). The common lizard *Z. vivipara*, exhibits crypsis with tail loss in response to a predator, and flees at shorter distances (Capizzi *et al.*, 2007); it is suggested that escape strategy may vary specifically between ground-dwelling and climbing species given the opposite response observed in the rupicolous lizard *Iberolacerta horvathi* within the same study. Locomotor responses by wild sand lizards have shown increased speed with tail autotomy, with shorter tails resulting in faster running speed; this could be due to physically lower body weight or perceived increased risk to life (Ekner-Grzyb *et al.*, 2013). Basic sprint speed has also been linked to number of offspring sired by male collared lizards *Crotaphytus collaris*, with no other measure of morphometrics, testosterone or corticosterone levels affecting this. This shows a direct link between whole-animal performance and reproductive success (Husak *et al.*, 2006).

Immediate response to release in a novel environment by sand lizards will be impacted by individual behavioural syndromes, akin to 'personalities', that have been identified for this species in captivity as repeated behavioural responses (Jordan, 2018). Such behavioural response was considered at a demographic level in Chapter 5. The importance of including behaviour in applied wildlife conservation is increasingly recognised (Merrick and Koprowski, 2017; Berger-Tal *et al*., 2019). It has been one of the least represented topics in reintroduction (Seddon *et al.*, 2007) despite being reported as the cause of a third of the problems encountered by practitioners (Berger-Tal *et al*., 2019). Previous research indicates repeatable behavioural responses (equating to syndromes or personalities) are present within lizards (Jordan, 2018).

6.1.2 Aims

The overarching aim of this chapter is: **to determine the effect of individual variation on post-release movement and post-winter survival of reintroduced sand lizards, and consequent implications for reintroduction protocols.** Its objectives are:

1. To assess spatio-temporal observations of individual sand lizards post-release
2. To determine if morphometrics, locomotive fitness and response to release are predictors of sand lizard
    i. survival through first winter,
    ii. distance travelled from release location.
3. To assess the effect on sand lizard locomotive performance of
    i. body weight,
    ii. body condition in terms of tail autotomy and toe loss.

6.2 Methods

6.2.1 Morphometrics

Details of morphometric measurements taken are provided in 5.2.3. Any additional notable features such as scarring, and loss of tail or toes were also recorded. As noted, where every effort was made to catch up all lizards to obtain the repeat morphometric data, several were typically missed on each occasion, resulting in inconsistent sample sizes in results.

6.2.2 Photography and individual identification

Individual sand lizards were all uniquely identifiable by their dorsal lines and spots, or ocelli. These markings are retained throughout their lives, although some brightening, darkening and stretching of features does occur. Ocelli patterns typically fell into three broad categories: spotty, stripy and most typically somewhere between the two. Pattern types differed within clutches (Figure 36).

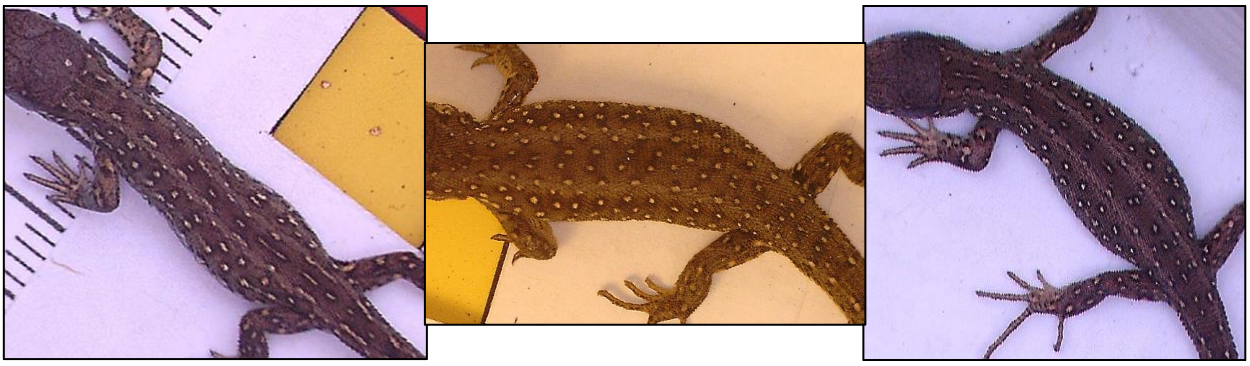


Figure 36 Three hatchling sand lizards showing the three different dorsal pattern types (left to right: stripy, spotty, a combination).

Most photographs in the captive environment were taken using a computer-attached (USB) light microscope and software QMicroCapture Pro. For lizards older than one year, a digital SLR camera (Canon EOS 1100D) with a macro-lens (EF 75-300 mm f/4-5.6 III USM) were used instead. Lizards were placed on a white background and adjacent to a scale and ColourCard, allowing calibration of images in different lighting conditions, should this be required to assist with identification.

All lizards were assigned a unique code and a database of ID photographs was created. An accompanying spreadsheet detailed all data collected for each individual pre- and post-release. Identification software (I3S Pattern+ version 4.1) was looked into to assist with matching photographs (Dodd, 2016). However, the time taken to upload and prepare each image was comparably lengthier than manually matching images in this instance where the number of lizards totalled less than 300, and different age groups narrowed down possible options to far fewer than this.

Photographs in the field at the point of release and during post-release monitoring (Figure 37) were taken using the Canon camera and lens (as above). Patience was required to obtain photographs of sufficient clarity, quality and proximity to identify lizards post-release, and without causing undue disturbance to them. The multiple high-quality, whole-body photographs taken of lizards in captivity meant that in instances where post-release images only detailed a section of a lizard's back due to vegetation cover, there was still a high likelihood that the individual could be identified.

Figure 37 Photographs of a) two yearling and b) two hatchling sand lizards taken in the field, showing obvious differences between spot patterns and suitable photograph quality to allow individual identification; c) a yearling (L) and a hatchling (R) photographed immediately pre-release.

6.2.3 Locomotor performance

A locomotion test was undertaken when individuals were returned to vivaria after collecting pre-release photographs and morphometric data, or, for yearlings/subadults, after summer measurements in 2017, (Vanhooydonck and Van Damme, 2003; Ekner-Grzyb *et al.*, 2013). This test had been used to assess locomotor performance in adult sand lizards at Marwell Zoo in a study examining behaviour and fitness of breeding adults (Govier, 2017); assistance with collecting the locomotor data in 2017 was kindly given by B. Govier. Lizards were placed in turn at one end of an open-ended half-round pipe, covered with a thin layer of sand, in an area of the research vivarium and encouraged to move along this by manually sweeping a brush at a steady pace behind them; this aspect was undertaken by the main researcher across all years to avoid experimenter effects (Figure 38). For hatchling lizards, the total length traversed was 40 cm, with ten segments of 4 cm analysed separately along the pipe; for subadult lizards, the total length was 100 cm, with ten segments of 10 cm; segments were measured and overlain onto the footage manually during analysis. The difference in pipe lengths equated roughly to the differences in size between hatchling and subadult lizards.

Figure 38 Paused video footage of the locomotor performance test on a subadult sand lizard.

Each lizard was placed through the run once (as pre-determined by ethical limitations, ERGO II IDs 27600 / 23009) and a video recorder (Sony Handycam) was set to record each test. Locomotor tests were consistently undertaken on days of good lizard activity with partial cloud cover and temperatures between 20 – 25 °C. Lizards were held in groups in plastic terraria in a semi-shaded area prior to having morphometrics and photographs taken (~ 2 minutes per lizard) and then undergoing the locomotor test, maintaining as comparable a pre-test environment as possible for each lizard. Videos were subsequently examined using VLC Media Player, where the video was converted to frames (30 frames per second) and the number of frames for the individual to traverse each segment calculated; a segment was classed as entered once the lizard's snout had passed the segment line. Time taken for each lizard to traverse its fastest five segments was calculated as the single measure of locomotor performance; this, as far as possible, removed the effect of occasional pauses which the majority of lizards had. On several occasions, lizards either did not traverse the whole length of the pipe (stopped or tried to climb out) or did not move at any speed down it (i.e. paused repeatedly the whole way down). These lizards were not included in subsequent locomotion analyses.

6.2.4 Immediate response of sand lizards to release into a novel environment

The response of sand lizards to release was recorded as detailed in Chapter 5 (5.2.1).

6.2.5 Post-release monitoring

Post-release survey methodologies are detailed in Chapter 3 (3.2.2) and Chapter 5 (5.2.2).

6.3 Analysis

All statistical analyses were undertaken in RStudio 1.1.463 (R version 3.5.3)

Time-series line charts for post-release spatial observations of lizards included all observations (both during surveys and *ad hoc* sightings outside survey time). Measures were calculated based on where that specific lizard was released (locations / release squares 1-8) and from release date of each individual (accounting for the 2018 release over two dates). Cumulative minimum distance travelled, and maximum distance reached from the point of release for each lizard were calculated using trigonometry by assuming the shortest possible distance traversed between observations. The route between observations was unlikely to have been direct; however the measure offers as comparable a representation as possible of the variation in post-release movement.

The effect of lizard body mass, locomotor performance and response to release (exploration, activity and time taken to leave release square) on apparent survival post-winter (survived / unknown) were analysed using Mann-Whitney U tests and student's *t*-tests; time data were log transformed. The effect of body mass, locomotor performance and response to release on distance travelled by lizards observed at least once post-release pre-winter, were analysed using linear models and Spearman's Rank; time data were log transformed. This series of testing allowed the small sample sizes to be maximised for each aspect (where not all individuals had the full data suite, as previously discussed) in order to pinpoint any single key factors that may be indicative of post-release behaviour and apparent survival, with the potential for this to be assessed in a captive environment pre-release. Correlations between body mass and locomotor performance were tested using Spearman's Rank. Differences in locomotor performance by individuals suffering tail loss (classified as anything $\geq$ 10 % anticipated original tail length) and toe loss (> 1 toe) were assessed using Mann-Whitney U tests. All data were displayed as scatterplots or boxplots as appropriate.

6.4 Results

6.4.1 Spatiotemporal observations of lizards in relation to release location

A variety of spatiotemporal behaviours were shown in Figure 39, Figure 40, Figure 41 and Figure 42; colours correspond for individuals between adjacent figures; includes sightings outside surveys.)Pre-winter in 2017 (Figure 39, Figure 40), there was a lot of activity within 30 m of the release site. Eight yearlings were not seen beyond 10 m from their release point. The movements of one yearling and two hatchlings were particularly large and quick. Eight yearlings travelled more than 15 m from their release point, then returned to within 5 m. In 2018, the 2017 cohort yearlings observed were within 40 m of their release site. One hatchling was observed, within 10 m of its release, and found dead on 30/03/2018. In 2019, three yearlings from the 2017 release were seen $\geq$ ~ 40 m from the release site; one male (light green, Figure 40a), repeatedly looped back to the release area.

The 2018 release cohort was not observed with as great a frequency as the 2017 cohort pre-winter post-release (Figure 41, Figure 42). However, within nine days post-release, three yearlings had been found further than any yearlings from the 2017 cohort were seen to reach in the same time. Of the 12 hatchlings located before winter, all but three were observed more than 20 m from their point of release and of these, two were found more than 60 m away. Following winter 2018-19, two 2-year olds from the 2018 cohort were observed. Ten yearlings were observed including one female consistently within 30 m of her release site and looping back to within 20 m (orange, Figure 42; the same female shown digging in Figure 31). Six further yearlings were consistently within 40 m of their release site; again showing looping return patterns. The five 2018 hatchlings observed post-winter were a wide variety of distances from the release site.

Across both release cohorts, release site return (fidelity) is more apparent in yearlings than hatchlings. Minimum distance travelled is broadly comparable between hatchlings and yearlings (including within-season), with the few greater distance exceptions being yearling lizards.

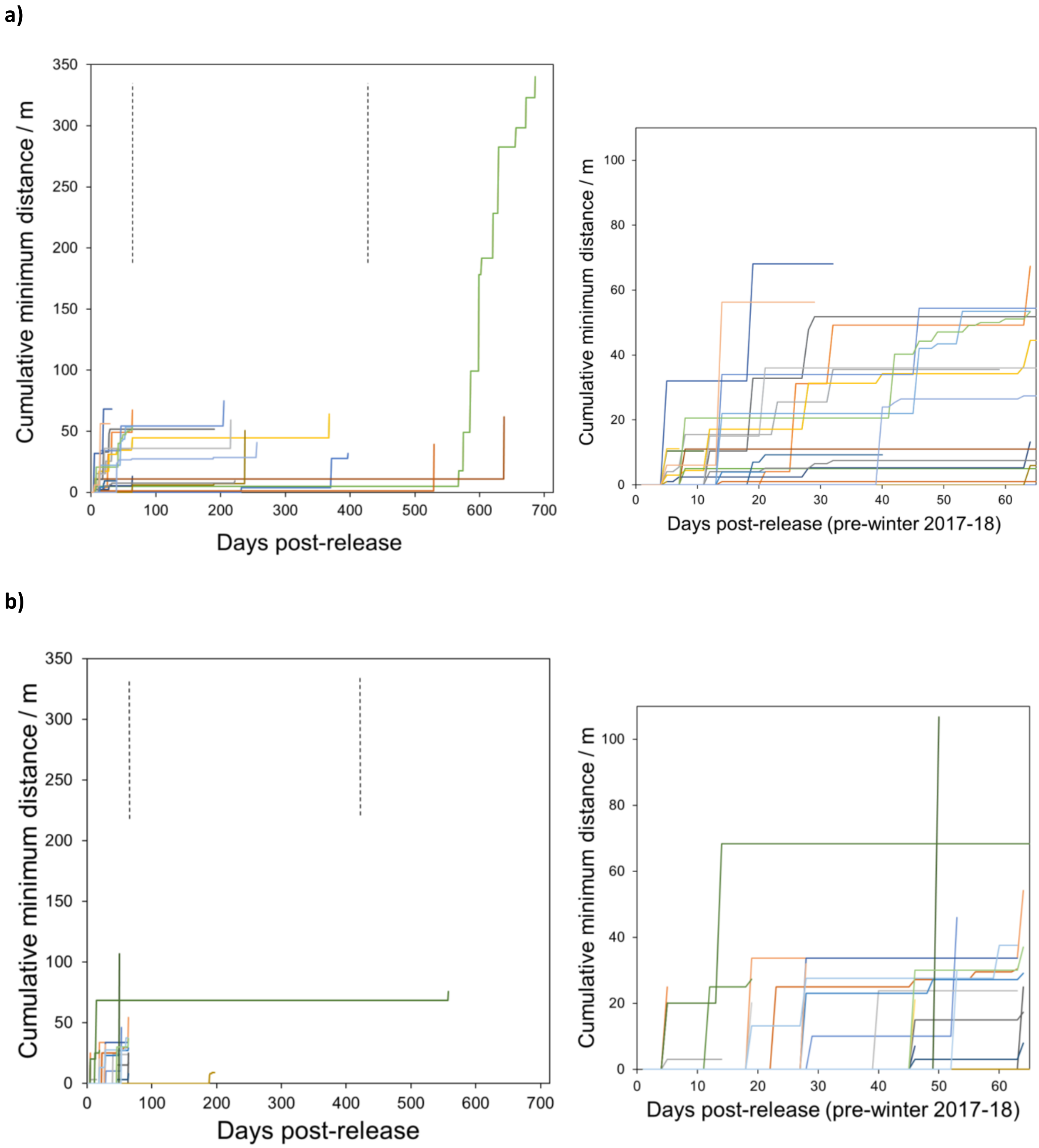


Figure 39 2017 release cohort cumulative minimum distance traversed by a) yearling sand lizards (total released n = 26, observed at least once post release n = 23); and b) hatchling sand lizards (total released n = 54, observed at least once post-release n = 24) including all observations on left, and detailed study on right of immediate post-release, pre-winter observations. End of season observations are indicated using dashed lines.

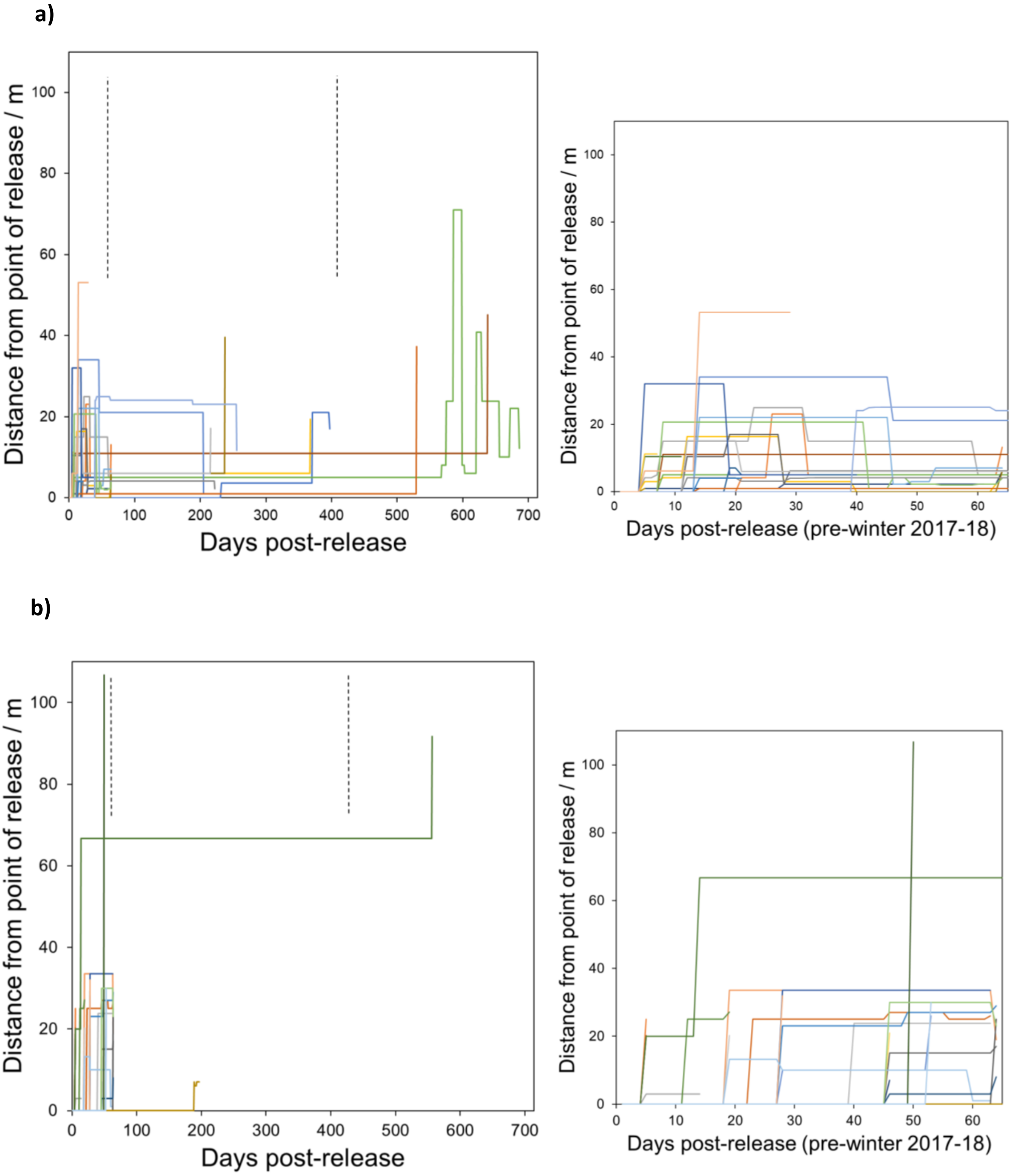


Figure 40 2017 release cohort distance from release site of each observation of a) yearling sand lizards (total released n = 26, observed at least once post release n = 23); and b) hatchling sand lizards (total released n = 54, observed at least once post-release n = 24) including all observations on left, and detailed study on right of immediate post-release, pre-winter observations. End of season observations are indicated using dashed lines.

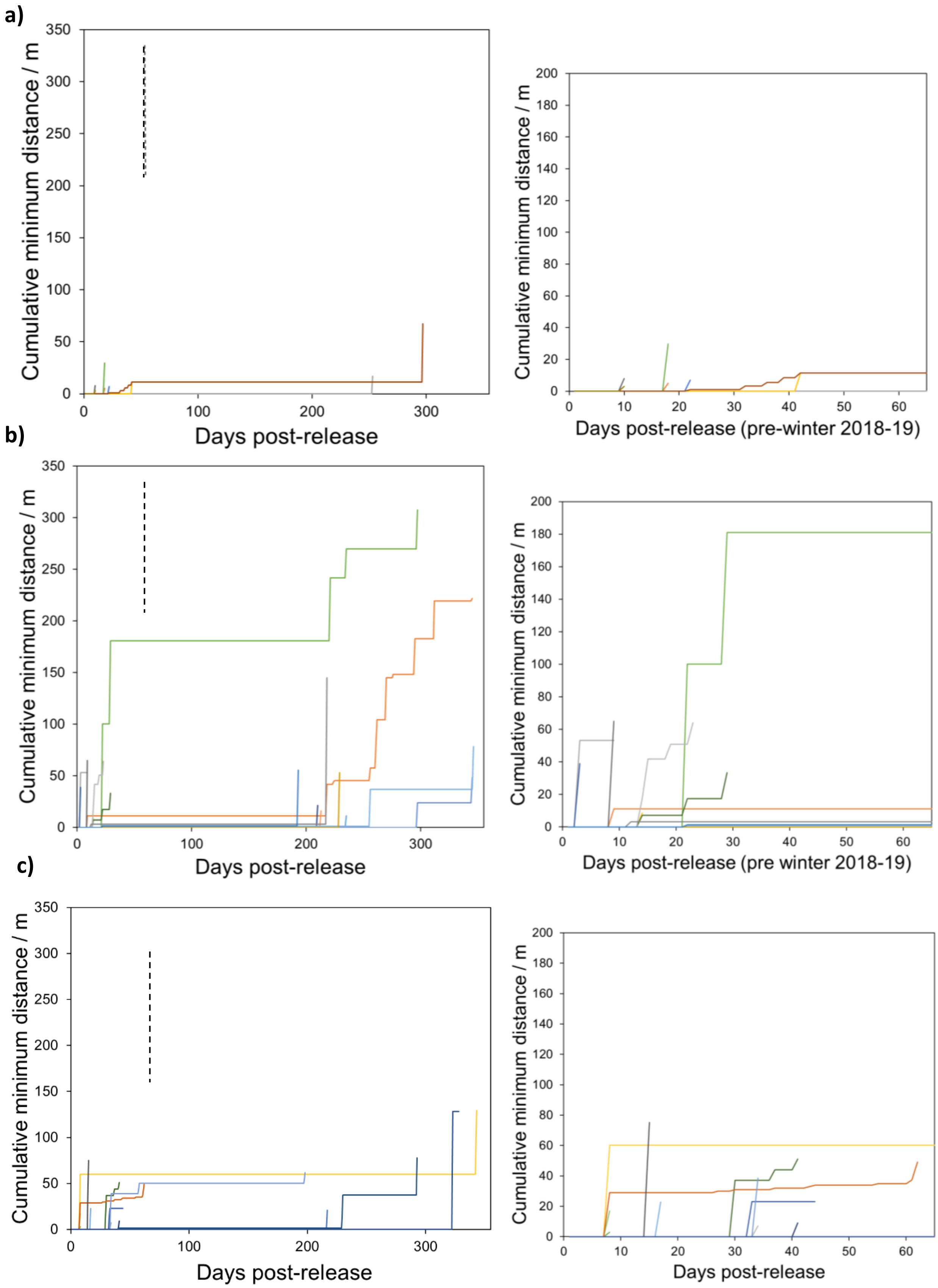


Figure 41 2018 release cohort cumulative minimum distance traversed by a) 2-year old sand lizards (total released n = 10, observed at least once post release n = 8); b) yearling sand lizards (total released n = 25, observed at least once post release n = 16); and c) hatchling sand lizards (total released n = 51, observed at least once post-release n = 14) including all observations on left, and detailed study on right of immediate post-release, pre-winter observations. End of season observations are indicated using dashed line.

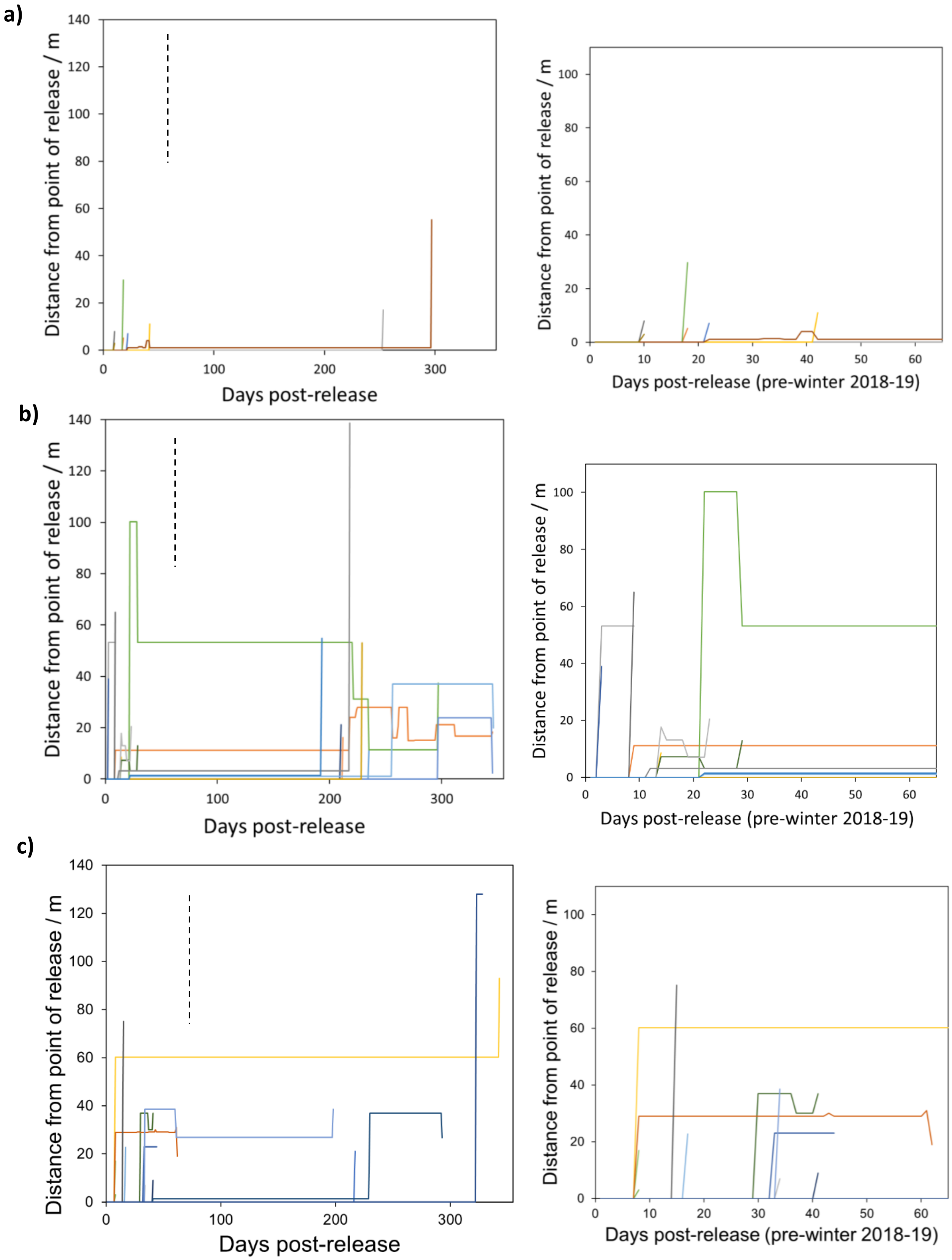


Figure 42 2018 release cohort distance from release site of each observation of a) 2-year old sand lizards (total released n = 10, observed at least once post release n = 8); b) yearling sand lizards (total released n = 25, observed at least once post release n = 16); and c) hatchling sand lizards (total released n = 51, observed at least once post-release n = 14) including all observations on left, and detailed study on right of immediate post-release, pre-winter observations. End of season observations are indicated using dashed line.

6.4.2 Effects of morphology, locomotor performance and release response on survival and movement

6.4.2.1 Yearling sand lizards

There was no difference in weight of yearlings known to have survived the winter (mean: 5.006, 95% CI[4.045, 6.205]) and those that were not seen after it (mean: 4.473, 95% CI[3.827, 4.803]) (t_{44} = 0.955, p = 0.348) (Figure 43). The median weight of the yearlings known to survive (5.13g) was almost equal to the upper quartile of those whose survival is unknown (5.17g). The individual known to have died pre-winter weighed 4.31 g.

There was no correlation between yearling lizard body mass and furthest distance reached when fitting a linear regression ($F_{1,34}$ = 1.406, p = 0.244, R^2 = 0.040) (Figure 43).

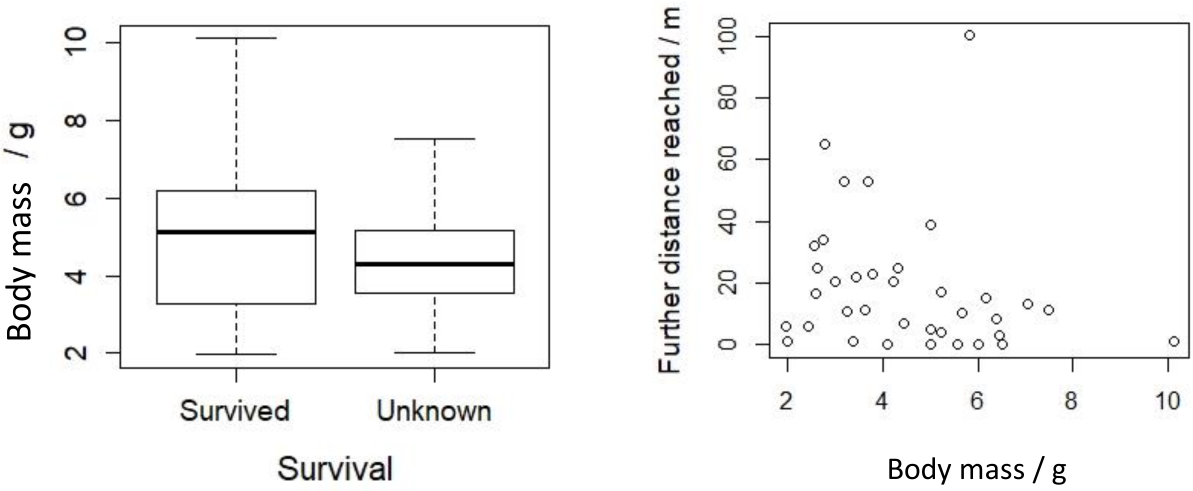


Figure 43 Weights of yearling lizards that survived the winter (n = 18) and those that were not seen after it (n = 28); and weights and corresponding furthest distance reached by yearlings that were observed at least once post-release (n = 36). Boxplot highlights the median, interquartile range, extreme values and outliers.

Yearlings that survived the winter were significantly faster in the locomotion test compared to those that were not observed post-winter ($U_{17,23}$ = 121.0, p = 0.043) (Figure 44). The individual that died performed the locomotion test in 1.27 seconds. There was no correlation between locomotor performance and furthest distance reached by yearling lizards pre-winter ($F_{1,29}$ = 2.039, p = 0.164, R^2 = 0.066) (Figure 44).

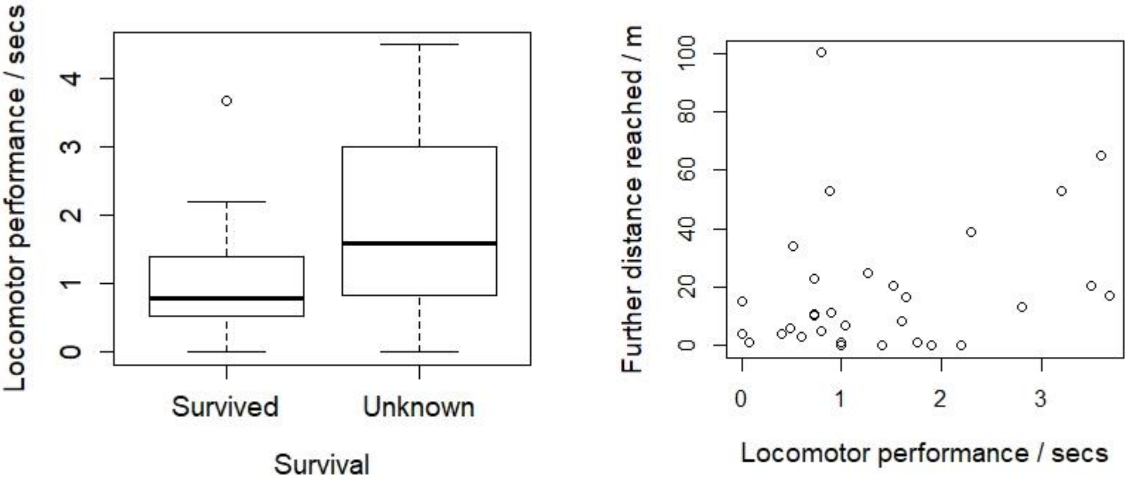


Figure 44 Locomotor performance of yearling lizards that survived the winter (n = 17) and those that were not seen after it (n = 23); and locomotor performance and corresponding distance reached by yearlings that were observed at least once post-release (n = 31). Boxplot highlights the median, interquartile range, extreme values and outliers.

Individuals that were confirmed to have survived through the first winter were significantly less exploratory ($U_{19.29}$ = 148.50, p = 0.006) and less active ($U_{19,29}$ = 152.50, p = 0.008) in response to release compared to those individuals who were not encountered. There was no significant difference in time taken to leave the release square (t_{46} = -0.845, p = 0.403) and survival over-winter (Figure 45a). The single deceased yearling found pre-winter had exploration and activity scores of 4 and took 184.1 seconds to leave the release square. Yearlings that travelled furthest from their release location pre-winter showed greater exploratory (rs = 0.476, p = 0.003, n = 37) and active (rs = 0.533, p < 0.001, n = 37) behaviour at release. There was not a significant relationship between time to leave the release square and distance reached ($F_{1,35}$ = 3.124, p = 0.086, R^2 = 0.082) (Figure 45b).

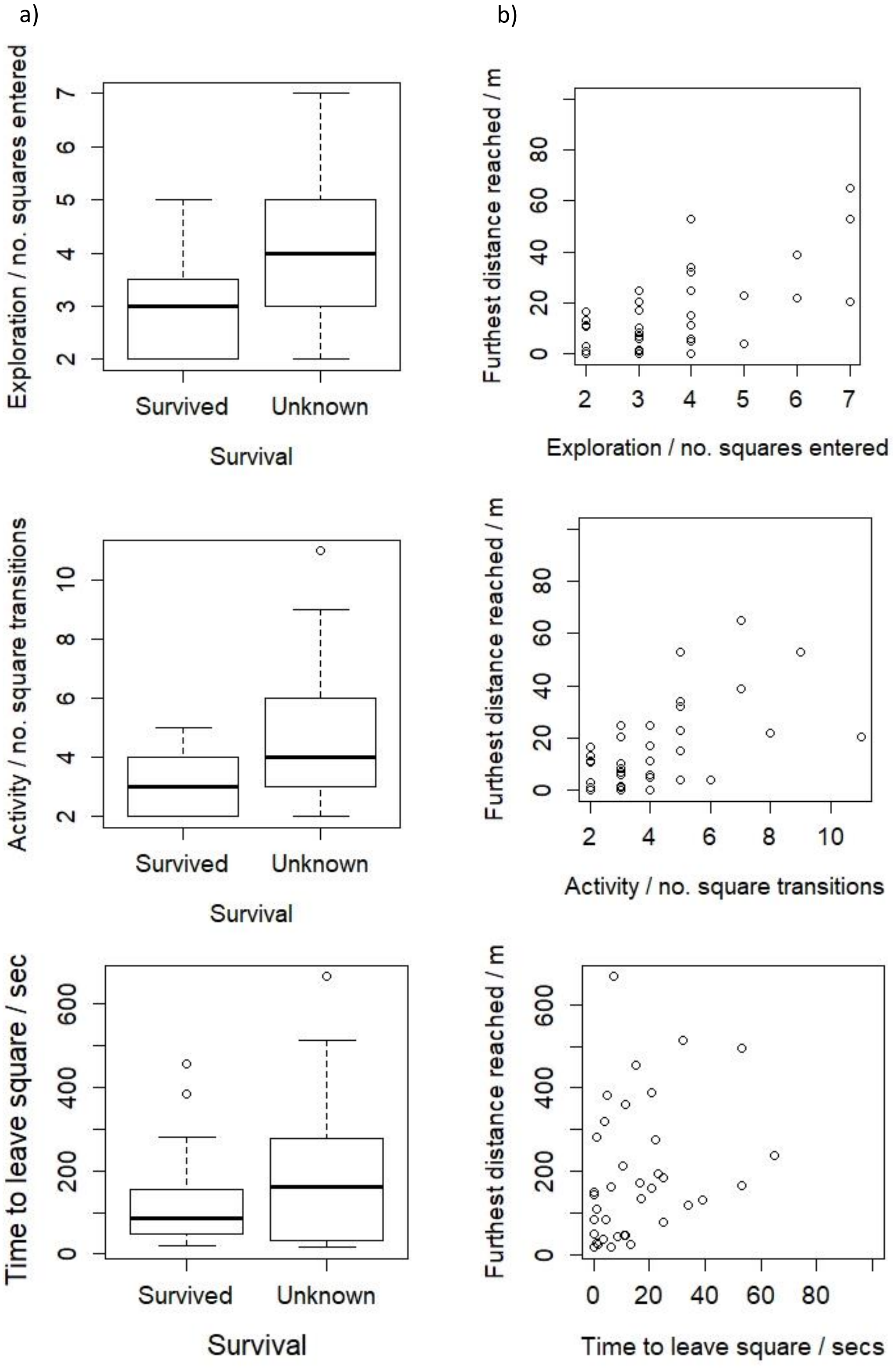


Figure 45 a) Measures of exploration, activity and time taken to leave the release square for yearlings that were known to have survived the winter (n = 19) and those that were not seen after it (n = 29); and b) furthest distance observed at pre-winter and corresponding measures of exploration, activity and time taken to leave the release square for yearlings that were observed post-release (n = 37). Boxplots highlight the median, interquartile range, extreme values and outliers.

6.4.2.2 Hatchling and two-year old sand lizards

There were no significant effects of morphology, locomotor performance and release response on survival and movement of hatchling sand lizards (released n = 105). Full results can be found in Appendices E.1, E.2 and E.3).

The sample size of two-year-old sand lizards was too small (released n = 10) for robust statistical analyses.

6.4.3 The effect of body mass and body condition on locomotor performance

There was a negative correlation between lizard body mass and locomotor time in hatchling sand lizards (*rs* = -0.200, *p* = 0.021, n = 134) (Figure 46a). There was a positive correlation between lizard mass and locomotor time in yearling sand lizards (*rs* = 0.262, *p* = 0.031, n = 68) (Figure 46b).

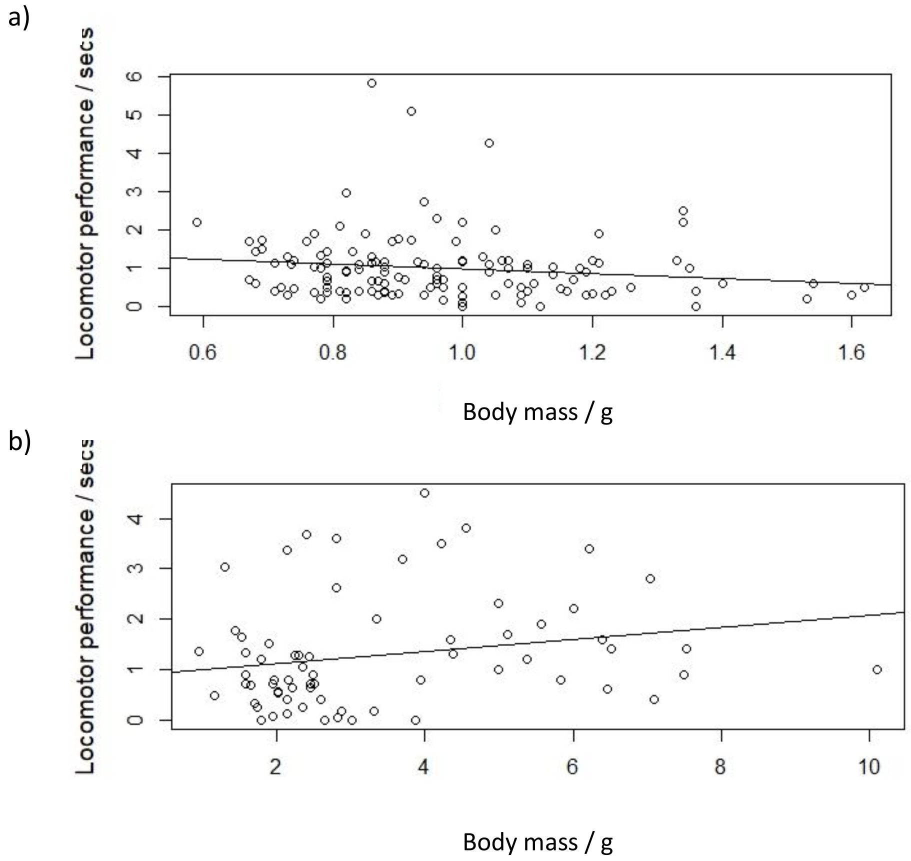


Figure 46 a) Lizard body mass and corresponding locomotor performance for a) hatchling (n = 134) and b) yearling (n = 68) sand lizards. Increased time equates to decreased locomotor performance.

In hatchlings, there was no effect of tail loss on locomotor performance ($U_{25,109}$ = 1218.0, *p* = 0.411) (Figure 47a). Toe loss was not recorded for any hatchlings at the time of locomotor testing. In yearlings there was no effect of tail loss on locomotor performance ($U_{5,63}$ = 153.0, *p* = 0.925). There was an effect of toe loss (> 1 toe) on locomotor performance ($U_{13,55}$ = 217.0, *p* = 0.029); individuals with > 1 toe lost performed significantly slower (Figure 47b).

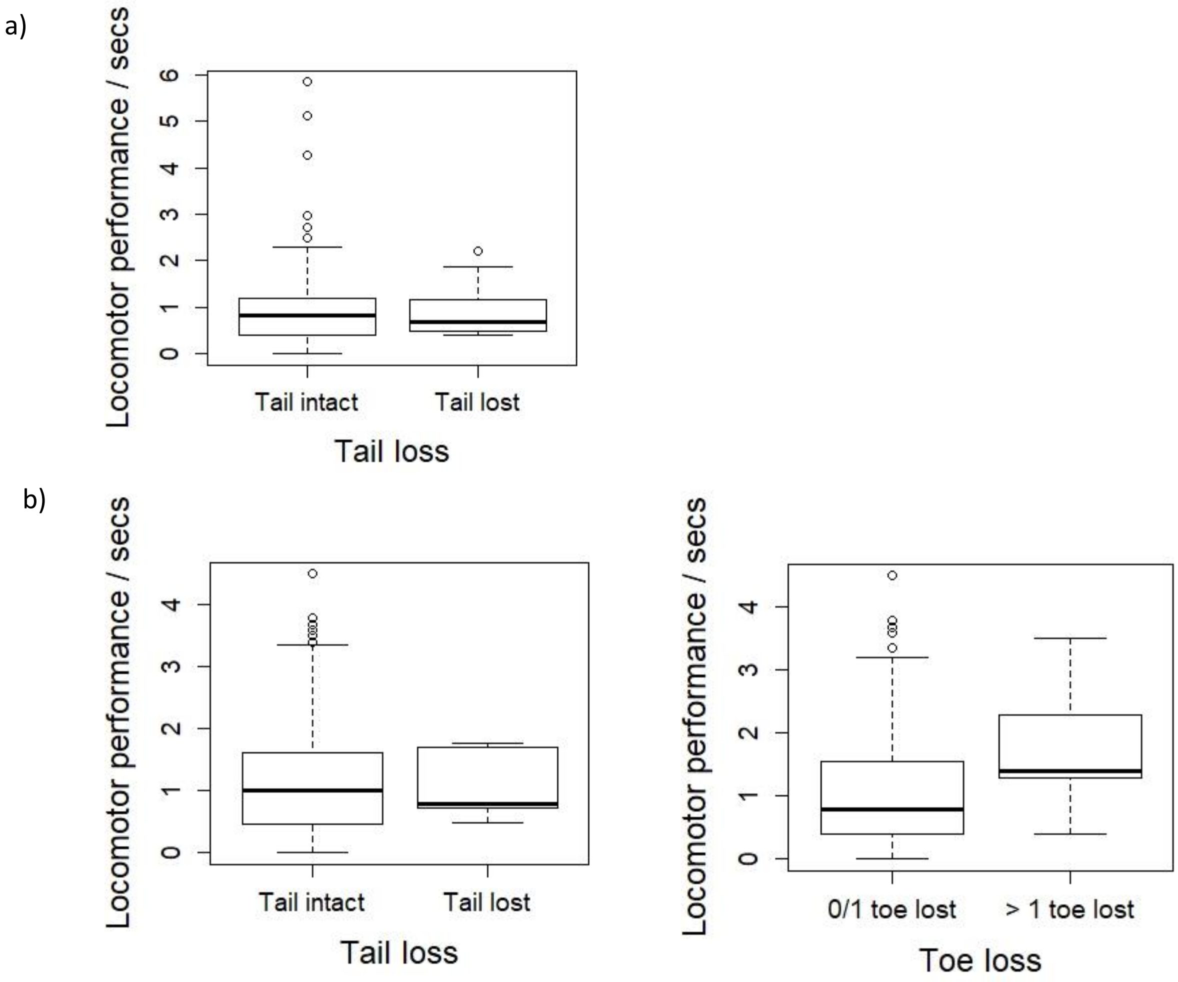


Figure 47a) The effect of tail loss (> 10%) (intact n = 109, lost n = 25) on locomotor performance in hatchling sand lizards; b) the effect of tail loss (> tip) (intact n = 63, lost n = 5) and toe loss (> 1) (0/1 lost n = 55, > 1 lost n = 13) on locomotor performance in yearling sand lizards. Increased time is decreased locomotor performance. Plots highlight the median, interquartile range, extreme values and outliers.

6.5 Discussion

6.5.1 Post-release movement of sand lizards

As with many studies that have evaluated post-release movement of species, variability is large, with many individuals remaining around the immediate release site, but others travelling great distances away and some within a matter of days or weeks (Foster *et al.*, 2003; Santos *et al.*, 2009; Roe *et al.*, 2010). Cumulative distances also vary greatly, with some individuals reaching a location and being repeatedly observed there, and others constantly moving around. This has implications for spatial management of a site to suit the variety of movements observed and suggests the radial distance from the point of release that ought to be considered. The apparent 'homing' nature observed in many sand lizards back to the release location, notably predominantly yearlings, is a behaviour observed in wild studies of this species, with already established home ranges. During one such study, of individuals translocated 70 m outside their home range, 81.5 % return, across areas they are not previously seen to utilise; in 100 m displacements, 66.7 % return; however those moved 150 m away fail to return (Strijbosch *et al.,* 1983*)*. Similar patterns are seen during mitigation translocations, where lizards unhelpfully return to their point of capture when moved to nearby safe and suitable habitat (Russell and Foster, 2017). Observing comparable behaviour in reintroduced cohorts of sand lizards over multiple seasons to their first point of reference when released in a novel environment, as opposed to an established home range, highlights the importance of the release location itself being of optimal habitat, offering ample *foci* for the species to utilise post-release and into future years. It is also noted in previous research that some sand lizards appear to be 'wanderers' that do not ever settle but move constantly within and sometimes out of their natal population (Rose, 1982; Blanke and Fearnley, 2015), as opposed to maintaining a home range. Site management must also take this into consideration, ensuring that a site still offers sand lizard-specific qualities and *foci* towards the limits of its bounds.

(Although not a key element of analysis, spatial distribution mapping (Appendix C.10) did not show a key directional trend westwards from the release site at Eelmoor Marsh, as has been suggested in the immediate post-release phase for sand lizards (ARC Trust, pers. comm.). Post-release movement was evident in all directions and the furthest limits were observed in an easterly and north-westerly direction. There was a lack of extensive southerly movement.)

6.5.2 Effects of morphology, body condition, locomotor performance and release response on survival and movement

There was no effect of body mass on hatchling sand lizard survival or distance reached pre-winter. For yearlings, lizards that survived the winter displayed a range of body mass values that were not statistically different from those for which survival was unknown, however the median value, upper quartile and upper limit were higher for those that survived. A trend towards increased body weight leading to improved survival is observed in a range of species (Janzen *et al.*, 2000; Nagy *et al.*, 2015). There is indication of the lightest and heaviest individuals travelling only short distances from the release location before winter. This broadly agrees with research into common lizards suggesting individuals in most optimal body condition favour dispersal (Meylan *et al.*, 2002). Immediate post-release movement is a trade-off in energy loss and locating optimal habitat for biological functioning, and in relation to conspecifics (Cote and Clobert, 2007). The extremes seen could represent individuals of low body mass/poorest condition limiting their loss of energy stores during this key pre-brumation period. At the other end of the spectrum, the heavier individuals – likely also more sexually mature (Bischoff, 1984) – may be establishing themselves at the release site in good habitat, already showing signs of more dominant territorial behaviour (Govier, 2017). Lizards of low-middling weight took more variable approaches, with both sufficient body mass and a wider variety of behavioural syndromes present amongst them (Meylan *et al.*, 2002; Haage *et al.*, 2017).

Post-release behaviour differs in a number of species by age group and there are variable trends between older and younger animals (Dunham, 2000; Letty *et al.*, 2007; Ryckman *et al.*, 2010; Le Gouar *et al.*, 2012). There is evidence of development and shifting of behavioural traits with age in many species (Stamps and Groothuis, 2010; Petelle *et al.*, 2013; Sakai, 2018); age-related differences are observed between yearling and two-year-old sand lizards (Jordan, 2018). In this research, there was a shift from finding no significant behavioural trends in release response, post-release movement and survival in hatchling lizards; to post-release movement and survival being predictable for yearlings, as detailed below. This may highlight the behavioural development occurring in sand lizards between the ages of hatchling and one year old.

There were no significant effects of locomotor performance or release response on hatchling survival or distance reached pre-winter. Yearlings that were known to have survived performed significantly quicker in the locomotion test and were significantly less exploratory and active immediately post-release; they also took less time to leave the release square although this relationship was not significant. More exploratory and more active individuals at release also travelled significantly further. This indicates that movement of yearlings post-release can be predicted by their response at release, as can their survival. Those that were more exploratory and active immediately, went on to be more exploratory in their wider habitat, travelling greater distances and potentially moving out of the most optimal habitat, ultimately exhibiting poorer survival. Those that stayed nearer the release area were less exploratory and active and showed greater overwinter survival, in ideal habitat. Faster individuals in the locomotion test were also more likely to survive, likely due to their greater fleeing ability. It may have been expected that individuals that left the release square quickly would also be those that travelled furthest, along with being more exploratory and active. However, the cause of this quick movement may have been movement away from conspecifics they were released in immediate proximity to. Although the sand lizard is a broadly non-territorial, “communal species” (Moulton and Corbett, 1999; Blanke and Fearnley, 2015), defence of favourable *foci* and basking spots, and clear hierarchical structures observed in captivity (Govier, 2017; Chapter 2, this thesis) show that, even during the autumn months, survival and sexual selection pressures operate on individuals. As discussed, yearlings are near to sexual maturity and these pressures may be beginning to act on them as they begin to establish themselves at a release site.

Heavier hatchlings were faster in the locomotion test whereas lighter yearlings were faster. This could reflect factors of increased muscle mass improving hatchling sprint speed or increased stride length in relation to body mass. The transition to opposing subadult behaviour from hatchling behaviour is then apparent, here in relation to escape theory. A perceived threat leads to a delayed response from lighter individuals that have been shown to have a shorter fleeing distance in order to conserve energy (Hawlena *et al.*, 2009), followed by a consequent need to flee quickly when the threat is confirmed, as is observed here. Concurrently, heavier individuals have less need to conserve energy and so flee earlier as a threat becomes apparent, but move more slowly.

Tail loss did not have a significant effect on locomotor performance, for either hatchlings or yearlings. This contradicts previous research where sand lizards with autotomy were found to run significantly faster (Ekner-Grzyb *et al.*, 2013). This former study was however conducted on wild-caught sand lizards and as the authors state, the result may be an artefact of the individuals that had dropped their tails being the fastest in order to have escaped predation in the first place. In a study of the captive adult population at Marwell Zoo using the same methodology as in this research, no difference was found in speed of lizards based on caudal autotomy either (Govier, 2017). Even though locomotion appears unaffected by tail loss in this instance, there is still a survival cost of having lost this anti-predatory escape mechanism. There were a relatively high number of hatchlings with tail autotomy in 2018, corresponding with the summer heatwave, which could be a biological response to these extreme temperatures. If this link is accurate, climate change leading to hotter summers could result in detrimental effects on the ability of sand lizards to survive multiple predatory attacks. This is in addition to more broad intolerance to extreme heat and the apparent inability of lizard species to raise their critical thermal maximum temperature (CT_{max}), which overall may place them in a particularly vulnerable position (Dayananda *et al.*, 2017).

Toe loss was found to significantly reduce locomotor performance in yearlings. This supports concerns that losing toes has a detrimental effect on individuals (Parris *et al.*, 2010). Although toe clipping is advocated by some to be a preferred individual marking mechanism, measures have not always accounted for its direct effect on behaviour and animals' ability to perform optimally in a predatory situation, instead observing it in light of other marking techniques (Guimarães *et al.*, 2014) or using measures of stress as a proxy (Langkilde and Shine, 2006). This research indicates that not only are there broad welfare issues raised by toe loss, but researchers may no longer be observing true natural behaviour in their subjects, somewhat defeating its point as a technique. Considered here as a natural occurrence in the context of reintroduction, it is apparent that toe loss may result in sand lizards being able to flee threats less effectively.

6.5.3 Efficacy of post-release monitoring of sand lizards

Post-release observations showed that monitoring sand lizards using walking transects and refugia was a viable approach to evidence the range of post-release movements both pre- and post-winter by released individuals. Given the need for further improvement to remote monitoring at the micro-scale for cryptic species in complex environments, this traditional approach (although requiring high survey effort and with modification using multi-refugium arrays) returned meaningful and informative data.

The detectability of different individuals and the same individuals over time was highly variable. There were those that are not seen for a whole spring-autumn season only to be observed the following year, and then those seen up to nine or ten times during the same period. It is suggested that varying observation frequencies may be reflective of real differences in sand lizard activity, where in previous research particularly high numbers of sightings indicated animals lack of survivorship to the following year (Rose, 1982). This is inferred as attributed to underlying health or fitness conditions (Blanke, 2006), however depending on the behaviour of the animal during observation, it could in fact be increased detectability putting the individual at greater risk of predation. For example, the male sand lizard from the 2017 release, observed repeatedly in 2019, seemed to actively draw attention to himself during most observations, by for example, moving loudly over dead bracken vegetation in full visibility; he was then observed having lost his tail part-way through the year.

The data highlighted the difficulties around conclusive population estimates for this species (Fearnley, 2009) but showed the long-advocated benefit of individually identifying animals in post-release monitoring to better infer survivorship, highlighting where the same individual was observed a disproportionate number of times. For example, here 19/39 observations (almost 50%) during surveys in spring-summer 2019 were of the same two individuals. In another mark-recapture study, repeat sightings of just 29% of individually marked beetles *Anoplophora glabripennis* enable beetle movement to be attributed to beetle density, weather, individual size and tree size; techniques are considered to be optimal to date for quantifying dispersal for this cryptic species (Bancroft and Smith, 2018). The post-release monitoring at Eelmoor Marsh exceeds this recapture percentage, offering informative, quantitative data on post-release movement of the sand lizard for the very first time.

6.5.4 Conservation implications of research and future directions

Individual sand lizards showed a wide range of post-release movement, indicating site management should focus predominantly around the release area but also across the release site to offer suitable resources for wider ranging, or ‘wanderer’, individuals. Yearling sand lizards showed a more predictable response to release, in relation to subsequent survival and movement. This could be used to select individuals for release to sites of specific size, for example. It would suit the intentions of minimising post-release movement away from the release site before winter, in order to keep lizards within optimal, prepared habitat with good brumation opportunities.

Surveying continued after the final sand lizard release at Eelmoor Marsh to cover autumn 2019 and much of the 2020 summer season. It is intended that this multi-year dataset be used to undertake the first capture-mark-recapture analysis of this species following reintroduction, to offer survival estimates in the population-establishment phase and further inform reintroduction protocols.

This chapter has highlighted the difficulties associated with monitoring a cryptic species, but also the necessity of doing so. Evaluating projects as comprehensively as possible through post-release monitoring is the only way to assess protocols and suggest potential improvements to optimise success and resources. As has been reiterated, quantifying what occurs post-release is a vital component of reintroduction initiatives (Armstrong and Seddon, 2008; Taylor *et al.*, 2017). This study has maximised the research opportunities of a project that spans *in situ* and *ex situ* conservation, through pre-release studies and data collection and post-release monitoring, tying together these two complementary approaches (Zegeye, 2017). In the light of emerging climatic changes and continued habitat loss and fragmentation in many parts of the world, but alongside increased conservation measures to protect and restore locally threatened species, it is vital that an understanding of post-release biology for species exists at the basic level. Only by doing this can informed, evidence-based decisions be made as scope and priorities of translocation efforts undoubtedly shift in light of change at the global-scale. Such shifts include towards ecosystem restoration and rewilding at multi-species scales (Corlett, 2016); researching assurance populations held in captivity to assess their adaptability to current, and unknown, threats (Harding *et al.*, 2016); and using climatic predictions in assessment of best-placed population recovery efforts (Molloy *et al.*, 2020).

6.6 Conclusions

- Lizards released as yearlings, showing greater survival than hatchlings (Chapter 5), were also more predictable in their response to release and more likely to remain closer to the point of release for their first brumation.
    - Yearlings observed post-winter were faster and showed less exploratory and active behaviour in response to the novel release site. The furthest travelled yearlings showed a more exploratory and active response to release.
- Reintroduced lizards showed strong release site fidelity and 'homing' behaviour, highlighting the importance of this area being in optimal condition and providing species-specific requirements, such as good edge habitat *foci* and sand for oviposition.
- The wider site should also be managed to offer suitable *foci* to support 'wanderers' as well as further-ranging hatchlings (Chapter 5), and to allow for spatial growth of the population as population recruitment occurs naturally.
- Protocols for species reintroduction require continual assessment in order to optimise resources and project success. Cryptic species raise difficulties; however, methodologies are continually open to assessment and improvement in order to make beneficial, evidence-based recommendations going forward.

Chapter 7 General Discussion

7.1 Post-release monitoring of reintroduced species and the wider community

7.1.1 Sand lizard spatiotemporal behaviour and implications for species monitoring

As with all reptile species, sand lizard reliance on the surrounding environment for thermoregulation predominantly drives daily and seasonal activity (House *et al.*, 1980; Castilla *et al.*, 1999; Edgar *et al.*, 2010; Blanke and Fearnley, 2015). This has led to survey recommendations around optimal time of day to undertake monitoring, that shift seasonally (ARC, unpubl.; Froglife, 1999; Moulton and Corbett, 1999). Visibility of juvenile sand lizards and the majority of adult sand lizards in this research was predicted by environmental variables, as would be expected. However, social factors were shown to play a key role.

A dominance hierarchy was observed in adult males, and indication of a hierarchy in females in this study outside of breeding season, with more dominant individuals utilising a greater area and being visible for longer periods. The activity of subordinate individuals was more restricted and appeared to be in less optimal conditions. This comparable use of resources is well-established in behavioural ecology for many species including lizards (Kaufmann, 1983; Borgmans *et al.*, 2020), and has been observed to be relaxed depending on specific conditions at a given time, such as density of individuals and resource availability (Strickland *et al.*, 2016). Although key indicators of adult visibility collectively centred around temperature and humidity, broadly in agreement with previous findings (Inns, 1996; Fearnley, 2009; Blanke and Fearnley, 2015), the individual differences observed suggest social factors play a part in whether lizards are active during ‘ideal’ conditions. Unlike previous studies into sand lizard behaviour within a captive setting (House *et al.*, 1980; Fearnley, 2009; Kraft, 2012), this experimental set-up optimised detectability, whereby if a lizard was above ground and not fully obstructed by vegetation (i.e. had any potential to be observed), then there was almost complete certainty that it would be seen. Enclosure size, accessibility and habitat complexity in previous studies have limited detectability to that more replicate of surveying this species in the wild (House *et al.*, 1980; Fearnley, 2009; Kraft, 2012). This study has therefore enabled causes of differing visibility between individuals to be observed beyond environmental conditions, which are due consideration for monitoring efforts. It offers some way of an explanation for the differences in activity times noted between sand lizards previously (Olsson and Shine, 1997a) and highlights that observations of just a few individuals from a known large population may not be solely due to poor detectability of visible lizards, but social factors that may be driving visibility in the first place. Dominance hierarchies ought also to be considered in captive breeding populations with the purpose of reintroduction, with regards to dominance being linked to breeding success (Neumann *et al.*, 2018) and the impact of releasing offspring with potentially limited genetic diversity.

Juvenile sand lizards in captivity showed a very marked reduction in visibility as the day progressed, indicating time of day to be a more accurate predictor in observation of this age group. Their sociality, observed as aggregatory tendencies and inferred to raise body temperatures through kleptothermy, suggests that their relationship with environmental variables was more indicative of preferred conditions. Key defining variables were temperature and humidity (as broadly observed in adults) and increased solar radiation. The latter is highlighted as strongly associated with sand lizards in particular, and reasoning behind their being termed 'shuttling heliotherms' (Spellerberg, 1976; Blanke and Fearnley, 2015).

The captive study informed the approach taken in the field to monitor both the reintroduced sand lizard population, and the wider reptile assemblage. Despite wood substrate being noted as a prime basking location within wider habitat for sand lizards (House *et al.*, 1980; Blanke and Fearnley, 2015), this did not appear transferable to wooden (plywood) refugia. As this was so seldom used in the captive study, it was replaced with felt in the wild experimental design, alongside the same additional materials of tin, slate and tile. Optimal refugium materials for sand lizards shifted from slate and tile in a captive population to slate and felt in the lizards post-release. Due to the change in materials offered, it is difficult to suggest whether there is an aversion to tile in a wild setting, or whether felt may have been preferred to tile in a captive setting as well. The higher use of tin over tile in the wild suggests that tile is disfavoured in this setting specifically. This could be a result of its comparable high visibility (R. Gardner, personal observation) compared to other materials in a wild environment, potentially leading to increased predation risk if selected for basking, or its weight bearing down on vegetation limiting rapid escape opportunity if selected for refuging. The actual and perceived predation risk in captivity is eliminated, therefore tile may be utilised preferentially here, purely in relation to its thermal characteristics. This finding offers evidence that additional components around refugium selection, as opposed to just thermal properties, need consideration for monitoring in the wild. It also offers a suggestion that released lizards may have responded to their new environment in the light of its increased risks.

Both the captive and wild studies highlighted the role of structural heterogeneity in determining sand lizard use of space. Higher diversity is particularly key in the wild with use of felt refugia. This adds weight to the argument that material selection will also consider factors beyond thermal properties. The rapid heating and cooling of felt offers sand lizards the high temperature gradients they require for thermoregulation and heliothermic shuttling between microhabitats (Spellerberg, 1976; House and Spellerberg, 1983; Blanke and Fearnley, 2015). However, it is a light material that bears little weight on vegetation, with greater sub-material airflow, and may be perceived as unreliable cover. The corresponding need for high structural diversity in surrounding vegetation places lizards in an ideal position to flee to high cover if required, with low pathways also being present and therefore assisting in this process. Weightier slate, capable of reaching the highest temperatures, was most utilised by sand lizards and slightly favoured to felt.

Key microclimate conditions were linked with sand lizard use of refugia in captivity and the field. Many environmental variables were closely correlated as shown in Chapter 2 and were also predictable by wider weather variables, shown in Chapter 3. As with visibility, adult use of refugia in captivity was driven by social dynamics, resulting in a variety of key environmental predictors across individuals. In the wild, higher humidity and lower lighting were key predictors of use of refugia in general, indicating their use when key ambient conditions were not meeting thermal requirements in natural microhabitat. This corresponds with the constant shifting spatial behaviours of sand lizards to adjust their body temperature accordingly (Spellerberg, 1976; House and Spellerberg, 1983; Blanke and Fearnley, 2015). It suggests that surveying this species using refugium techniques may be most optimal outside of those conditions considered good predictors of sand lizard visibility. Refugia were utilised in preference to natural habitat when weather and microclimatic conditions were sub-optimal, in low lighting conditions and high humidity. Specific measures of environmental variables (both microclimate and microhabitat) associated with use of different material types are included in this thesis and can be used to further inform refugium deployment and best survey conditions.

Sand lizards were still comparably poor users of refugia in comparison to all other species, and in comparison to the time they were visible for in captivity. However, offering a variety of refugia was of benefit over traditional tin refugia, which are seldom used (Reading, 1997; Sewell *et al.*, 2013). In this research, multiple-material arrays have been seen to provide a more measured, objective method for monitoring this species, which should be considered alongside walking transects, where alternative monitoring techniques such as radio tracking (Godfrey and Bryant, 2003; Rowley and Alford, 2007; Winkel, 2008) currently still fall short for this species in this habitat. This combination of a combined refugium-array grid, with transect walks between arrays, optimises detectability in a quantitative, spatiotemporally comparable manner minimising observer bias as far as possible.

This research has highlighted the importance of re-evaluation of monitoring techniques and the need for scientific rigour and evidence-driven recommendations.

7.1.2 Monitoring of the wider species community

The importance of optimal monitoring of both the released species and wider community is highlighted in order to detect knock-on effects of releasing a species into a community within which it has not existed for some time (IUCN, 2013; Robinson *et al.*, 2020).

This research did not aim to address and discuss the ecophysiology and behavioural variability of the more common reptile species occupying dry heathland in the same detail that was undertaken for the sand lizard in Chapters 2 and 3. However, in order to offer an all-encompassing monitoring methodology to assess both the sand lizard and the wider community post-release, it was able to make surveying recommendations on a species-level basis based on refugia, microhabitat and microclimate variables across a variety of habitats for the slow worm and common lizard, and with a larger dataset in dry heath habitat for the adder and grass snake in addition. Chapter 4 highlighted that survey methodologies offered very different estimates of relative species abundance, and that preferred refugium materials differed between species, with a variety of key microclimate and microhabitat predictors associated with observing each species. This information can be applied to reptile monitoring according to the species in hand, or the assemblage holistically and methods used objectively as with the sand lizards, to ensure spatiotemporal comparability of recorded data. Where resources are limited and there is a target species being monitored, optimal refugium types can be deployed accordingly, in optimal microhabitat for that material and then surveyed in optimal weather (and associated microclimate) conditions to improve the likelihood of observations. For common lizards that, like sand lizards, are poor users of traditional tin refugia, felt was found particularly optimal, and common lizards were observed at mixed-material refugium arrays in dry heath habitat approximately 2.5 times as often as on walking transects. This showed this method may offer a best-practice monitoring tool for common lizards, as well as encompass the variety of optimal refugium scenarios for the reptile assemblage as a whole.

7.2 Sand lizard reintroduction protocols

Within each of the different age-groups released in this study, body mass was not found to be predictive of post-release movement or apparent survival, a relationship that is observed in some species (Janzen *et al.*, 2000; Nagy *et al.*, 2015) but not all (Charland, 1989). Current release protocols involve the release of juvenile lizards in their first autumn, however post-release indications from this research suggested that overwintering lizards in optimal conditions in captivity and releasing them as yearlings improved apparent survival. In addition to this, these yearling lizards showed a more predictable response to release, in relation to individual differences in exploratory and active behaviours relating to distances travelled and apparent survival. An increase in personality variation across individuals and development of personality traits has been observed with increased age in a number of species (Stamps and Groothuis, 2010; Petelle *et al.*, 2013; Jordan, 2018; Sakai, 2018), although partially shifting personalities across life stages are also shown (Stamps and Groothuis, 2010; Wuerz and Krüger, 2015). In a study of the clonal gecko species *Lepidodactylus lugubris* (in which personality differences can only be attributable to environmental pressures), small juveniles were composed of just bold, low explorative individuals where larger juveniles and adults had a range of personality types, likely the result of learning and a boldness/growth syndrome (Sakai, 2018). The linking of release response and subsequent post-release factors in yearling sand lizards indicates a development of personality variation in this species through early ontogeny.

Sand lizard yearlings that showed lower exploratory and less active behaviour at the time of release were more likely to be observed following the winter and travelled the least distance from their point of release pre-winter. Response at the time of release, into a novel environment, therefore appears to be replicable for individuals at a larger spatiotemporal scale than the immediate point of release, building on previous research showing that sand lizards exhibited an activity-exploration dispersal syndrome in a captive setting (Govier, 2017) through Open Field Tests (Hall and Ballachey, 1932; Perals *et al.*, 2017). These relationships could go on to be assessed in captive breeding facilities pre-release to determine the likely patterns of post-release movement and predict survival rates. For example, if a release cohort constituted a number of particularly exploratory individuals, site preparation or post-release management could be tailored to account for the further distances these individuals are likely to travel. Movement behaviour is a good indicator of reintroduction progress, with individuals responding according to their increased knowledge gain at a site following release (Berger-Tal and Saltz, 2014). Recommendations to include behaviour, and particularly movement behaviour, in making post-release management decisions have been suggested in relation to site fidelity, recurring locations, proximity to other individuals and individual variation in movement (Berger-Tal and Saltz, 2014). These factors that have been considered through the course of this research.

Monitoring herein was during the establishment phase post-release at Eelmoor Marsh. Further monitoring will be essential in order to determine if the trends seen continue (Bernardo *et al.*, 2011; Parker *et al.*, 2013). Detectability of juvenile and subadult sand lizards is likely to vary demographically as well as between individuals as has been shown in adult lizards. It is intuitive to think larger animals are more detectable, but there is suggestion that hatchlings, being more numerous and active, may be more likely to be seen (Blanke and Fearnley, 2015). Observing the trend of age-related survivorship into the future is of interest to further inform release demographic protocols.

Homing behaviours have been studied in sand lizards, showing the propensity for individuals to relocate themselves in former localities following displacements. Observations of individual sand lizards post-release showed that released yearlings had a tendency to 'home' back to their point of release, over both the short-term pre-winter, and the long term, with one particular individual looping tens of meters away and back to within 10 m of his release site repeatedly. Habitat maintenance of the immediate release site vicinity is therefore imperative to ensure it continues to provide the necessary sand lizard requirements, and suggests it is a good location to manage sand areas for oviposition.

Sand lizard conservation guidance suggests broad timing of reintroduction releases between mid-April to early September (Moulton and Corbett, 1999), and more specifically in August and early September or late spring following overwintering, to try and improve survival rate (Corbett and Moulton, 1998). The majority of translocations currently occur in the autumn, with the timing of the releases herein comparable to other sites over these years, in the first part of September. August is often hot, which is poor for surveys, and consequently causes quick movement away from the optimal habitat of the release area, where contingency overwintering burrows are also created (ARC Trust, pers. comm.; Langham, S., pers. comm.). Exact timing each year is based on weather forecasts showing a run of a few relatively dry days to time release at the start of and sunny and warm weather on the day (ARC Trust, 2016; ARC Trust, pers. comm.). Captive breeders of this species typically aim for this early September timing; where there has been limited capacity to retain young lizards for long periods (ARC Trust, pers. comm.).

Monitoring discussed in Chapter 3 showed that individuals remained above ground and active very late into the season and in sub-optimal weather conditions; this was highlighted in the differences seen between common lizard and sand lizard microclimate predictors of observations. It suggests that timing might benefit from being shifted earlier in the year to allow individuals to better establish themselves and behave more naturally in response to the onset of winter conditions. Individuals observed very late in the season are often not seen to survive and exhibit poor body condition (Blanke, 2006; Blanke and Fearnley, 2015). This should, however also be weighed against the rapid growth rates seen in subadults between May/June and September following overwintering, suggesting late summer (August) may be preferential over both spring and autumn for yearling releases. The homing shown by those lizards that did move further away indicates that individuals had strong release site fidelity. Any concerns over the release cohorts moving away from optimal habitat and re-distributing themselves more widely, and the negative consequences that may have for breeding in the crucial population establishment phase, may be negated by this observation. This was further evidenced by the signs of breeding observed in the release vicinity.

Releasing yearling lizards that have overwintered in optimal conditions in captivity, obtained good body mass and whose response to release can be anticipated, would favour more rapid population recruitment at a release site. Individuals would be more likely capable of breeding within a year of release, as thanks to head-starting in captivity, they would be in optimal condition to do so (Bischoff, 1984). The strong release site fidelity of yearlings and apparent homing behaviour if they do initially move away, further supports this.

For sand lizards, climate change may have positive initial effects in this northern part of its range in the UK, by optimising egg incubation in warmer conditions (Dunford and Berry, 2013; Blanke and Fearnley, 2015). However, changes in sea level will affect coastal dune habitats, and remaining strongholds in southerly lowland heaths are threatened by the low levels of resilience of these heath vegetation communities to the projected shifts in climate (Berry *et al.*, 2007; Dunford and Berry, 2013), as well as continued encroachment of human development and urbanisation (Hayhow *et al.*, 2019). This further loss of habitat for a species that has already suffered so much (Rose *et al.*, 2000; Edgar and Bird, 2006), would leave sand lizards in a dire position. Losing specialists, such as the sand lizard, from communities reduces resilience to further change, and biodiversity is lost in this cyclical manner (Olden *et al.*, 2004). Protecting and restoring habitats, creating linkages through habitat corridors and reintroducing species that are unable to return of their own accord, may offer the best chances of optimising ecosystem resilience at the population, species, community and landscape level. That is why it is vital that the efforts being poured into conservation initiatives, in this context reintroduction, are effective and optimising success and resources.

7.3 Conservation and management impacts

This research has direct application to conservation of the sand lizard in the UK and associated reintroduction protocols. It offers evidence-based recommendations for timing and nature of the release, indicating releasing overwintered individuals may holistically offer higher success rates. It also makes recommendations for ongoing site management to account for release site fidelity by many individuals and the far-travelled behaviour of a few.

This is an optimal time to reassess sand lizard reintroduction in the UK, where efforts have taken place for fifty years (Moulton, pers. comm.) (Corbett and Moulton, 1998; Moulton and Corbett, 1999). Reintroduction has predominantly been successful in returning the species to sites, as evidenced by ongoing presence in many cases (Woodfine *et al.*, 2017). Quantitative data however, is of great benefit, encompassing monitoring of released individuals and offering recommendations for monitoring of the wider reptile community into which they are released. Only through such rigorous research can recommendations be made to best optimise resources, be they the released lizards themselves, time, or monitoring efforts.

It is hoped that the results of this study will help inform sand lizard reintroduction protocols along with post-release monitoring methodologies. It offers baseline data and recommendations that can be assessed further, across multiple sites and multiple years. On consideration of the findings, conservation practitioners will need to also consider practicalities and logistics. For example, overwintering individuals and retaining them until their second autumn may be infeasible in captive breeding facilities as they stand. However, if there was capacity to overwinter and undertake a release just prior to the hatching of more juveniles, this may offer comparable benefit of optimal overwintering conditions and greater body mass than release as hatchlings. Repeating this research following this suggested scenario would be an obvious next step if retention capacity is not easy for a full additional year.

The research highlights that in the light of the many challenges being faced by species on a global level, and in particular the cryptic, less charismatic specialists (Gibbons *et al.*, 2000; Bonnet *et al.*, 2002; Afonso Silva *et al.*, 2017), the application of a rigorous, scientific approach is highly beneficial. Through this quantitative approach, focused data was obtained in the hope of informing conservation practitioners and their efforts going forward.

7.4 Future study

The following are broad recommendations encompassing all aspects of this research. Many areas of sand lizard ecology more generally, such as brumation and burrowing locations, and specific environmental requirements for oviposition, remain in need of further research efforts (Blanke and Fearnley, 2015).

Ex situ captive breeding environments offer unique opportunities to observe a population of known size and demographic; they offer an insight into species and individual behaviour that is challenging to gain from a wild population, especially for cryptic species. However, the cost of this – as with many behavioural studies – is small sample size. Undertaking further captive studies would confirm, or further expand on, the individual and demographic trends in spatiotemporal and social behaviour observed here.

It would be useful to repeat post-release monitoring of sand lizards at additional sites to highlight any site-specific effects. This could be based on the methods herein, perhaps with reduced refugium types of felt and slate and at decreased density in order to further refine technique. Monitoring more releases would also allow affirmation, or otherwise, of the trends observed.

Continued monitoring at Eelmoor Marsh would allow both the sand lizard population and wider reptile community to be assessed into the population growth and persistence phases of reintroduction. This research traded off the benefits of multiple field sites to allow focus at one site to the level of detail required to advance understanding for this cryptic species.

As mentioned, depending on the feasibility of retaining lizards until they are yearlings, emphasis might be placed on late spring/early summer releases, prior to the hatching of more juveniles. Monitoring release cohorts released at this time and using methods discussed in this thesis would allow direct comparison of behaviour, response to release and apparent survivorship between individuals of around 10-11 months old and those of around 15 months, the latter having had an additional full summer in captivity. Spring or early summer releases may be a good, practical compromise or may even show additional benefit over retaining individuals for a full year in captivity.

Emerging techniques around environmental DNA (eDNA) that have developed during the course of this research for terrestrial species, may offer an improved method of monitoring sand lizard distribution and spatiotemporal movement post-release as a cohort (although would not be capable of recognising individuals) (Ficetola *et al.*, 2019). Techniques would likely require quantification and optimisation in a captive setting for sand lizards before deployment in the wild.

Reintroduction initiatives should utilise all resources and tools available to them to optimise monitoring protocols and best inform wider protocols. The value of this is inherent in this research. Fieldwork-intensive monitoring techniques will be applicable to a number of cryptic species and optimisation of these will enable informed, evidence-based conservation measures to be better applied. The potential for such intensive efforts to reveal key data around under-studied species, is enormous and should not be underestimated in terms of the benefits it can bring to understanding species at the individual-level, through to monitoring metapopulations and modelling future species distributions in view of anticipated habitat and climatic changes (Molloy *et al.*, 2020).

7.5 Conclusions

This research concludes that re-evaluation of longstanding field-based monitoring techniques enabled increased understanding of the post-release response of a cryptic species. This in turn has allowed recommendations to be made for future reintroductions of the sand lizard and sets a baseline for future research and monitoring methods. The key findings summarised below.

Dominance hierarchies apparent in adult male, and to a degree female, sand lizards influenced their visibility and therefore potential of being observed. The aggregatory behaviour of juvenile lizards was observed earlier in the day when body temperatures require raising and was therefore likely indicative of kleptothermy as opposed to, or in addition to, other grouping benefits. These differences in sociality may influence post-release movement when individuals are released collectively, and also influence their individual detectability in what are deemed 'optimal' surveying times.

Sand lizards showed preferences for felt and slate refugium types in a wild monitoring scenario and deployment should particularly consider increased structural heterogeneity for felt. Refugia were also used by sand lizards in less optimal microclimate conditions, with lower light levels and increased humidity. The wider reptile community had key detectability predictors, regarding refugia, microhabitat and microclimate, with common lizards as a competitor of the sand lizard also showing preference for felt and slate refugia in areas of diverse vegetation structure, as well as increased vertical cover for felt. For common lizards, the multi-material array greatly improved refugium use over walking transects. The overall preferences for different materials by different species, as well as variation in use in different environmental conditions, suggests a multi-material method is most optimal for monitoring the sand lizard within its wider reptile community following release.

Sand lizards showed age-dependent differences in post-release movement, with hatchlings travelling further than older animals. Yearlings had overall greatest survivorship post-winter. In yearlings, individual variation in behavioural traits of activity and exploration were linked to post-release movement pre-winter and survival post-winter with less active, less exploratory individuals remaining nearer the release location and showing greater survivorship. Yearlings that were faster in the locomotion test also showed greater survivorship. Within each age group, body mass did not predict post-release movement or apparent survival. Release site fidelity was observed in yearlings, through both retention in this area throughout observations and subsequent return to it by individuals that had travelled a distance away; a few individuals showed 'wandering' behaviour around a large area, as seen in this species previously.

A vast number of factors require consideration when undertaking species reintroduction (IUCN, 2013). This research has highlighted the importance of post-release monitoring the release species and the wider community. It has shown the benefit of utilising captive breeding facilities where possible for detailed studies. Adequate monitoring is often inadequately carried out across reintroduction initiatives for a variety of reasons and is particularly challenging for cryptic species. In addressing this, multiple factors have been highlighted to be predictive of establishment-phase reintroduction success for the cryptic example of the sand lizard. They provide evidence-based recommendations for reintroduction efforts going forward.

Appendix A Chapter 2

A.1 Captive study space use ethogram

Materials/vegetation:	S = Slate		R = using as refugium (under)			
	T = Tile		B = using as basking (on top of)			
	Tn = Tin		Rh / Bh = Refugium half under; Basking half on			
	W = Wood		f = flattened body position			
	BSo = Bare sand in open		n = normal body position			
	BSv = Bare sand sheltered by some veg					
	Under/within grass = UG					
	On grass = OG					
	Under heather = UH					
	On heather = OH Climbed grass = CG					
	Climber heather = CH					
	In burrow (confirmed) = Bw					
	Three of each material for adults, so left to right = 1,2,3					
	Split each material into 9 parts i.e.					
	1	2	3			
	4	5	6			
	7	8	9			
	best describe where bulk of animal's body is (1-3 = width of material; 1-7 = length)					
Location in viv:	Split whole viv into 18 parts* i.e.				*for hatchlings, 9 parts	
	A1	A2	A3	A4	A5	A6
	B1	B2	B3	B4	B5	B6
	C1	C2	C3	C4	C5	C6

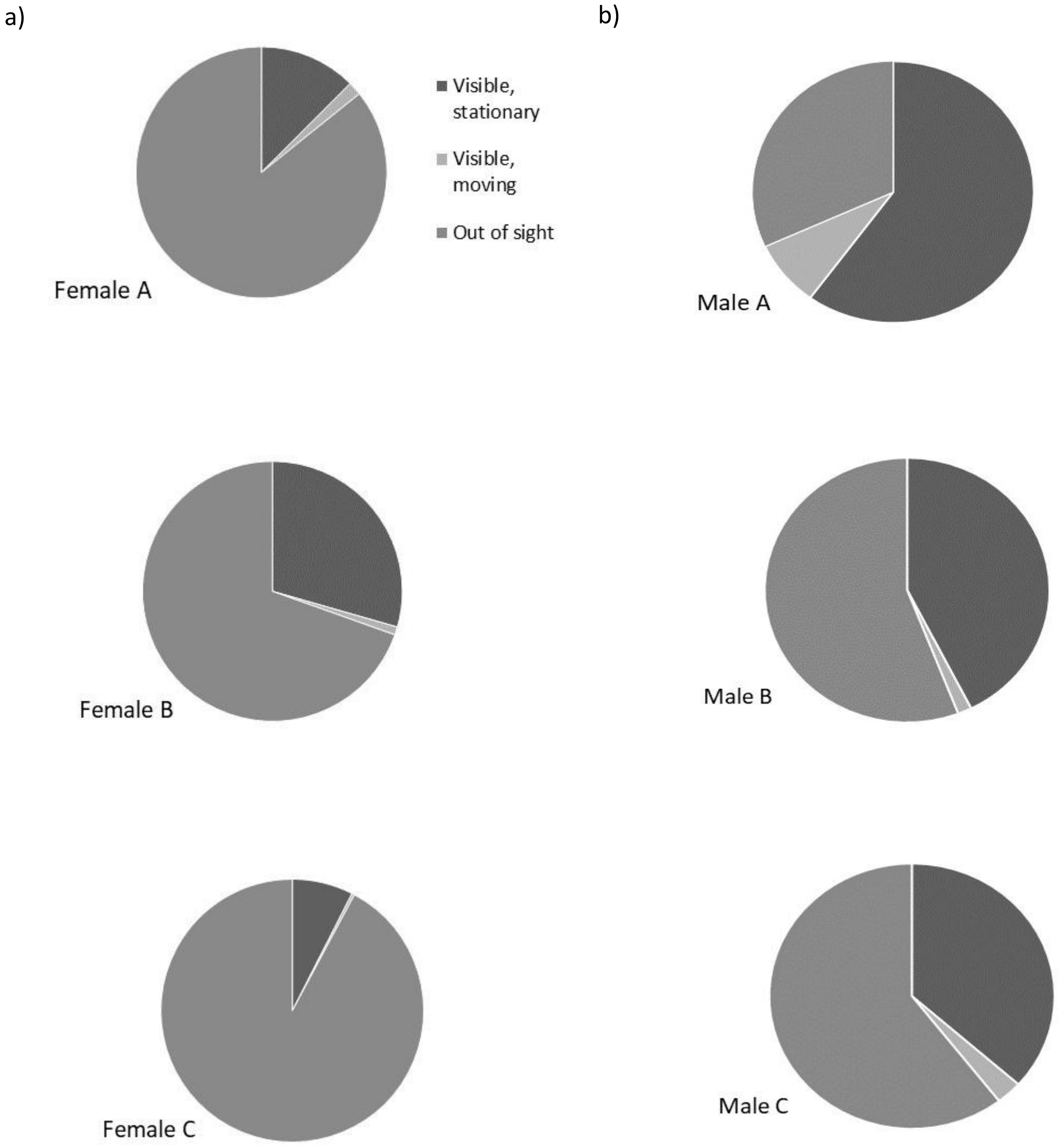
Extra details = obvious hunting, head protruding from under material, interaction with another lizard (adults)

- Note that location is quoted as where the animal's body (i.e. from neck to abdomen). If the head/tail are elsewhere (e.g. under a refugium when the rest of the animal is out), make a note in the 'Extra details' box.
- B/R denotes whether the lizard is on / under a refugium type. If the lizard has flattened itself on a material (f) it is likely actively basking; this would be written Bf. Use 'f' *whenever* a lizard is seen flattened (on refugium/veg/sand etc).
- BSo/BSv – the lizard is on bare sand. When looking vertically down on the animal, if fewer than three blades of vegetation cross its body (body = neck to abdomen), this is BSo. If three or more cross its body, it is recorded BSv.
- OG – the lizard is on grass (not bare sand) with fewer than three blades of vegetation crossing its body (body = neck to abdomen). (If more than three blades cross its body it is deemed UG.) The angle of the lizard's body is less than 45 degrees to the horizontal.
- UG – this covers the lizard being under (view of lizard highly obscured by) and within (view partially obscured by) grass (bare sand cannot be seen through the grass – this would result in BSv). If three or more blades of vegetation cross its body when viewed from above, it is deemed UG. The angle of the lizard's body is inconsequential.
- OH – the lizard is on any part of the heather. The angle of the lizard's body is less than 45 degrees to the horizontal.
- UH – the lizard is under heather (on ground level).
- CG/CH – the lizard is angled at 45 degrees or more to the horizontal on grass/heather. CG - there are fewer than three blades of vegetation crossing its body when viewed from vertically above (body = neck to abdomen) (were there more, this would be UG).

A.2 Agonistic interactions during 40 hours of observation of three male sand lizards

		WINS		
		A	**B**	**C**
DEFEATS	**A**		0	0
	B	6		2
	C	7	3	

A.3 Percentage of time, t (t = 40 hours), that a) female and b) male sand lizards were visible.



A.4 Chi square analysis of frequency of grouped grid cell use by two female sand lizards over 40 hours of observations.

Frequency = number of times lizard observed stationary in grid cell group). Female C was visible for too little time (at too low a frequency) to be included in analysis.

	Female A				**Female B**			
Grid cell	Observed frequency	Expected frequency	Pearson residuals	Contribution to χ^2 (%)	Observed frequency	Expected frequency	Pearson residuals	Contribution to χ^2(%)
A1 B1 C1	4	4.832	-0.378	0.118	8	7.168	0.311	0.079
A2 B2 C2	2	8.858	-2.304	4.369	20	13.142	1.892	2.945
A3 B3 C3	11	40.668	-4.652	17.809	90	60.332	3.820	12.005
A4 B4 C4	22	14.898	1.840	2.786	15	22.102	-1.511	1.878
A5 B5 C5	20	8.456	3.970	12.969	1	12.544	-3.259	8.742
A6 B6 C6	32	13.288	5.133	21.684	1	19.712	-4.215	14.616
χ^2	121.53							
df	5							
n	226							
p-value	<0.001							

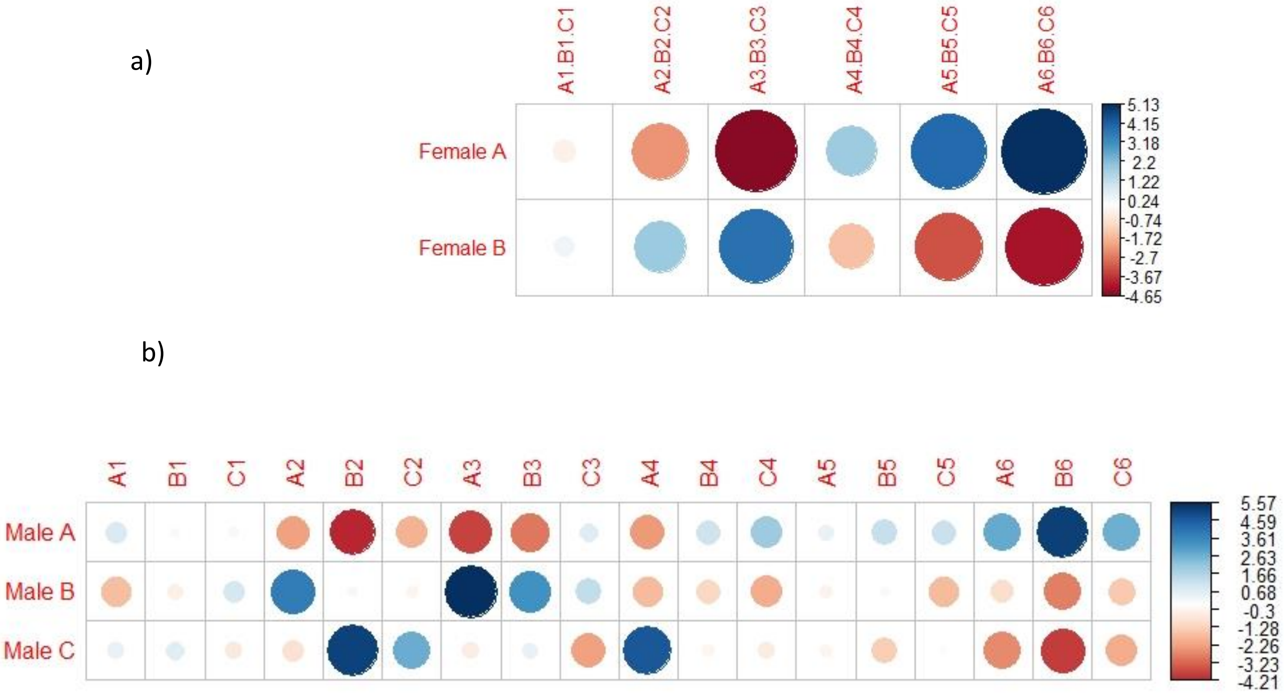
A.5 Chi square analysis of frequency of grid cell use by three male sand lizards over 40 hours of observations

(Frequency = number of times lizard observed stationary in grid cell).

Grid cell	*Male A*				*Male B*				*Male C*			
	Observed frequency	Expected frequency	Pearson residuals	Contribution to χ^2 (%)	Observed frequency	Expected frequency	Pearson residuals	Contribution to χ^2 (%)	Observed frequency	Expected frequency	Pearson residuals	Contribution to χ^2 (%)
A1	17	13.764	0.872	0.267	3	7.830	-1.726	1.045	10	8.407	0.550	0.106
B1	3	10.093	-2.233	1.748	15	5.742	3.864	5.236	4	6.165	-0.872	0.267
C1	6	24.775	-3.772	4.99	35	14.093	5.569	10.877	13	15.132	-0.548	0.105
A2	7	16.516	-2.342	1.923	4	9.396	-1.760	1.087	25	10.088	4.695	7.731
B2	22	19.728	0.512	0.092	10	11.223	-0.365	0.047	11	12.049	-0.302	0.032
C2	21	11.470	2.814	2.777	4	6.525	-0.988	0.343	0	7.005	-2.647	2.457
A3	14	14.681	-0.178	0.011	7	8.352	-0.468	0.077	11	8.967	0.679	0.162
B3	10	34.868	-4.211	6.22	21	19.835	0.262	0.024	45	21.297	5.136	9.253
C3	8	21.563	-2.921	2.992	24	12.266	3.350	3.936	15	13.170	0.504	0.089
A4	19	14.681	1.127	0.446	5	8.352	-1.160	0.472	8	8.967	-0.323	0.037
B4	9	5.964	1.243	0.542	3	3.393	-0.213	0.016	1	3.643	-1.385	0.672
C4	72	39.456	5.181	9.414	9	22.445	-2.838	2.825	5	24.099	-3.891	5.309
A5	24	25.234	-0.246	0.021	18	14.354	0.962	0.325	13	15.412	-0.614	0.132
B5	4	10.093	-1.918	1.29	5	5.742	-0.310	0.034	13	6.165	2.753	2.658
C5	17	14.223	0.736	0.19	12	8.091	1.374	0.662	2	8.687	-2.269	1.805
A6	39	28.445	1.979	1.374	8	16.181	-2.034	1.451	15	17.374	-0.569	0.114
B6	24	18.810	1.197	0.502	5	10.701	-1.743	1.065	12	11.489	0.151	0.008
C6	18	9.635	2.695	2.547	2	5.481	-1.487	0.775	1	5.885	-2.014	1.422
χ^2	285.13											
df	34											
n	728											
p-value	<0.001											

A.6 Pearson residuals of χ^2 test for frequency of occurrence in each vivarium grid cell group by a) female sand lizards A and B, and b) all male sand lizards.

Positive (blue) and negative (red) relationships between each lizard and cell. Female C had too low a frequency of observations to be included (total *n = 27*), indicative of her being visible for only 3 hours and 7 seconds of the total 40 hours of observations.



A.7 Overarching predictors of use of space by adult sand lizards

Proportion of time adult sand lizards spent in a location visible was modelled with binomial GLMMs; ‘Session within Date’ was included as a random effect. Individual was nested in Sex and Refugium Type was nested in Location. Female, Session 1, Vegetation (Location), Vegetated microhabitat, Male A, Female C and Wood were the reference levels of the categorical fixed effects included. Time spent visible was significantly predictable by Sex, Location (Refugia/Vegetation), one particular individual (Female B), and the use of Slate and Tile Refugia. Fixed effects of Microhabitat and Session were removed in Models 2 and 3 successively, with Model 3 proving optimal using both AIC scores and Likelihood Ratio tests as the minimal adequate model. No further variables could be removed due to their inclusion of significant categories.

	Model 1 / Maximal				**Model 2**				**Model 3 / Optimal**			
Coefficients	**Estimate**	**St. Err.**	***t* value**	***P***	**Estimate**	**St. Err.**	***t* value**	***P***	**Estimate**	**St. Err.**	***t* value**	***P***
(Intercept)	-2.988	0.489	-6.108	**<0.001**	-3.026	0.494	-6.119	**<0.001**	-3.133	0.476	-6.589	**<0.001**
Sex (Male)	2.290	0.496	4.618	**<0.001**	2.311	0.501	4.613	**<0.001**	2.320	0.503	4.618	**<0.001**
Location (Refugia)	-5.223	0.742	-7.036	**<0.001**	-5.212	0.704	-7.404	**<0.001**	-5.196	0.700	-7.427	**<0.001**
Female B	1.576	0.516	3.056	**0.002**	1.603	0.520	3.080	**0.002**	1.612	0.522	3.091	**0.002**
Refugia : Slate	2.047	0.737	2.777	**0.005**	2.049	0.737	2.779	**0.005**	2.024	0.734	2.759	**0.006**
Refugia : Tile	2.174	0.732	2.969	**0.003**	2.166	0.733	2.956	**0.003**	2.159	0.728	2.964	**0.003**
Male C	-0.512	0.291	-1.758	0.079	-0.500	0.291	-1.717	0.086	-0.503	0.290	-1.732	0.083
Session 2	0.355	0.268	1.326	0.185	0.376	0.268	1.403	0.161				
Session 3	-0.370	0.306	-1.210	0.226	-0.354	0.307	-1.154	0.248				
Session 4	-0.280	0.302	-0.929	0.353	-0.264	0.302	-0.876	0.381				
Microhabitat (Open)	-0.437	0.460	-0.950	0.342								
Microhabitat (Semi-veg)	0.339	0.379	0.892	0.372								
Male B	-0.240	0.277	-0.865	0.387	-0.233	0.277	-0.843	0.399	-0.239	0.277	-0.863	0.388
Female A	0.841	0.556	1.514	0.130	0.873	0.560	1.559	0.119	0.876	0.561	1.561	0.119
Refugia: Tin	-0.791	1.238	-0.638	0.523	-0.717	1.209	-0.593	0.553	-0.735	1.208	-0.608	0.543
AIC	881.7				881.0				882.1			
									Deviance	**d.f.**	***p***	
Likelihood ratio test								**Model 1 : Model 2 (remove Microhabitat)**	-3.385	-2	0.184	
								Model 1 : Model 3 (remove Session)	-10.453	-5	0.063	

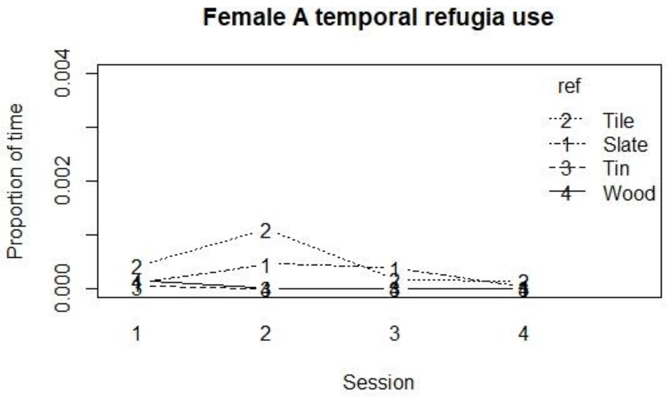
A.8 Adult sand lizard visibility

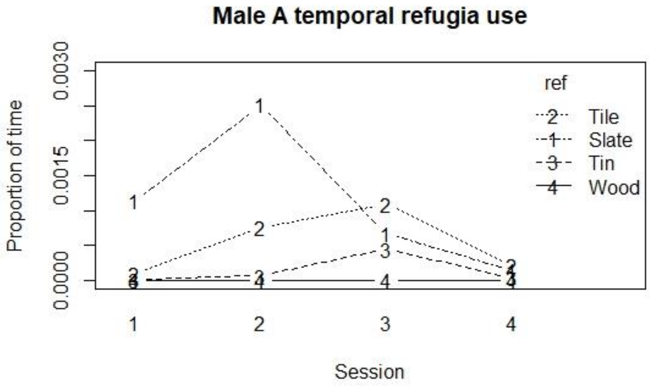
The effect of Session and Individual Female/Male sand lizard (fixed effects) on Visibility as a proportional response was modelled with binomial GLMMs; ‘Session number within Date’ was included as a random effect. Reference level of Individual was Female A / Dominant Male (A); reference level of Session was Session 1. For females, Model 1 included an interaction term, Model 2 did not. Model 1 was the optimal model, using AIC values and Likelihood Ratio Tests. For males, Model 1 was optimal showing significant interaction terms.

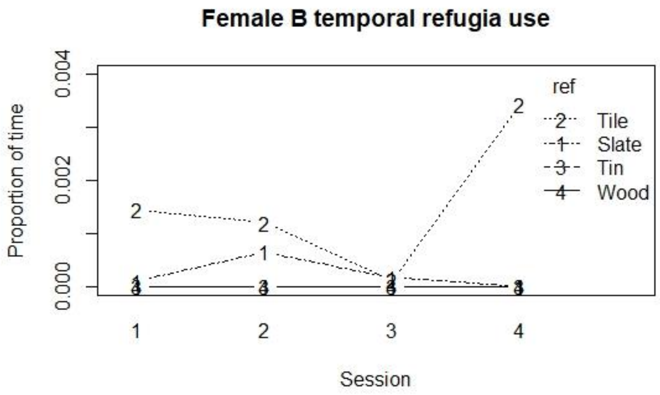
Females	Model 1 (optimal model)				Model 2 (interaction removed)			
Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***	**Estimate**	**Std. Err.**	**z value**	***P***
(Intercept)	-2.626	1.493	-1.759	0.079	-1.639	0.583	-2.813	**0.005**
Session 2	0.971	1.595	0.608	0.543	-0.216	0.692	-0.313	0.754
Session 3	0.154	1.724	0.089	0.929	-1.619	0.909	-1.780	0.075
Session 4	-0.456	1.803	-0.253	0.800	-0.542	0.624	-0.868	0.385
Female B	2.697	1.478	1.825	0.068	1.076	0.462	2.326	**0.020**
Female C	-19.272	260.559	-0.074	0.941	-1.127	0.600	-1.879	0.060
Session 2 * Female B	-1.956	1.734	-1.128	0.259				
Session 3 * Female B	-10.883	24.013	-0.453	0.650				
Session 4 * Female B	-0.262	1.873	-0.140	0.889				
Session 2 * Female C	18.329	260.558	0.070	0.944				
Session 3 * Female C	19.273	260.561	0.074	0.941				
Session 4 * Female C	10.604	275.085	0.039	0.969				
AIC	202.3				217.6			
Likelihood Ratio Test	**Deviance**	**d.f.**	***P***					
Model 1 : Model 2	27.32	-6	**<0.001**					

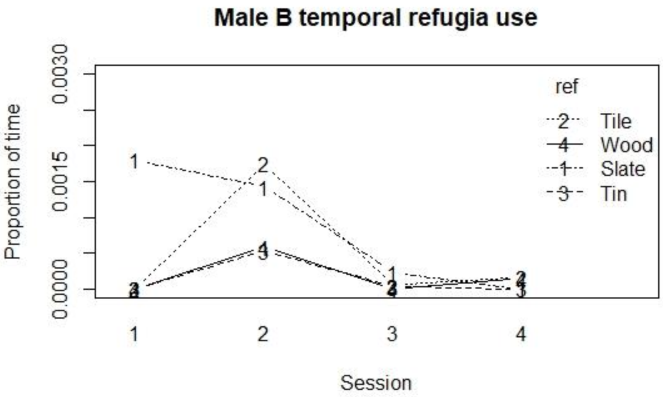
Males	Model 1 (optimal model)			
Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
(Intercept)	-1.039	0.745	-1.395	0.163
Session 2 * Mid-rank	-2.485	1.188	-2.092	**0.037**
Session 3 * Mid-rank	-5.037	2.014	-2.501	**0.012**
Session 3 * Subord.	-5.427	2.519	-2.155	**0.031**
Session 2	2.140	0.970	2.206	**0.027**
Session 3	2.812	1.398	2.012	**0.044**
Session 4	0.042	0.930	0.045	0.964
Mid-rank (Male B)	1.017	0.851	1.195	0.232
Subord. (Male C)	0.032	0.832	0.038	0.969
Session 4 * Mid-rank	-0.302	1.106	-0.273	0.785
Session 2 * Subord.	-0.663	1.146	-0.578	0.563
Session 4 * Subord.	-0.332	1.117	-0.297	0.766
AIC	313.7			

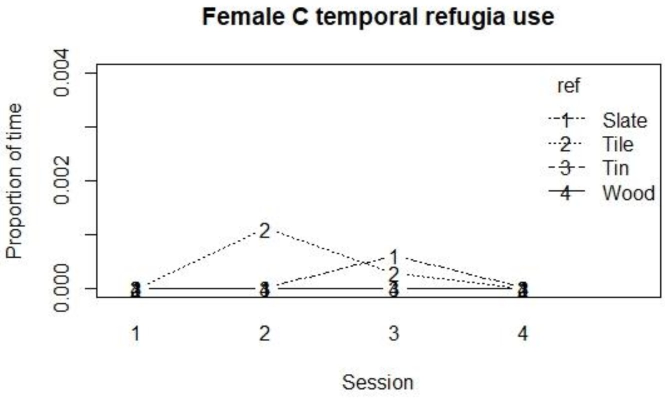
A.9 Proportion of time spent by each individual utilising four different refugium types across the day. (Sessions: early/mid-morning, late morning/noon, early afternoon, late afternoon/evening)

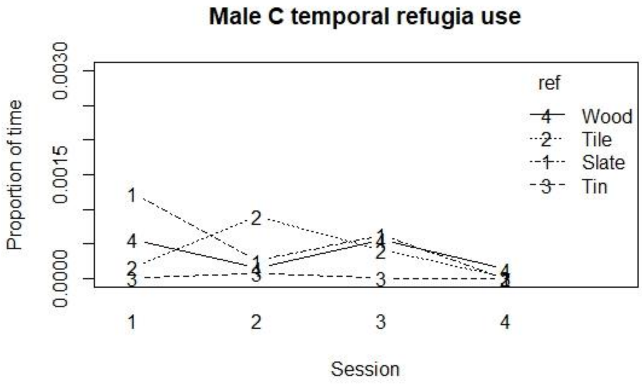




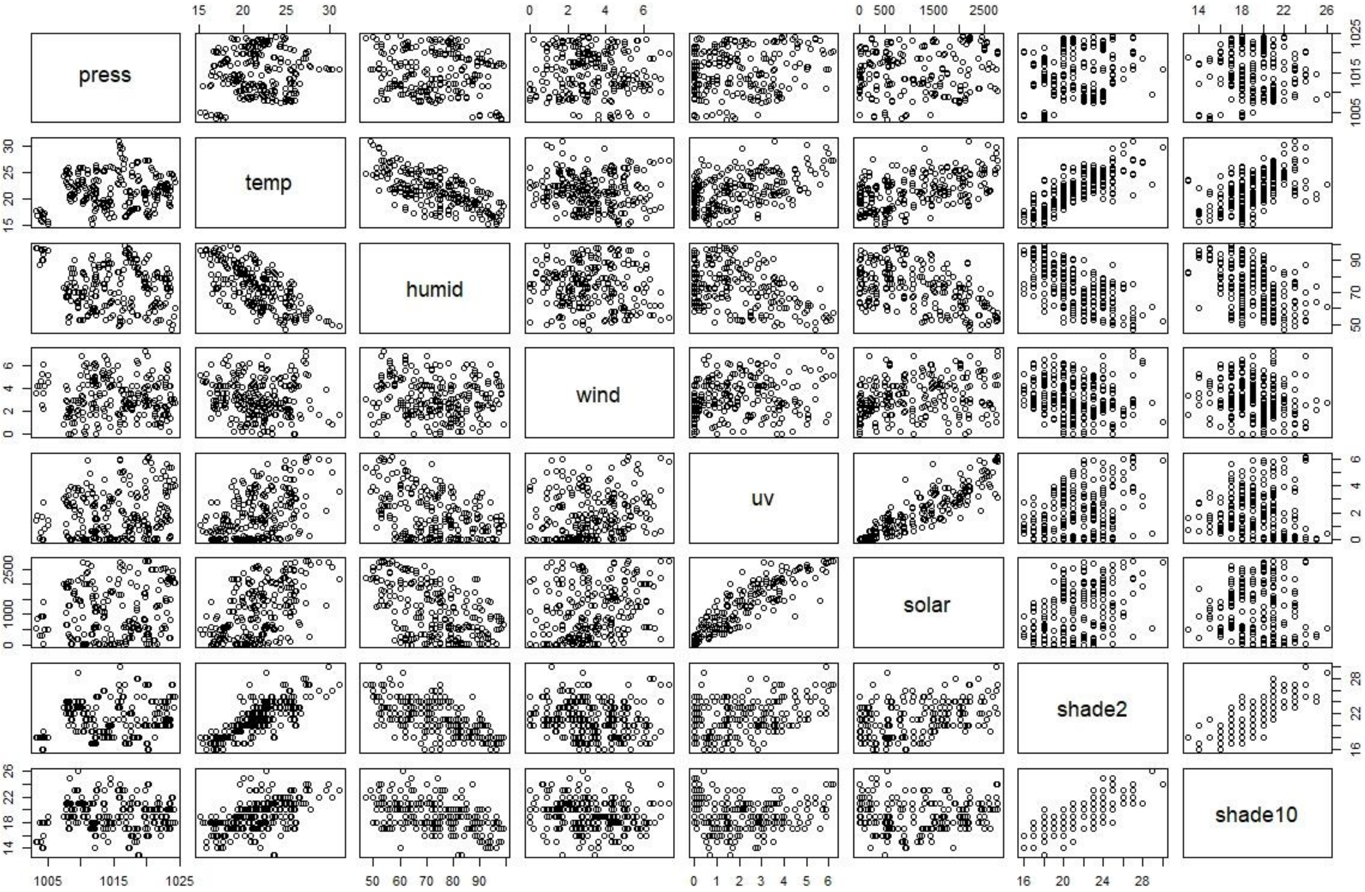




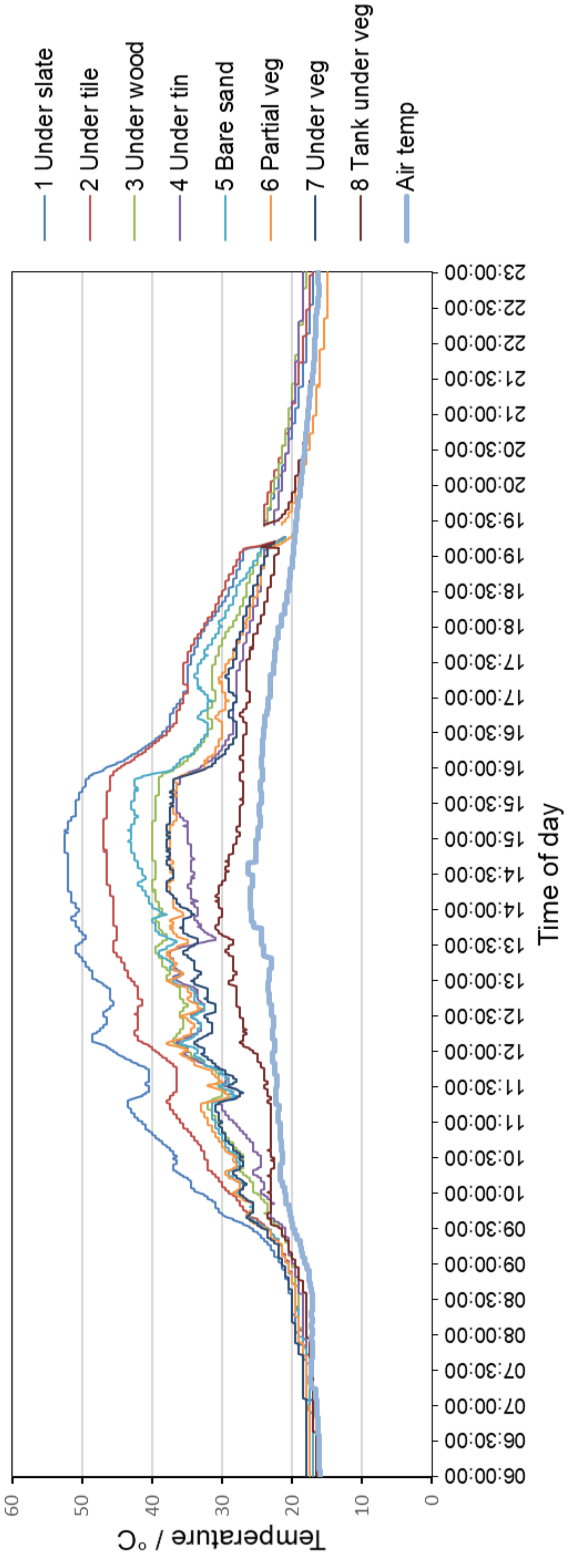




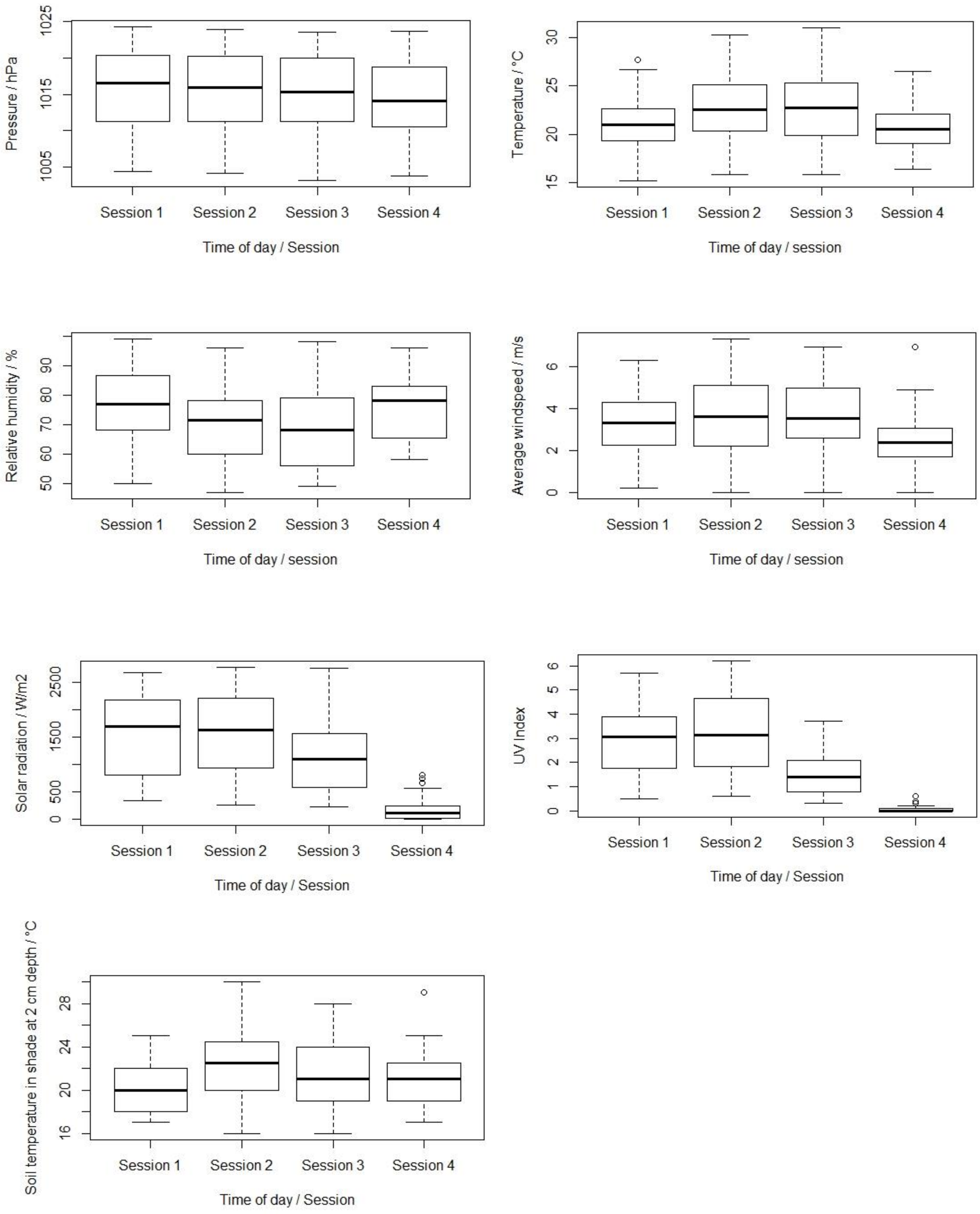
A.10 Plots of weather variables for all sessions in Chapter 2 (males, females, juveniles) (n = 240)



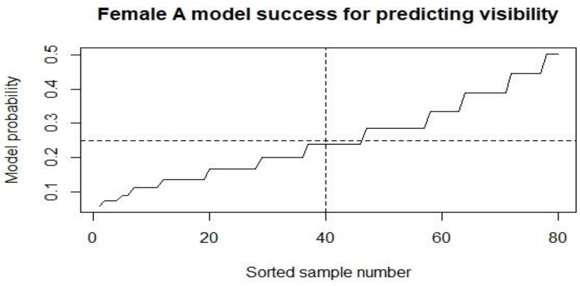
A.11 Refugium heating properties in vivaria (°C). Remote temperature logger (iButtons) data recorded every 10 minutes, example from 18/08/2016.

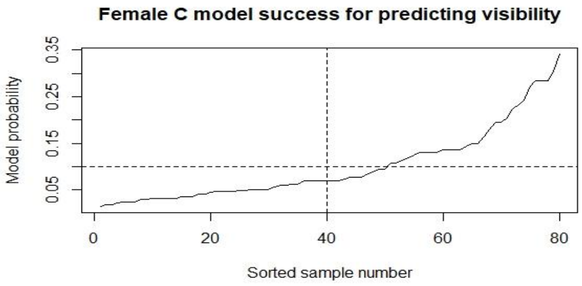


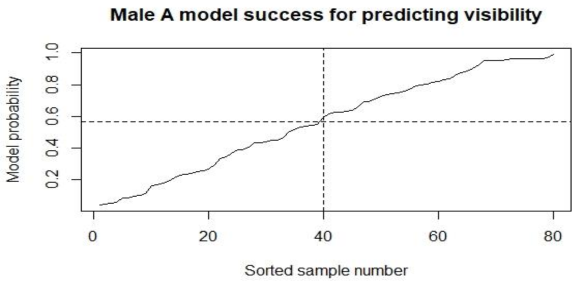
A.12 Variation of environmental variables with Session (time of day), from all 30-minute observation sessions August-September 2016 (total session n = 240). Plots highlight the median, interquartile range, extreme values and outliers.

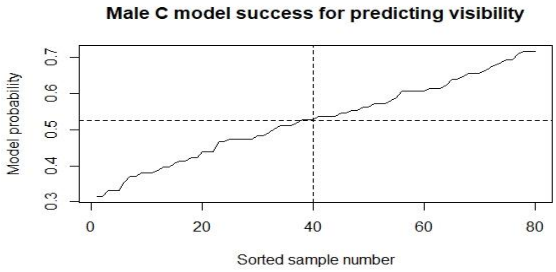


A.13 Success of environmental variable models at predicting Female A and C, and Male A and C visibility (including guidelines for mean probability and mean of the sample number).

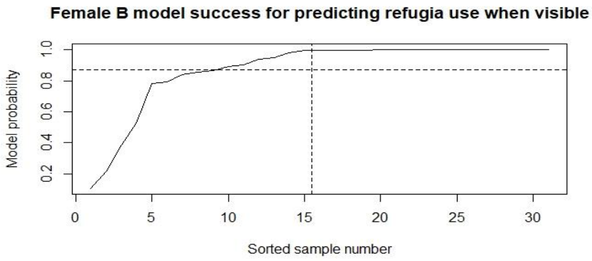

Female A model success for predicting visibility

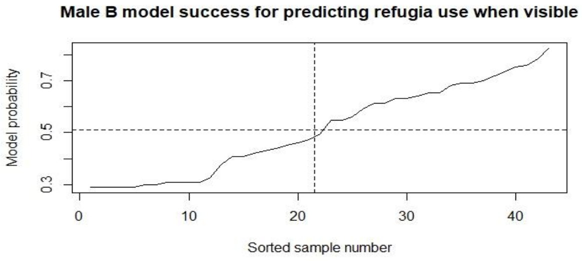

Female C model success for predicting visibility

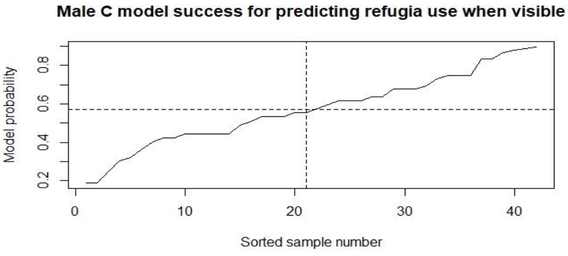

Male A model success for predicting visibility


Male C model success for predicting visibility

A.14 Success of environmental variable models at predicting refugium use when visible for Female B, and Males B and C (including guidelines for mean probability and mean of the sample number).


Female B model success for predicting refugia use when visible


Male B model success for predicting refugia use when visible


Male C model success for predicting refugia use when visible

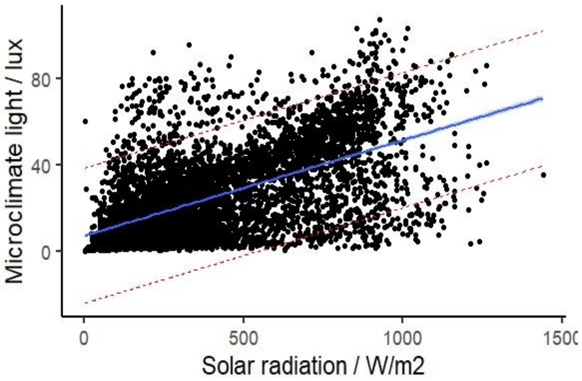
A.15 Juvenile sand lizard visibility

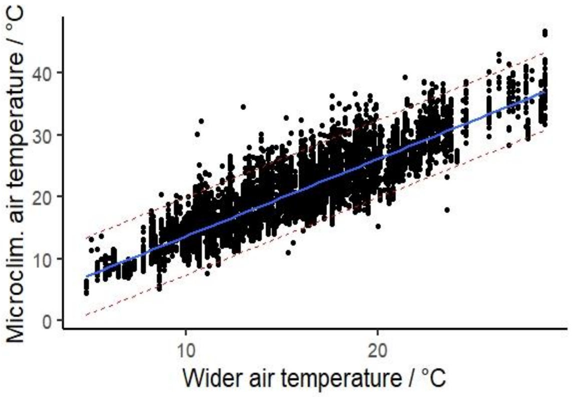
Collective juvenile sand lizard visibility as a proportional time response in a binomial GLMM did not vary significantly between Sessions. ‘Session 1’ was the reference level; ‘Session number within Date’ was included as a random effect. Sand lizards were (non-significantly) less visible in Sessions 2, 3 and 4 compared to the reference level of Session 1.

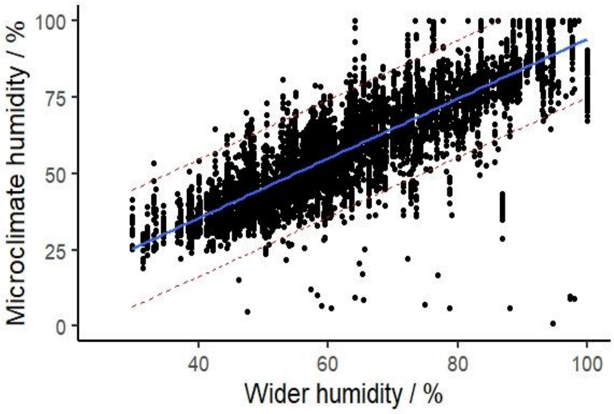
Coefficients	**Estimate**	**Std. Err.**	***t* value**	***P***
(Intercept)	-1.128	0.379	-2.973	**0.003**
Session 2	-0.824	0.618	-1.333	0.183
Session 3	-0.809	0.617	-1.311	0.190
Session 4	-5.538	4.383	-1.264	0.206
AIC	133.7			

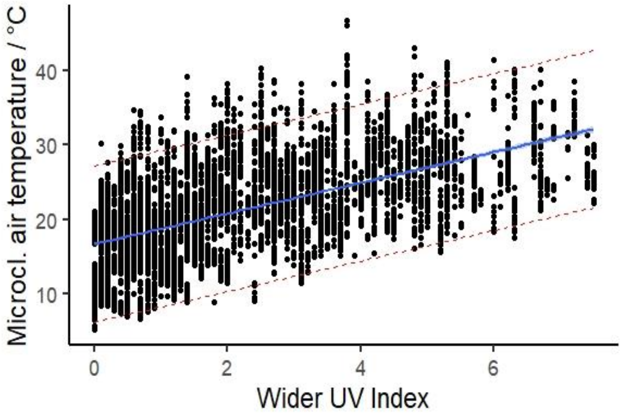
Appendix B Chapter 3

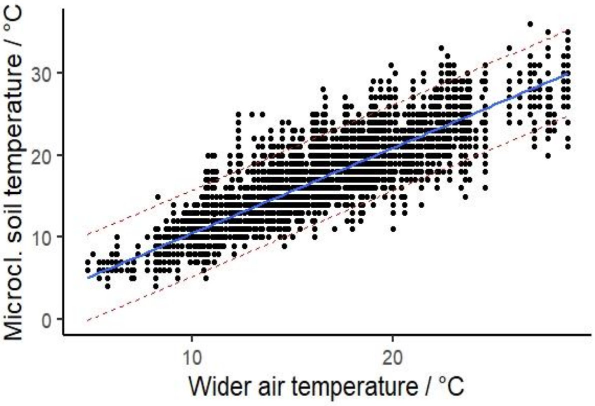
B.1 Weather variables and corresponding microclimate variables in dry heath (regression line blue, CI grey band, PB red dashed line).

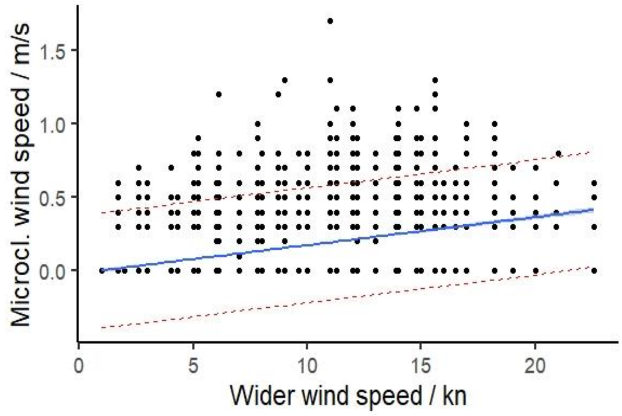












Appendix C Chapter 4

C.1 Total counts of four common reptile species across five broad habitat types of Eelmoor Marsh and respective refugium totals and transect distances in each habitat.

		Adder	Common lizard	Grass snake	Slow worm	Refugia/transect per habitat
New arrays	Dry heath	1	34	1	92	18
	Wet heath	0	9	0	36	4
	Grassland	0	9	0	33	5
	Mire	0	5	0	22	9
	Woodland	0	0	0	27	12
Old tins	Dry heath	6	8	3	108	17
	Wet heath	5	11	0	61	12
	Grassland	5	1	3	14	5
	Mire	2	4	0	37	8
	Woodland	0	2	0	38	6
Transects	Dry heath	2	11	2	1	*2537 m*
	Wet heath	0	3	0	0	*778 m*
	Grassland	0	3	0	0	*1078 m*
	Mire	0	10	0	1	*1141 m*
	Woodland	0	0	0	1	*1509 m*
Transect encounter rate	*Dry heath*	*0.788x10*$^{-3}$	*4.336x10*$^{-3}$	*0.788x10*$^{-3}$	*0.394x10*$^{-3}$	
	Wet heath	*0.0*	*3.856x10*$^{-3}$	*0.0*	*0.0*	
	Grassland	*0.0*	*2.783x10*$^{-3}$	*0.0*	*0.0*	
	Mire	*0.0*	*8.764x10*$^{-3}$	*0.0*	*0.876x10*$^{-3}$	
	Woodland	*0.0*	*0.0*	*0.0*	*0.663x10*$^{-3}$	
	TOTALS	**21**	**110**	**9**	**471**	**48 / 48 / *5902 m***

C.2 Observations of common lizards and slow worms utilising different refugium materials within each habitat type.

		Dry heath	Wet heath	Grassland	Mire	Woodland	Total
Common lizard	Tin	9	1	4	3	0	**17**
	Slate	3	1	1	2	0	**7**
	Felt	13	5	2	1	0	**21**
	Tile	8	2	2	0	0	**12**
	TOTAL	**33**	**9**	**9**	**6**	**0**	**57**
Slow worm	Tin	31	16	6	6	10	**69**
	Slate	31	11	9	11	2	**64**
	Felt	11	1	7	2	11	**32**
	Tile	18	9	11	3	4	**45**
	TOTAL	**91**	**37**	**33**	**22**	**27**	**210**

C.3 Common lizard use of habitat types and refugia

(Overleaf) Model simplification results for Poisson GLMM. As no variables were shown to be significant in the maximal model, the interaction term was removed initially. This reduced the AIC value to 561.97; a Likelihood Ratio Test (LRT) showed no significance between this simplified and the maximal model. By removing ‘habitat type’, the least significant variable, the AIC increased to 578.64 (by >2) and an LRT showed a significant difference between the maximal model and the refugium material only model, as well as between this and the refugium and habitat type model. ‘Tin’ was reference level for refugium type; ‘dry heath’ was reference level for habitat type; ‘array location’ was a random effect.

	Maximal model – Model 1				**OPTIMAL MODEL - Model 2**				**Model 3**			
Coefficients	Estimate	Std. Err.	z value	P value	Estimate	Std. Err.	z value	P value	Estimate	Std. Err.	z value	*P*
Intercept	-3.985	0.386	-10.318	**<0.001**	-3.896	0.346	-11.263	**<0.001**	-4.698	0.3675	-12.786	**<0.001**
Slate	-1.099	0.689	-1.595	0.111	-0.887	0.449	-1.976	**0.048**	-0.887	0.449	-1.976	**0.048**
Felt	0.368	0.448	0.821	0.412	0.211	0.326	0.648	0.517	0.211	0.3262	0.648	0.517
Tile	-0.118	0.502	-0.235	0.814	-0.348	0.377	-0.924	0.356	-0.348	0.3769	-0.924	0.355
Grassland	0.516	0.722	0.715	0.475	0.028	0.533	0.052	0.959	NA			
Mire	-0.365	0.766	-0.476	0.634	-0.971	0.539	-1.801	0.072				
Woodland	-18.022	3785.739	-0.005	0.996	-15.145	75.491	-0.201	0.841				
Wet heath	-0.698	1.159	-0.602	0.547	0.200	0.562	0.357	0.721				
Felt:Grassland	-1.061	1.002	-1.059	0.290	NA							
Slate:Grassland	-0.288	1.346	-0.214	0.831								
Tile:Grassland	-0.575	1.027	-0.560	0.575								
Felt:Mire	-1.467	1.314	-1.116	0.264								
Slate:Mire	0.693	1.195	0.580	0.562								
Tile:Mire	-17.275	3692.887	-0.005	0.996								
Felt:Woodland	0.398	4582.081	0.000	1.000								
Slate:Woodland	1.732	4684.061	0.000	1.000								
Tile:Woodland	0.715	4714.042	0.000	1.000								
Felt:Wet heath	1.242	1.211	1.025	0.305								
Slate:Wet heath	1.099	1.608	0.683	0.494								
Tile: Wet heath	0.811	1.354	0.599	0.549								
AIC				576.5				562.0				578.6
Likelihood Ratio Test	**χ²**	**d.f.**	**P**									
Maximal : M1	9.426	12	0.666									
Maximal : M2	34.088	16	**0.005**									
M1 : M2	24.663	4	**<0.001**									

C.4 Slow worm use of habitat types and refugia

Model simplification results for negative binomial GLMM. No interaction terms were significant, however three had p-values between 0.05 and 0.1. Dropping the interaction term resulted in an increase in AIC by 6.2, suggesting the altered model has limited support, as well as a significant deviance from the maximal model. Collectively this indicated all factors, including the interaction, should remain in the model. 'Tin' was reference level for refugium type; 'dry heath' was reference level for habitat type; 'array location' was a random effect.

	Maximal – OPTIMAL MODEL – Model 1				**Model 2**			
Coefficients	Estimate	Std. Err.	z value	*P*	Estimate	Std. Err.	z value	*P*
Intercept	-2.789	0.288	-9.681	**<0.001**	-2.813	0.265	-10.619	**<0.001**
Felt	-1.005	0.362	-2.778	**0.005**	-0.758	0.224	-3.385	**0.001**
Tile	-0.510	0.309	-1.651	0.099	-0.430	0.203	-2.120	**0.034**
Woodland	-0.934	0.524	-1.781	0.075	-1.008	0.427	-2.361	**0.018**
Slate	0.004	0.269	0.014	0.989	-0.085	0.186	-0.456	0.648
Grassland	-0.457	0.657	-0.696	0.486	0.129	0.510	0.254	0.800
Mire	-1.052	0.599	-1.755	0.079	-0.847	0.457	-1.852	0.064
Wet heath	0.963	0.591	1.628	0.103	0.696	0.530	1.311	0.190
Felt:Grassland	1.090	0.690	1.579	0.114	NA			
Slate:Grassland	0.336	0.621	0.541	0.588				
Tile:Grassland	1.093	0.623	1.753	0.080				
Gfelt:Mire	-0.092	0.902	-0.102	0.919				
Slate:Mire	0.595	0.588	1.012	0.311				
Tile:Mire	-0.187	0.782	-0.239	0.811				
Felt:Woodland	1.129	0.580	1.946	0.052				
Slate:Woodland	-1.595	0.829	-1.923	0.054				
Tile:Woodland	-0.391	0.678	-0.577	0.564				
Felt:Wet heath	-1.784	1.106	-1.613	0.107				
Slate:Wet heath	-0.379	0.505	-0.750	0.453				
Tile: Wet heath	-0.098	0.547	-0.179	0.858				
AIC				1568.0				1574.2
Likelihood Ratio Test	**χ^2**	**d.f.**	**P**					
Maximal : M1	30.142	12	**0.003**					

C.5 The effect of environmental variables on common lizard refugium use in multiple habitat types

Minimum adequate Poisson GLMM considering microclimate factors and material type selected by common lizards (none were retained in the final model). 'Tin' was the reference level for material type.

Coefficients	Estimate	Std. Err.	z value	*P*
Intercept	-4.821	0.389	-12.408	**<0.001**
Felt	0.272	0.332	0.820	0.413
Slate	-0.827	0.453	-1.824	0.068
Tile	-0.288	0.382	-0.753	0.451
AIC	568.6			

Minimum adequate Poisson GLMM considering microhabitat factors and material type selected by common lizards. 'Tin' was the reference level for material type.

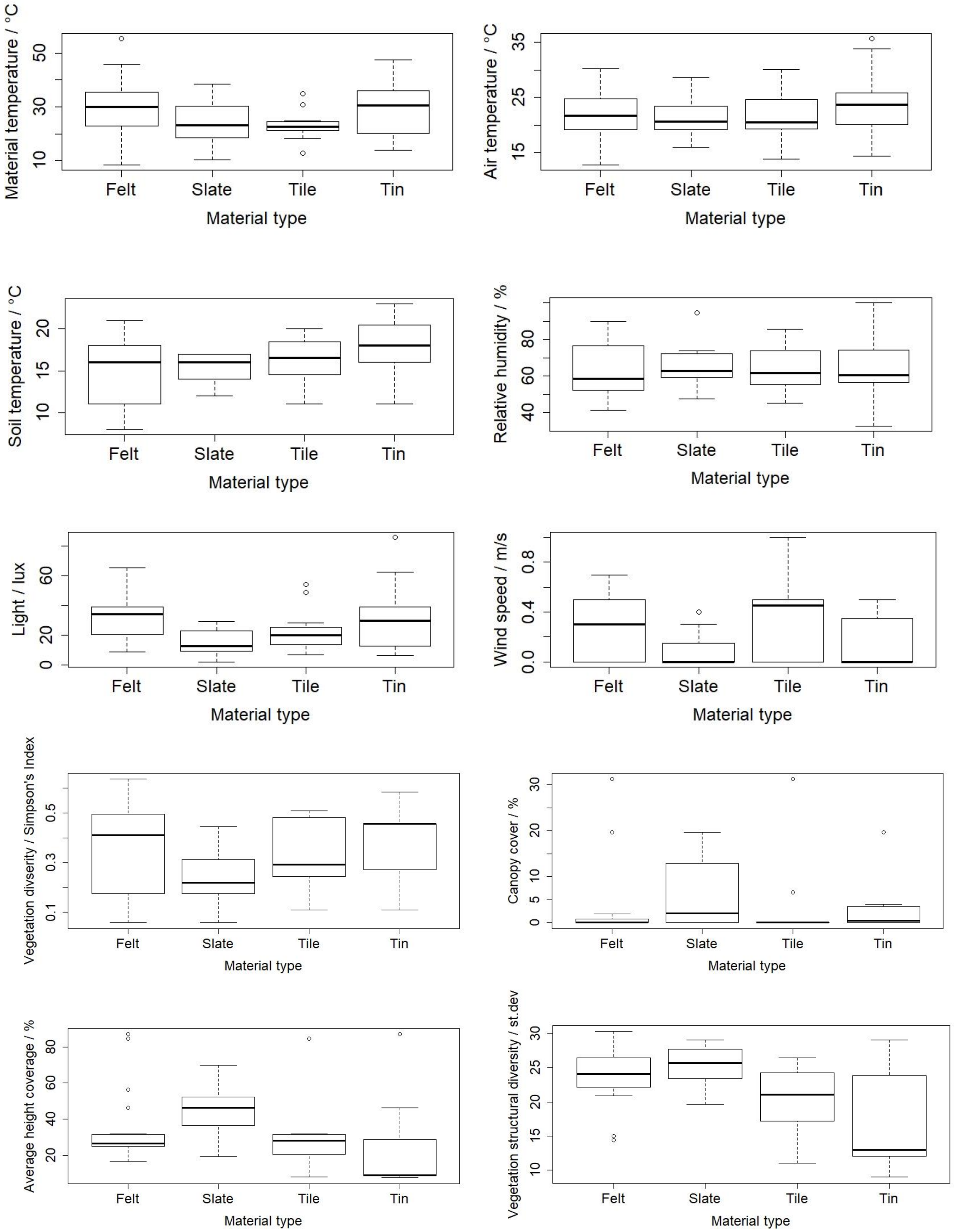
Coefficients	Estimate	Std. Err.	z value	*P*
Intercept	-1.838	0.940	-1.955	0.051
Felt: structural diversity	0.206	0.065	3.151	**0.002**
Slate: structural diversity	0.296	0.116	2.542	**0.011**
Felt	-4.035	1.445	-2.792	**0.005**
Slate	-7.349	2.869	-2.562	**0.010**
Canopy cover	-0.052	0.017	-3.111	**0.002**
Vertical veg cover	-0.020	0.009	-2.232	**0.026**
Tile	-1.896	1.258	-1.507	0.132
Structural diversity	-0.071	0.044	-1.627	0.104
Tile: structural diversity	0.085	0.062	1.372	0.170
AIC	536.5			

Environmental factors associated with **common lizard** material type use across wider Eelmoor Marsh SSSI, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are ***bold italicised*** and any significantly different material pairs following post-hoc testing are highlighted.

Common lizard

	Felt					**Slate**					**Tile**					**Tin**					**Main model (ANOVA/Kruskal-Wallis)**					
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	**F value**	**Kruskal-W χ^2**	**df**	**P value**	**Sig. pairs**	**Transform.**
MICROCLIMATE																										
Mat.temp °C	29.62	30.10	23.00	35.40	21	24.24	23.10	18.50	30.40	7	23.28	22.70	21.40	24.55	12	29.96	30.45	20.20	35.90	16	1.325		3,52	0.276	NA	NA
Air temp °C	21.31	21.70	19.10	24.80	21	21.49	20.60	19.15	23.50	7	21.84	23.70	20.15	25.90	12	23.85	23.70	20.15	25.90	16	0.945		3,52	0.426	NA	NA
Soil temp °C	15.19	16.00	11.00	18.00	21	15.29	16.00	14.00	17.00	7	16.33	16.50	14.50	18.50	12	18.00	18.00	16.00	20.50	16	2.230		3,52	0.096	NA	NA
Rel hum %	63.73	58.60	52.10	76.40	21	66.93	63.00	59.40	72.20	7	64.22	61.75	55.55	73.75	12	63.01	60.35	56.65	74.20	16	0.122		3,52	0.947	NA	NA
Light Lux	30.43	33.91	20.40	39.11	21	15.53	12.86	9.30	25.42	7	23.06	19.92	13.54	25.42	12	31.35	29.82	12.44	38.89	16	2.446		3,52	0.074	NA	Sq.rt.
Wind sp m/s	0.25	0.30	0.00	0.50	21	0.10	0.00	0.00	0.15	7	0.34	0.45	0.00	0.50	12	0.15	0.00	0.00	0.35	16		4.931	3	0.177	NA	NA
MICROHABITAT																										
Cover div SI	0.35	0.41	0.18	0.49	21	0.24	0.22	0.18	0.31	7	0.32	0.29	0.25	0.48	12	0.38	0.45	0.27	0.46	16	1.225		3,52	0.310	NA	NA
Struct st.dev	23.82	24.10	22.13	26.47	21	25.22	25.73	23.40	27.67	7	20.51	21.07	17.17	24.30	12	17.11	13.00	12.00	23.84	16	6.543		3,52	**<0.001**	Tin/Felt, Tin/Slate	NA
Height cover %	33.32	26.41	24.78	31.47	21	44.72	46.34	36.55	52.25	7	34.47	28.13	20.58	31.63	12	21.44	8.94	8.42	28.63	16	5.531		3,52	**0.002**	Tin/Felt, Tin/Slate	log10
Can cov %	2.77	0.00	0.00	0.78	21	6.80	2.08	0.00	12.94		3.15	0.00	0.00	0.00	12	2.63	0.46	0.00	3.51	16		4.159	3	0.245	NA	NA

Microclimate and microhabitat conditions associated with material use by common lizards across wider Eelmoor Marsh SSSI. Plots highlight the median, interquartile range, extreme values and outliers.



C.6 The effect of environmental variables on slow worm refugium use in multiple habitat types

Minimum adequate negative binomial GLMM considering microclimate factors and material type selected by slow worms. ‘Tin’ was the reference level for material type.

Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
Intercept	-6.465	0.806	-8.025	**<0.001**
Felt : lux	-0.037	0.014	-2.652	**0.008**
Felt : humidity	-0.038	0.015	-2.501	**0.012**
Soil temp	0.130	0.027	4.792	**<0.001**
Humidity	0.025	0.009	2.794	**0.005**
Felt	2.431	1.122	2.167	**0.030**
Lux	0.000	0.001	0.050	0.960
Slate	0.008	0.824	0.010	0.992
Tile	0.272	0.872	0.312	0.755
Material temp	-0.017	0.013	-1.299	0.194
Slate : humidity	-0.001	0.011	-0.130	0.896
Tile : humidity	-0.010	0.012	-0.843	0.399
Slate : lux	-0.003	0.004	-0.659	0.510
Tile : lux	-0.003	0.005	-0.556	0.578
AIC	**1547.3**			

Minimum adequate negative binomial GLMM considering microhabitat factors and material type selected by slow worms. ‘Tin’ was the reference level for material type.

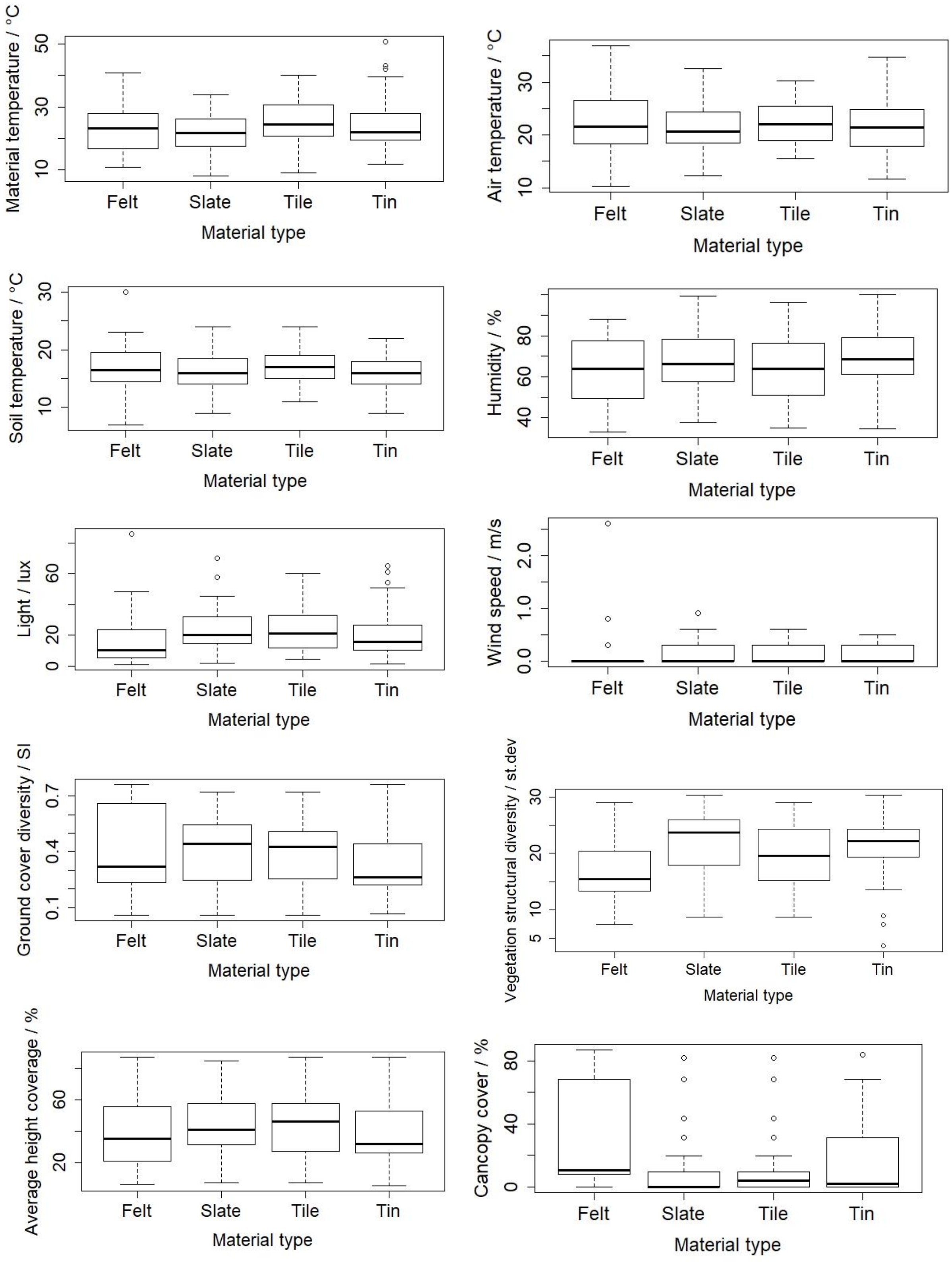
Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
Intercept	-3.607	0.808	-4.463	**<0.001**
Felt: canopy cover	0.021	0.008	2.762	**0.006**
Slate : canopy cover	-0.020	0.010	-1.982	**0.047**
Felt : ground cover diversity	2.439	1.193	2.044	**0.041**
Slate : ground cover diversity	2.371	0.956	2.481	**0.013**
Tile : ground cover diversity	2.354	1.026	2.295	**0.022**
Structural diversity	0.063	0.029	2.170	**0.030**
Felt	-2.138	0.549	-3.893	**<0.001**
Tile	-1.128	0.447	-2.526	**0.012**
Ground cover diversity	-1.619	1.033	-1.566	0.117
Canopy cover	-0.005	0.008	-0.563	0.574
Slate	-0.704	0.411	-1.715	0.086
Tile : canopy cover	-0.008	0.009	-0.899	0.369
AIC	**1561.3**			

Environmental factors associated with **slow worm** material type use across wider Eelmoor Marsh SSSI, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are ***bold italicised*** and any significantly different material pair

Slow worm

	Felt					**Slate**					**Tile**					**Tin**					**Main model (ANOVA/Kruskal-Wallis)**					
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	**F value**	**Kruskal-W χ^2**	**df**	**P value**	**Sig. airs**	**Transform.**
MICROCLIMATE																										
Mat.temp °C	23.67	23.20	16.85	27.90	32	21.68	21.70	17.60	26.30	64	24.72	24.40	20.70	30.70	46	23.81	22.00	19.45	27.85	68	1.821		3,206	0.144	NA	NA
Air temp °C	22.28	21.60	18.30	26.60	32	21.30	20.65	18.45	24.35	64	22.28	22.00	18.90	25.50	46	21.60	21.40	17.80	24.80	69	0.563		3,207	0.640	NA	NA
Soil temp °C	16.63	16.50	14.50	19.50	32	16.36	16.00	14.00	18.50	64	17.11	17.00	15.00	19.00	46	16.09	16.00	14.00	18.00	69	0.457		3,207	0.871	NA	NA
Rel hum %	62.62	63.90	49.40	77.35	32	67.18	66.30	57.60	78.10	64	64.55	63.75	51.00	76.40	46	68.47	68.40	61.00	79.10	69	1.451		3,207	0.229	NA	NA
Light Lux	16.93	10.28	5.34	23.76	32	22.98	20.32	14.83	31.74	64	24.52	20.93	11.90	33.07	46	19.59	15.83	9.94	26.64	68	5.195		3,206	**0.002**	Felt/Slate, Felt/Tile	log
Wind sp m/s	0.14	0.00	0.00	0.00	32	0.14	0.00	0.00	0.30	64	0.16	0.00	0.00	0.30	46	0.12	0.00	0.00	0.30	69		5.082	3	0.166	NA	NA
MICROHABITAT																										
Cover div SI	0.40	0.32	0.23	0.66	32	0.41	0.44	0.25	0.55	64	0.41	0.43	0.25	0.51	46	0.31	0.26	0.22	0.44	69	3.726		3,207	**0.012**	Tin/Slate	NA
Struct st.dev	16.19	15.47	13.30	20.42	32	21.97	23.75	17.96	26.00	64	19.68	19.60	15.17	24.28	46	20.72	22.13	19.37	24.28	69	8.243		3,207	**<0.001**	Felt/Slate, Felt/Tile, Felt/Tin	NA
Height cover %	36.48	35.17	20.84	55.64	32	42.36	41.09	31.47	57.56	64	44.18	45.94	26.88	57.56	46	37.66	31.78	26.25	52.81	69	1.720		3,207	0.164	NA	NA
Can cov %	33.95	10.47	8.06	68.51	32	7.74	0.00	0.00	9.36	64	11.40	4.16	0.00	9.36	46	16.21	2.08	0.00	31.20	69		22.016	3	**<0.001**	Felt/Slate, Felt/Tile, Felt/Tin	NA

Microclimate and microhabitat conditions associated with material use by slow worms across wider Eelmoor Marsh SSSI. Plots highlight the median, interquartile range, extreme values and outliers.

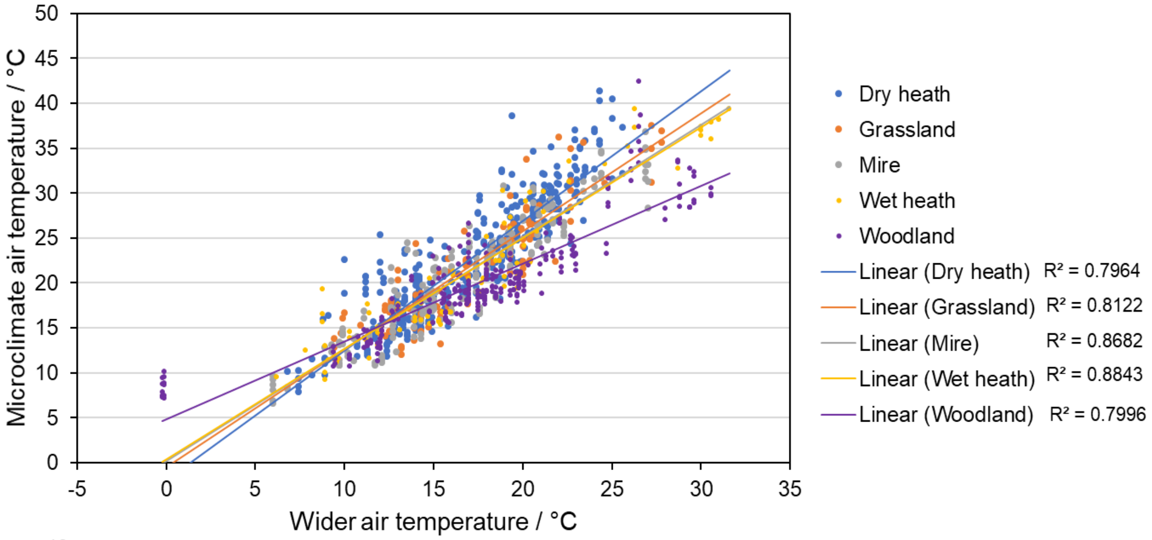


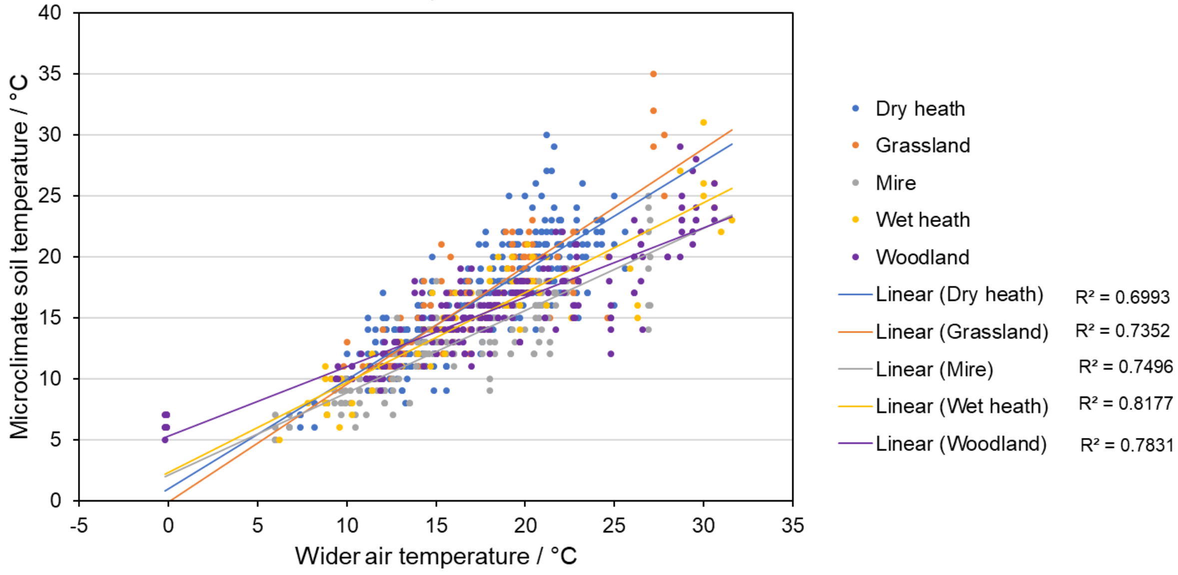
C.7 Linear regression analyses for weather (independent) and microclimate (dependent) across multiple habitats.

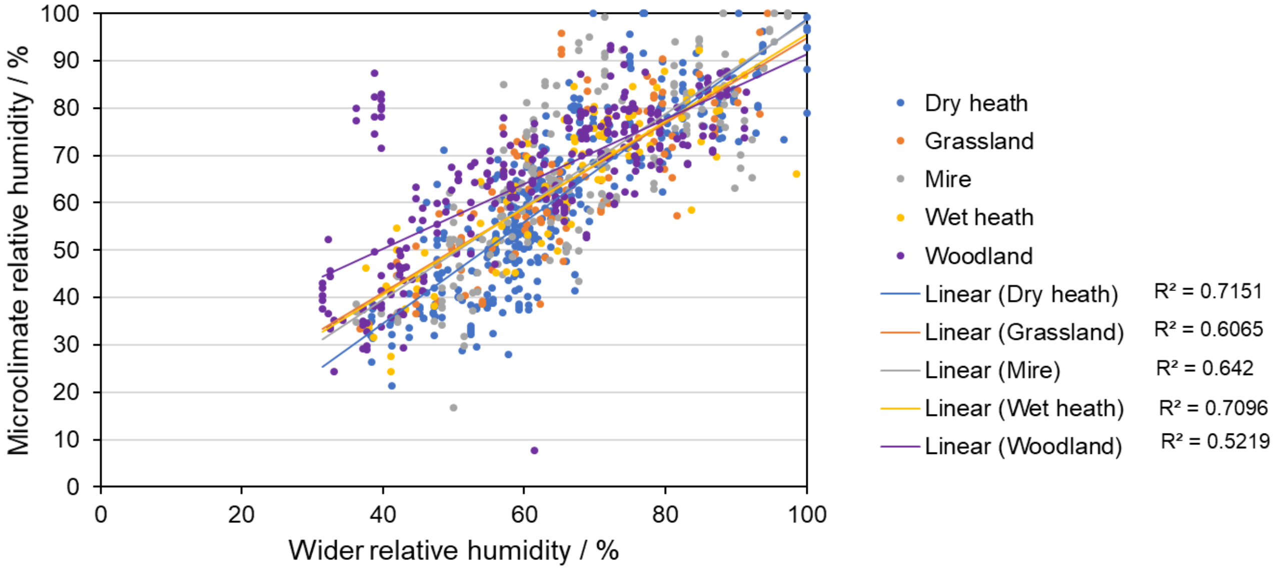
									Confidence Interval	
		Estimate	**Std. Err.**	***t* value**	***P***	**F-statistic**	**R^2**	**d.f.**	**2.5%**	**95%**
Microclimate air temperature ~ Wider air temperature										
Dry heath	Intercept	-1.973	0.673	-2.932	**0.004**					
	Air temp	1.444	0.039	37.471	**<0.001**	1404.00	0.796	1,359	1.368	1.520
Grass	Intercept	-0.493	1.101	-0.448	0.655					
	Air temp	1.312	0.062	21.106	**<0.001**	445.50	0.812	1,103	1.189	1.435
Mire	Intercept	0.145	0.607	0.239	0.811					
	Air temp	1.248	0.036	35.006	**<0.001**	1225.00	0.868	1,186	1.177	1.318
Wet heath	Intercept	0.373	0.900	0.415	0.679					
	Air temp	1.233	0.050	24.878	**<0.001**	618.90	0.884	1,81	1.135	1.332
Woodland	Intercept	4.858	0.502	9.676	**<0.001**					
	Air temp	0.866	0.027	32.207	**<0.001**	1037.00	0.800	1,260	0.813	0.919
Microclimate soil temperature ~ Wider air temperature										
Dry heath	Intercept	0.942	0.541	1.740	0.083					
	Air temp	0.896	0.031	28.890	**<0.001**	834.90	0.699	1,359	0.835	0.957
Grass	Intercept	-0.115	1.018	-0.113	0.910					
	Air temp	0.965	0.058	16.577	**<0.001**	274.80	0.735	1,99	0.850	1.081
Mire	Intercept	2.081	0.488	4.264	**<0.001**					
	Air temp	0.674	0.029	22.951	**<0.001**	526.80	0.750	1,176	0.616	0.732
Wet heath	Intercept	2.297	0.702	3.270	**0.002**					
	Air temp	0.738	0.039	19.060	**<0.001**	363.30	0.818	1,81	0.661	0.815
Woodland	Intercept	5.288	0.347	15.240	**<0.001**					
	Air temp	0.569	0.019	30.640	**<0.001**	938.70	0.783	1,260	0.533	0.606
Microclimate relative humidity ~ Wider relative humidity										
Dry heath	Intercept	-8.320	2.372	-3.508	**0.001**					
	Humidity	1.072	0.036	30.016	**<0.001**	900.90	0.715	1,359	1.002	1.143
Grass	Intercept	5.266	4.704	1.120	0.266					
	Humidity	0.896	0.071	12.600	**<0.001**	158.70	0.607	1,103	0.755	1.037
Mire	Intercept	0.432	3.731	0.116	0.908					
	Humidity	0.979	0.054	18.215	**<0.001**	331.80	0.642	1,185	0.873	1.085
Wet heath	Intercept	3.991	4.356	0.916	0.362					
	Humidity	0.916	0.065	14.070	**<0.001**	198.00	0.710	1,81	0.786	1.046
Woodland	Intercept	22.871	2.597	8.806	**<0.001**					
	Humidity	0.686	0.041	16.848	**<0.001**	283.80	0.522	1,260	0.606	0.766

Continued overleaf

									Confidence Interval	
		Estimate	**Std. Err.**	***t* value**	***P***	**F-statistic**	**R^2**	**d.f.**	**2.5%**	**95%**
Microclimate light ~ Wider solar radiation										
Dry heath	Intercept	8.201	1.636	5.012	**<0.001**					
	Sol rad	0.049	0.003	17.372	**<0.001**	301.80	0.457	1,358	0.043	0.055
Grass	Intercept	11.624	2.803	4.148	**<0.001**					
	Sol rad	0.031	0.005	5.634	**<0.001**	31.74	0.237	1,102	0.020	0.042
Mire	Intercept	7.552	1.991	3.792	**<0.001**					
	Sol rad	0.028	0.004	6.559	**<0.001**	43.02	0.192	1,181	0.019	0.036
Wet heath	Intercept	8.058	2.484	3.244	**0.002**					
	Sol rad	0.054	0.006	9.854	**<0.001**	97.11	0.542	1,82	0.043	0.065
Woodland	Intercept	2.646	1.519	1.742	0.083					
	Sol rad	0.017	0.003	5.924	**<0.001**	35.09	0.119	1,261	0.011	0.022
Microclimate air temperature ~ Wider UV Index										
Dry heath	Intercept	18.365	0.518	35.450	**<0.001**					
	UV	1.567	0.154	10.180	**<0.001**	103.60	0.223	1,356	1.264	1.870
Grass	Intercept	18.994	0.827	22.958	**<0.001**					
	UV	1.490	0.298	5.005	**<0.001**	25.05	0.196	1,103	0.900	2.081
Mire	Intercept	16.900	0.670	25.211	**<0.001**					
	UV	1.778	0.235	7.577	**<0.001**	57.41	0.243	1,179	1.315	2.241
Wet heath	Intercept	18.526	1.275	14.532	**<0.001**					
	UV	1.294	0.390	3.316	**0.001**	10.99	0.124	1,78	0.517	2.071
Woodland	Intercept	16.518	0.508	32.500	**<0.001**					
	UV	1.650	0.162	10.180	**<0.001**	103.70	0.298	1,244	1.331	1.969
Microclimate wind speed ~ Wider wind speed										
Dry heath	Intercept	-0.019	0.026	-0.734	0.464					
	Wind sp	0.020	0.003	6.559	**<0.001**	43.02	0.112	1,341	0.014	0.026
Grass	Intercept	-0.031	0.056	-0.560	0.577					
	Wind sp	0.020	0.006	3.443	**0.001**	11.86	0.108	1,98	0.008	0.031
Mire	Intercept	-0.015	0.034	-0.438	0.662					
	Wind sp	0.011	0.003	3.763	**0.001**	14.16	0.075	1,176	0.005	0.017
Wet heath	Intercept	-0.040	0.060	-0.668	0.506					
	Wind sp	0.025	0.005	4.631	**<0.001**	21.44	0.222	1,75	0.014	0.035
Woodland	Intercept	-0.057	0.071	-0.800	0.425					
	Wind sp	0.032	0.006	4.924	**<0.001**	24.24	0.093	1,236	0.019	0.044







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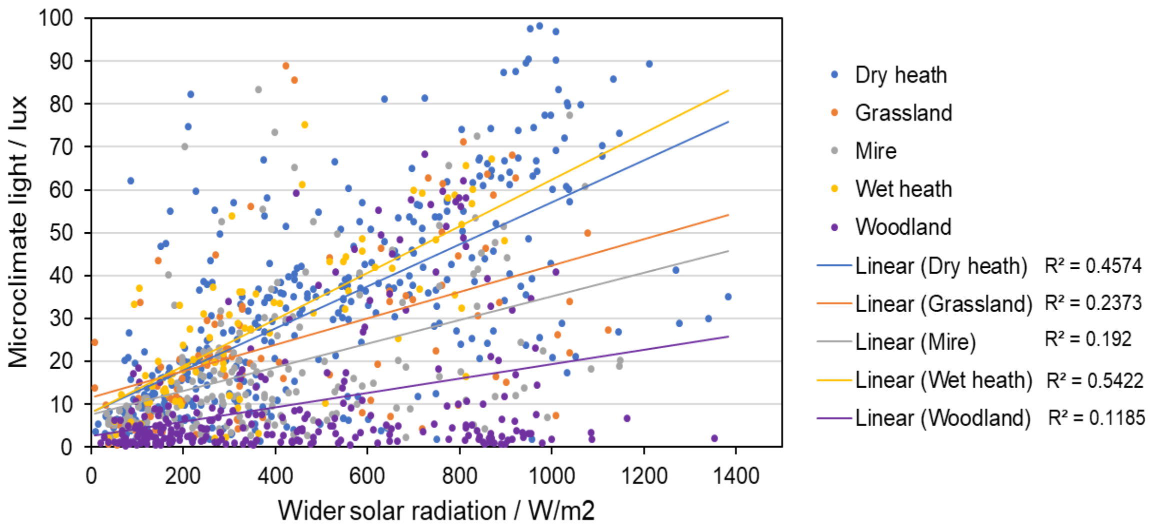
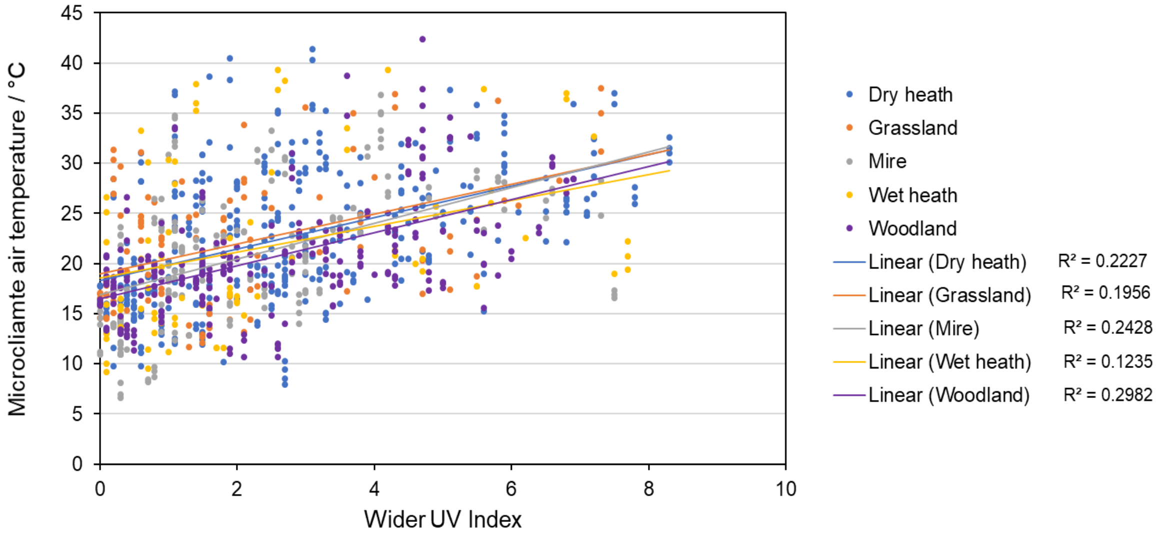
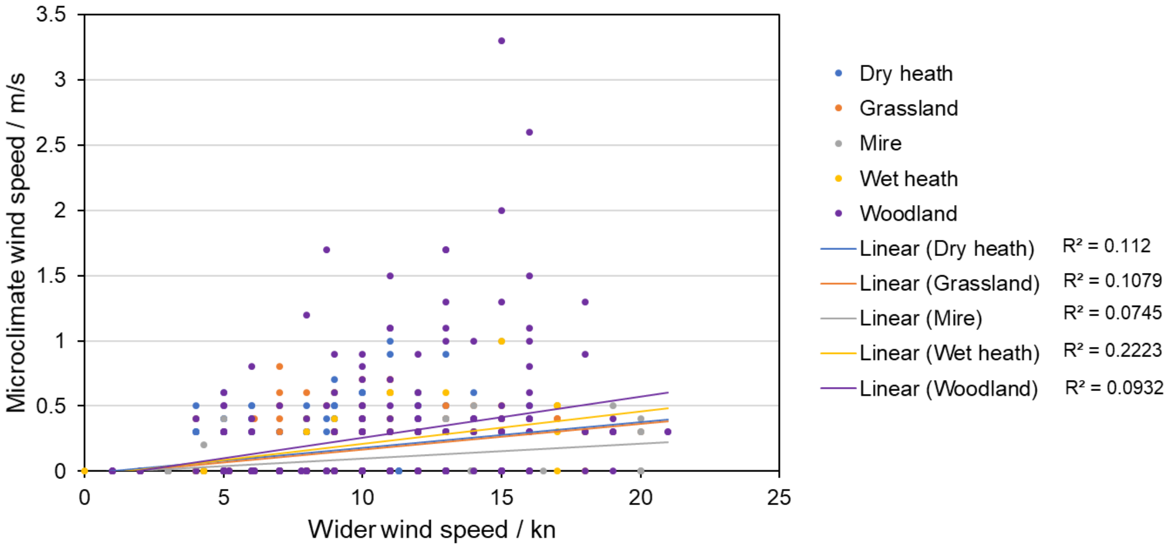
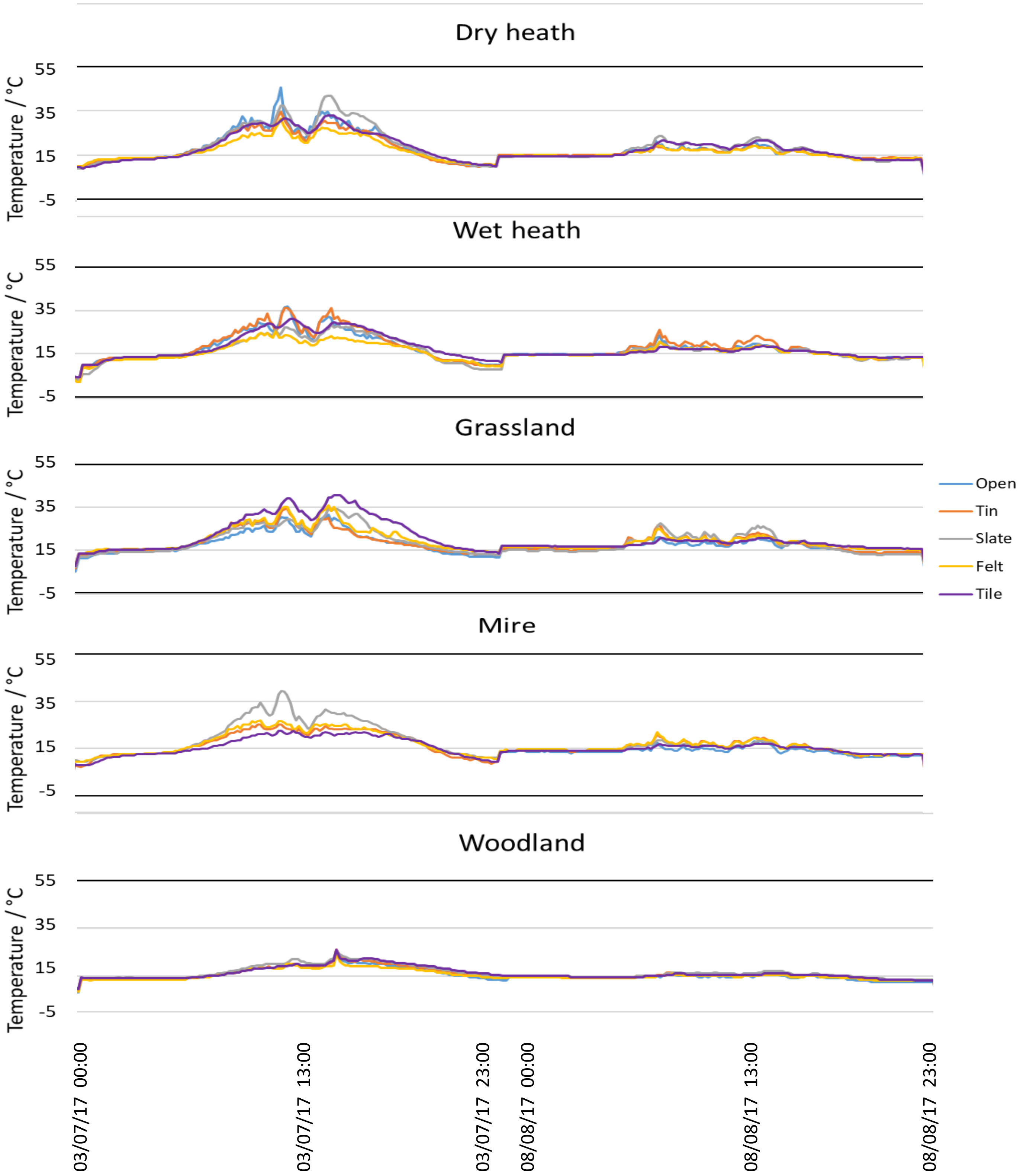

Microclimate light / lux
100
90
80
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
1200
1400
Wider solar radiation / W/m2
● Dry heath
● Grassland
● Mire
● Wet heath
● Woodland
Linear (Dry heath) R² = 0.4574
Linear (Grassland) R² = 0.2373
Linear (Mire) R² = 0.192
Linear (Wet heath) R² = 0.5422
Linear (Woodland) R² = 0.1185


Microcliamte air temperature / °C
45
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
Wider UV Index
● Dry heath
● Grassland
● Mire
● Wet heath
● Woodland
Linear (Dry heath) R² = 0.2227
Linear (Grassland) R² = 0.1956
Linear (Mire) R² = 0.2428
Linear (Wet heath) R² = 0.1235
Linear (Woodland) R² = 0.2982

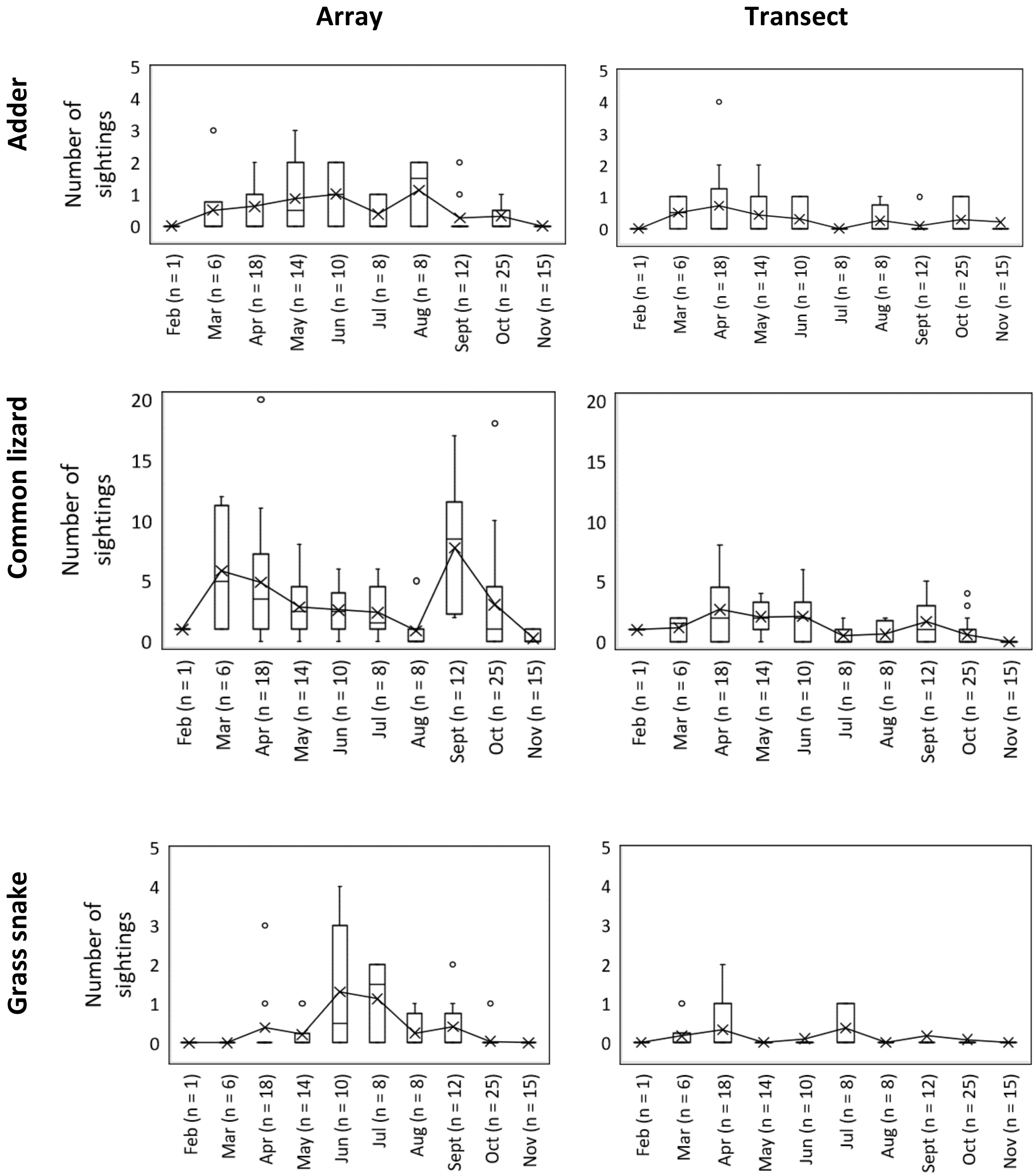

Microclimate wind speed / m/s
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Wider wind speed / kn
● Dry heath
● Grassland
● Mire
● Wet heath
● Woodland
Linear (Dry heath) R² = 0.112
Linear (Grassland) R² = 0.1079
Linear (Mire) R² = 0.0745
Linear (Wet heath) R² = 0.2223
Linear (Woodland) R² = 0.0932

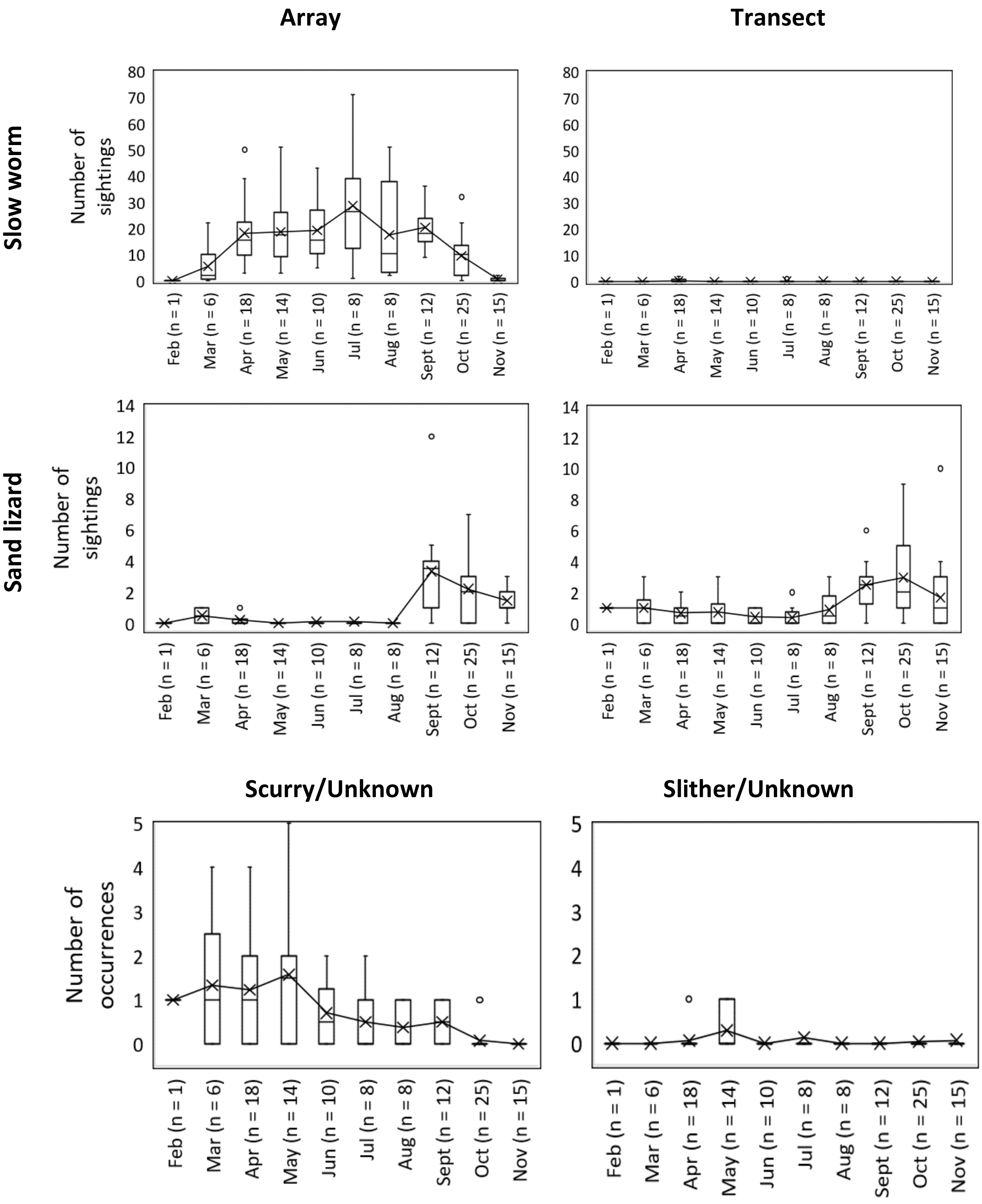
C.8 iButton temperature readings on 03/07/2017 and 08/08/217 in open air and under tin, slate, felt and tile at five habitat type-site arrays at Eelmoor Marsh SSSI.



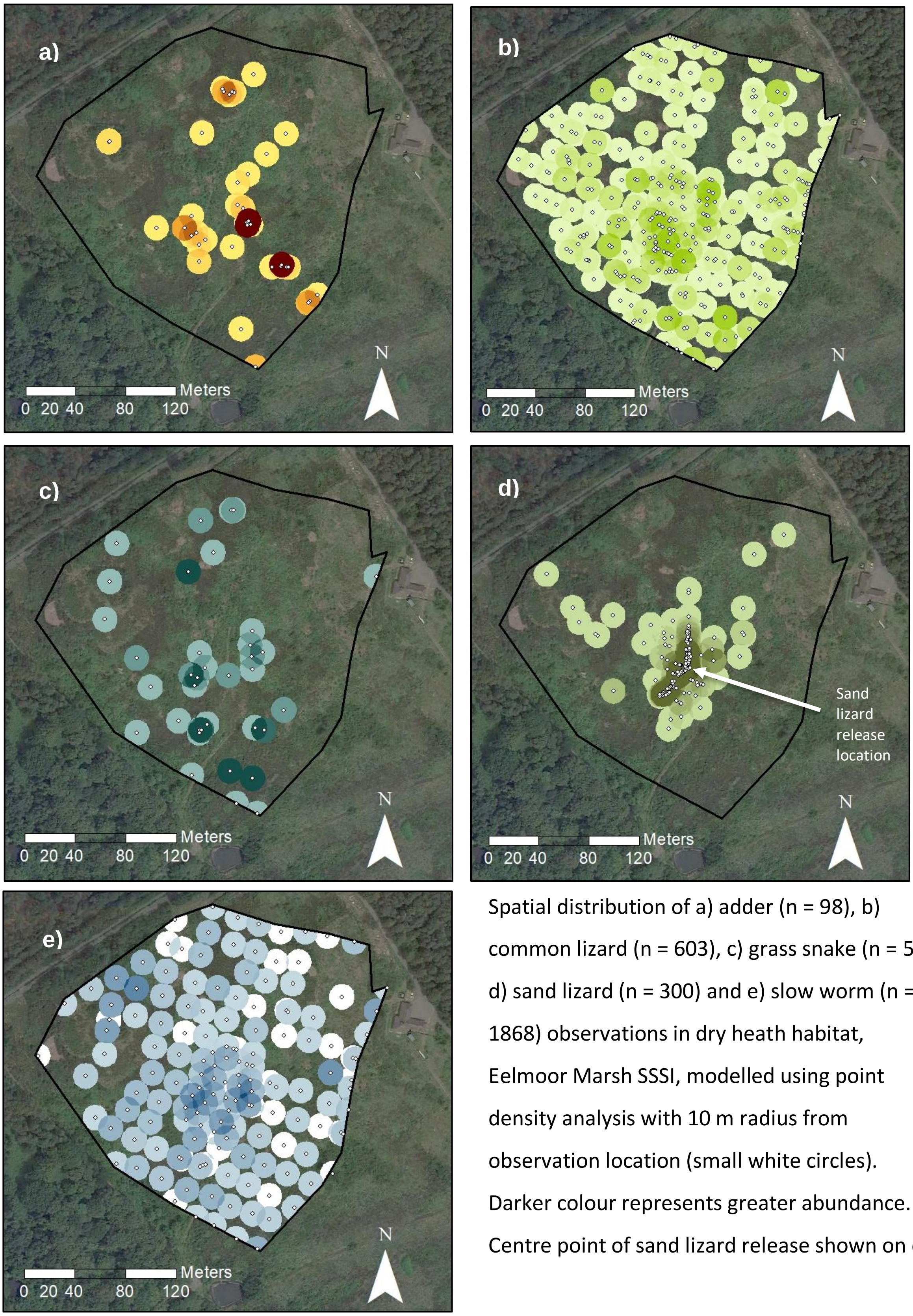
C.9 Seasonal variation in reptile observations in dry heath

Observations of reptile species per survey by month in dry heath in original survey area (100 x 100 m grid). Number of surveys per month (2017-2019) in brackets. Box and whisker plots show median, mean, upper and lower quartiles, minimum and maximum data points and outliers. Continues overleaf.




Array
Transect
Slow worm
Number of sightings
0
10
20
30
40
50
60
70
80
Sand lizard
Number of sightings
0
2
4
6
8
10
12
14
Feb (n = 1)
Mar (n = 6)
Apr (n = 18)
May (n = 14)
Jun (n = 10)
Jul (n = 8)
Aug (n = 8)
Sept (n = 12)
Oct (n = 25)
Nov (n = 15)
Scurry/Unknown
Slither/Unknown
Number of occurrences
0
1
2
3
4
5

C.10 Spatial distribution of reptile species in dry heath habitat



Spatial distribution of a) adder (n = 98), b) common lizard (n = 603), c) grass snake (n = 58), d) sand lizard (n = 300) and e) slow worm (n = 1868) observations in dry heath habitat, Eelmoor Marsh SSSI, modelled using point density analysis with 10 m radius from observation location (small white circles). Darker colour represents greater abundance. Centre point of sand lizard release shown on d).

C.11 The effect of environmental conditions on refugium selection by adders in dry heath

Minimum adequate negative binomial GLMM considering microclimate factors and material type selected by adder. 'Tin' was the reference level for material type.

	Estimate	**Std. Err.**	**z value**	***P***
Intercept	-19.145	3.139	-6.099	**<0.001**
Tile : air temp	-0.311	0.136	-2.283	**0.023**
Air temp	0.212	0.100	2.122	**0.034**
Tile	7.001	3.191	2.194	**0.028**
Open	5.727	2.760	2.075	**0.038**
Mat temp	0.039	0.028	1.382	0.167
Soil temp	-0.097	0.064	-1.515	0.130
Felt	5.240	2.717	1.928	0.054
Slate	4.402	2.970	1.482	0.138
Felt: air temp	-0.143	0.094	-1.530	0.126
Open : air temp	-0.168	0.097	-1.724	0.085
Slate : air temp	-0.151	0.106	-1.431	0.152
AIC	**425.80**			

Minimum adequate negative binomial GLMM considering microhabitat factors and material type selected by adder (none were retained in the final model). 'Tin' was the reference level for material type; variables were re-scaled before analysis.

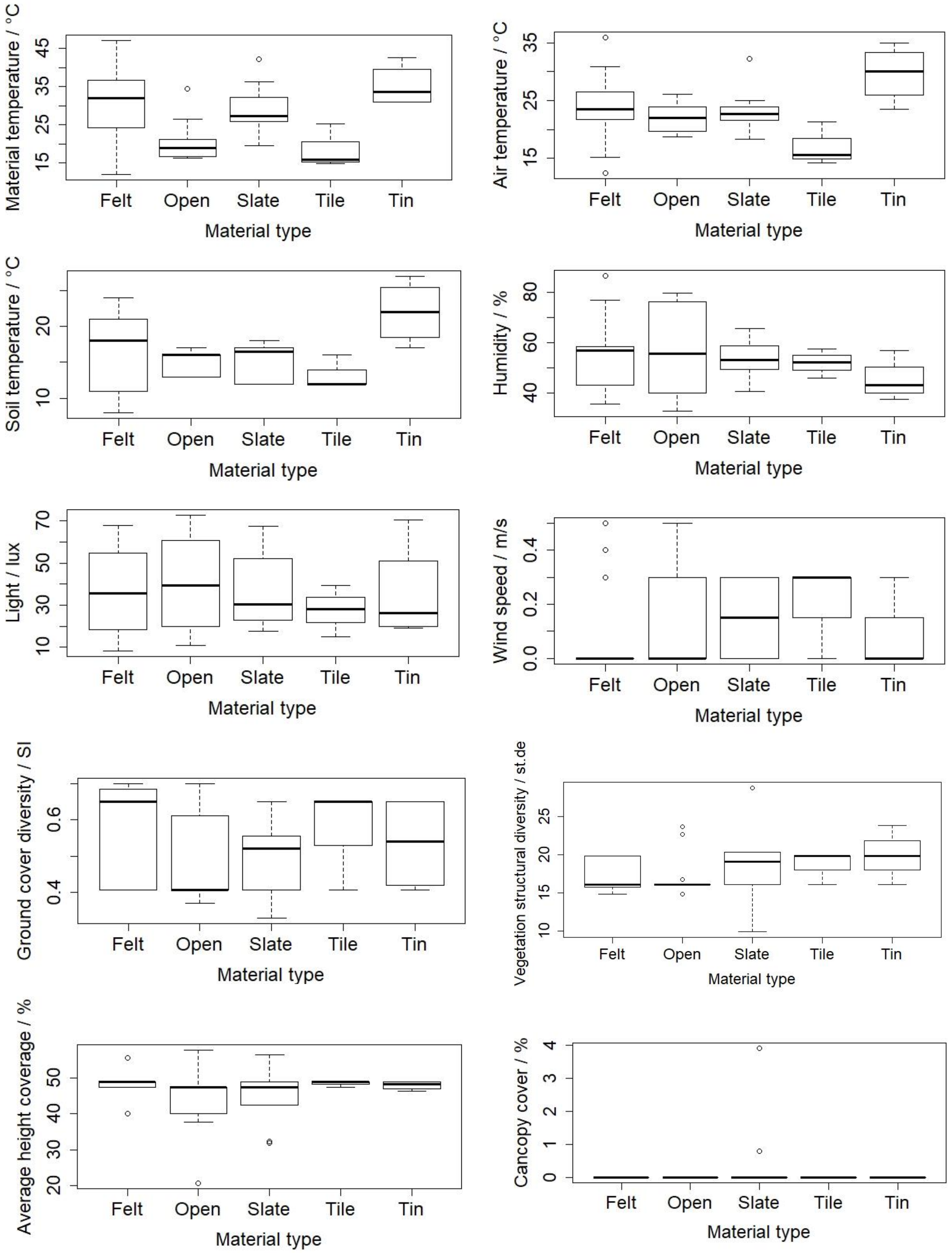
	Estimate	**Std. Err.**	**z value**	***p***
Intercept	-14.540	1.102	-13.192	**<0.001**
Felt	1.659	0.525	3.161	**0.002**
Open	1.250	0.549	2.277	**0.023**
Slate	1.468	0.537	2.733	**0.006**
Tile	-0.289	0.734	-0.394	0.694
AIC	**674.00**			

Environmental factors associated with **adder** material type use in dry heath habitat, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are ***bold italicised*** and any significantly different material pairs following post-hoc testing are highlighted.

Adder

	Felt					**Open**					**Slate**					**Tile**					**Tin**					**Main model (ANOVA/Kruskal-Wallis)**					
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	**F value**	**Kruskal-W χ^2**	**df**	**P value**	**Sig. pairs**	**Transform.**
MICROCLIMATE																															
Mat.temp °C	30.77	32.00	24.20	36.60	17	20.28	18.95	16.70	21.15	12	29.03	27.25	25.75	32.25	8	18.60	15.80	15.30	20.50	3	35.23	33.65	30.90	39.55	4	5.986		4,39	**<0.001**	Open/Felt, Open/Tin, Tin/Tile	NA
Air temp °C	23.66	23.50	21.80	26.50	17	21.80	21.95	19.65	23.90	12	23.36	22.75	21.55	23.90	8	17.03	15.60	14.90	18.45	3	29.65	30.10	26.00	33.30	4	3.842		4,39	**0.010**	Tin/Open, Tin/Tile	NA
Soil temp °C	16.82	18.00	11.00	21.00	17	15.00	16.00	13.00	16.00	12	15.33	16.50	12.00	17.00	6	13.33	12.00	12.00	14.00	3	22.00	22.00	18.50	25.50	4	2.978		4,37	0.032	Tin/Open	NA
Rel hum %	54.59	56.70	42.90	58.30	17	57.25	55.60	40.00	76.45	12	53.54	52.95	49.40	58.80	8	51.80	52.10	48.90	54.85	3	45.08	43.15	39.80	50.35	4	0.571		4,39	0.685	NA	NA
Light Lux	37.74	35.72	18.50	54.80	17	39.71	39.50	19.74	60.75	12	36.94	30.27	22.89	52.12	8	27.55	28.27	21.70	33.76	3	35.51	26.17	19.90	51.13	4	0.220		4,39	0.926	NA	NA
Wind sp m/s	0.09	0.00	0.00	0.00	17	0.14	0.00	0.00	0.30	12	0.15	0.15	0.00	0.30	8	0.20	0.30	0.15	0.30	3	0.08	0.00	0.00	0.15	4		1.943	4	0.746	NA	NA
MICROHABITAT																															
Cover div SI	0.59	0.65	0.41	0.68	21	0.48	0.41	0.41	0.61	14	0.50	0.52	0.41	0.56	17	0.57	0.65	0.53	0.65	3	0.53	0.54	0.42	0.65	4	2.423		4,54	0.059	NA	NA
Struct st.dev	17.49	16.09	15.77	19.87	21	16.97	16.09	16.09	20.33	14	18.29	19.10	16.09	20.33	17	18.61	19.87	17.98	19.87	3	19.91	19.87	17.98	21.85	4	1.027		4,54	0.402	NA	NA
Height cover %	48.16	48.84	47.50	48.84	21	44.55	47.50	40.03	47.50	14	45.51	47.50	42.53	48.84	17	48.40	48.84	48.17	48.84	3	47.88	48.17	46.91	48.84	4		7.440	4	0.114	NA	NA
Can cov %	0.00	0.00	0.00	0.00	21	0.00	0.00	0.00	0.00	14	0.28	0.00	0.00	0.00	17	0.00	0.00	0.00	0.00	3	0.00	0.00	0.00	0.00	4		5.026	4	0.285	NA	NA

Microclimate and microhabitat conditions associated with material use by adders in dry heath. Plots highlight the median, interquartile range, extreme values and outliers.



C.12 The effect of environmental conditions on refugium selection by common lizards in dry heath

Minimum adequate Poisson GLMM considering microclimate factors and material type selected by common lizard. ‘Tin’ was the reference level for material type.

Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
Intercept	-5.693	0.621	-9.166	**<0.001**
Open: soil temp	0.127	0.058	2.197	**0.028**
Air temp	0.084	0.030	2.826	**0.005**
Soil temp	-0.137	0.046	-2.987	**0.003**
Felt	1.881	0.712	2.641	**0.008**
Open	-2.733	1.095	-2.495	**0.013**
Slate	1.615	0.761	2.123	**0.034**
Mat temp	0.017	0.012	1.367	0.172
Tile	-1.640	1.304	-1.258	0.208
Felt: soil temp	-0.073	0.044	-1.680	0.093
Slate: soil temp	-0.082	0.048	-1.732	0.083
Tile: soil temp	0.025	0.075	0.334	0.739
AIC	**1853.80**			

Minimum adequate negative binomial GLMM considering microhabitat factors and material type selected by common lizard. ‘Tin’ was the reference level for material type.

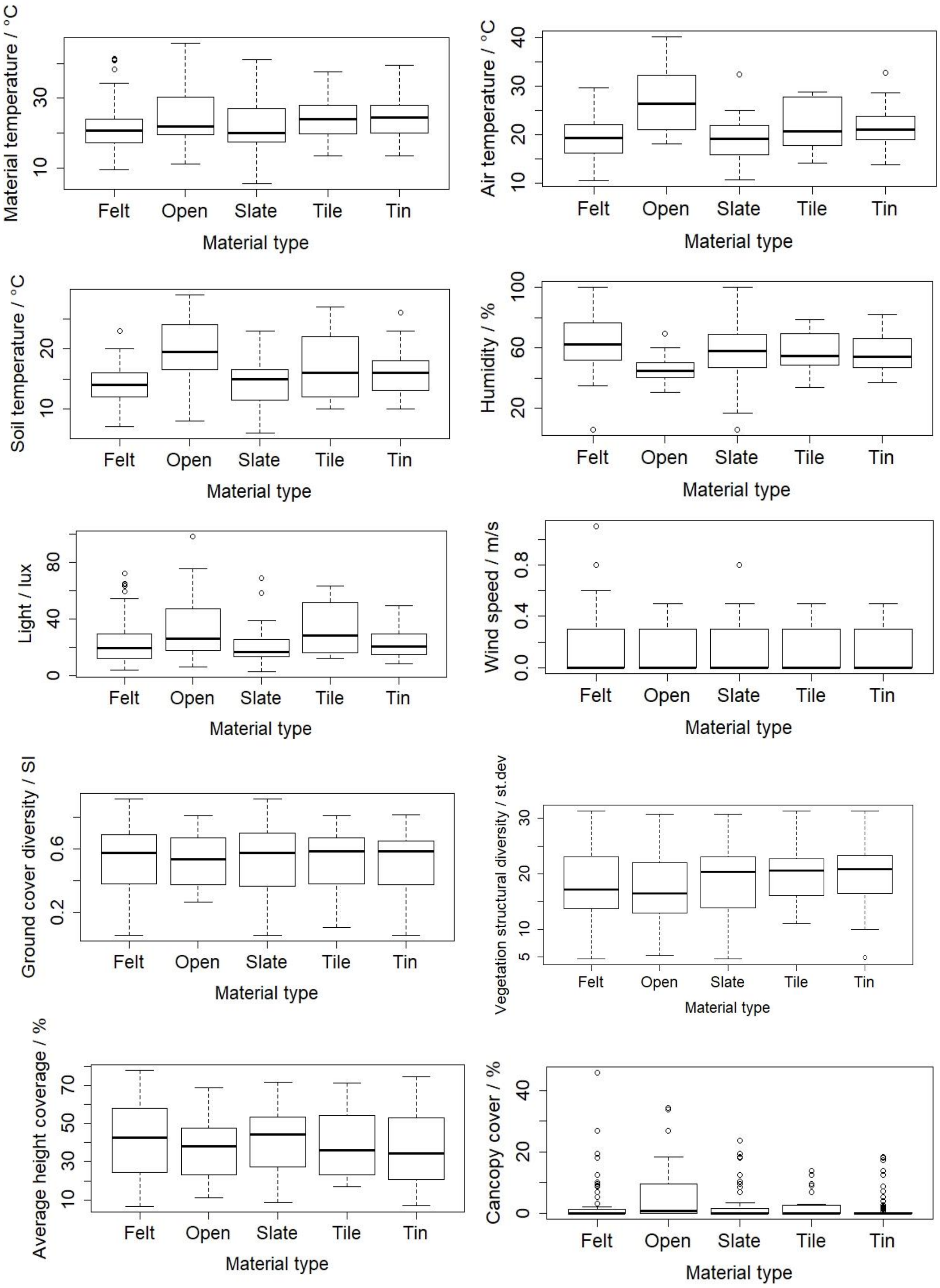
	Estimate	**Std. Err.**	**z value**	***P***
Intercept	-6.816	0.540	-12.626	**<0.001**
Felt: structural diversity	-0.065	0.023	-2.774	**0.006**
Open: canopy cover	0.076	0.032	2.365	**0.018**
Felt: vertical cover	0.017	0.008	2.196	**0.028**
Structural diversity	0.080	0.024	3.370	**0.001**
Felt	1.351	0.529	2.554	**0.011**
Canopy cover	-0.053	0.029	-1.836	0.066
Vertical cover	-0.007	0.008	-0.899	0.369
Open	0.101	0.787	0.129	0.898
Slate	0.471	0.585	0.805	0.421
Tile	-0.630	0.746	-0.844	0.399
Felt: canopy cover	-0.001	0.031	-0.020	0.984
Slate: canopy cover	0.027	0.031	0.885	0.376
Tile: canopy cover	0.030	0.037	0.799	0.424
Open: vertical cover	-0.002	0.012	-0.179	0.858
Slate: vertical cover	0.015	0.009	1.767	0.077
Tile: vertical cover	0.005	0.011	0.454	0.650
Open: structural diversity	-0.067	0.037	-1.827	0.068
Slate: structural diversity	-0.043	0.026	-1.682	0.093
Tile: structural diversity	-0.013	0.032	-0.395	0.693
AIC	**5180.5**			

Environmental factors associated with **common lizard** material type use in dry heath habitat, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are ***bold italicised*** and any significantly different material pairs following post-hoc testing are highlighted.

Common lizard

	Felt					Open					Slate					Tile					Tin					Main model (ANOVA/Kruskal-Wallis)					
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	**F value**	**Kruskal-W χ^2**	**df**	**P value**	**Sig. pairs**	**Transform.**
MICROCLIMATE																															
Mat.temp °C	21.29	20.80	17.20	24.10	61	25.45	21.90	19.55	30.40	16	22.20	20.00	17.45	27.15	43	24.35	23.95	19.95	27.95	8	24.71	24.40	20.00	28.10	29	1.753		4,152	0.141	NA	NA
Air temp °C	19.03	19.20	16.20	22.10	63	27.03	26.45	21.00	32.30	16	19.22	19.10	15.80	21.90	46	21.57	20.60	17.70	27.80	10	21.33	21.00	19.00	23.70	29	10.640		4,159	**<0.001**	Op/Fe, Op/Sl, Op/Tl, Op/Tn	NA
Soil temp °C	14.22	14.00	12.00	16.00	63	19.56	19.50	16.50	24.00	16	14.09	15.00	11.50	16.50	43	16.60	16.00	12.00	22.00	10	16.10	16.00	13.00	18.00	29	7.551		4,156	**<0.001**	Op/Fe, Op/Sl, Op/Tn	NA
Rel hum %	62.62	62.30	51.90	76.60	63	45.95	44.55	40.10	50.05	16	57.53	57.80	47.10	68.60	46	56.36	54.35	48.50	69.40	10	55.69	54.30	46.80	66.30	29	4.066		4,159	**0.004**	Op/Fe	NA
Light Lux	24.57	19.42	12.29	29.39	63	35.70	26.14	17.82	47.07	16	20.49	16.67	13.05	25.17	46	32.68	28.13	15.96	51.70	10	22.65	20.18	15.09	29.51	29	2.501		4,159	**0.045**	**NA**	log
Wind sp m/s	0.17	0.00	0.00	0.30	63	0.13	0.00	0.00	0.30	16	0.14	0.00	0.00	0.30	46	0.16	0.00	0.00	0.30	10	0.12	0.00	0.00	0.30	29		1.042	4,159	0.806	NA	NA
MICROHABITAT																															
Cover div SI	0.52	0.57	0.38	0.69	178	0.53	0.54	0.38	0.67	30	0.51	0.57	0.36	0.70	108	0.52	0.58	0.38	0.67	43	0.51	0.59	0.37	0.65	81	0.095		4,435	0.984	NA	NA
Struct st.dev	18.27	17.21	13.77	23.00	178	17.07	16.43	12.90	21.97	30	18.86	20.32	13.85	23.00	108	19.79	20.58	16.06	22.72	43	20.37	20.87	16.45	23.35	81	2.702		4,435	**0.030**	**NA**	NA
Height cover %	42.64	42.47	24.34	57.75	178	36.45	38.08	23.34	47.50	30	42.19	44.16	27.14	53.23	108	39.28	35.88	23.34	54.23	43	37.99	34.25	20.72	53.16	81	1.521		4,435	0.195	NA	NA
Can cov %	1.99	0.00	0.00	1.17	178	6.38	0.78	0.00	9.49	30	2.66	0.00	0.00	1.43	108	2.46	0.00	0.00	2.47	43	1.58	0.00	0.00	0.26	81		11.135	4	**0.025**	**NA**	NA

Microclimate and microhabitat conditions associated with material use by common lizards in dry heath. Plots highlight the median, interquartile range, extreme values and outliers.



C.13 The effect of environmental conditions on refugium selection by grass snakes in dry heath

Minimum adequate Poisson GLMM considering microclimate factors and material type selected by grass snake. 'Tin' was the reference level for material type.

	Estimate	Std. Err.	z value	*P*
Intercept	-12.529	1.849	-6.775	**<0.001**
Felt	-2.108	1.062	-1.985	**0.047**
Open	-1.755	1.076	-1.631	0.103
Slate	0.333	0.465	0.716	0.474
Tile	-2.073	1.061	-1.955	0.051
Mat temp	0.054	0.031	1.750	0.080
Soil temp	0.042	0.059	0.716	0.474
Light	-0.024	0.014	-1.637	0.102
AIC	**310.1**			

Minimum adequate negative binomial GLMM considering microhabitat factors and material type selected by grass snake. 'Tin' was the reference level for material type.

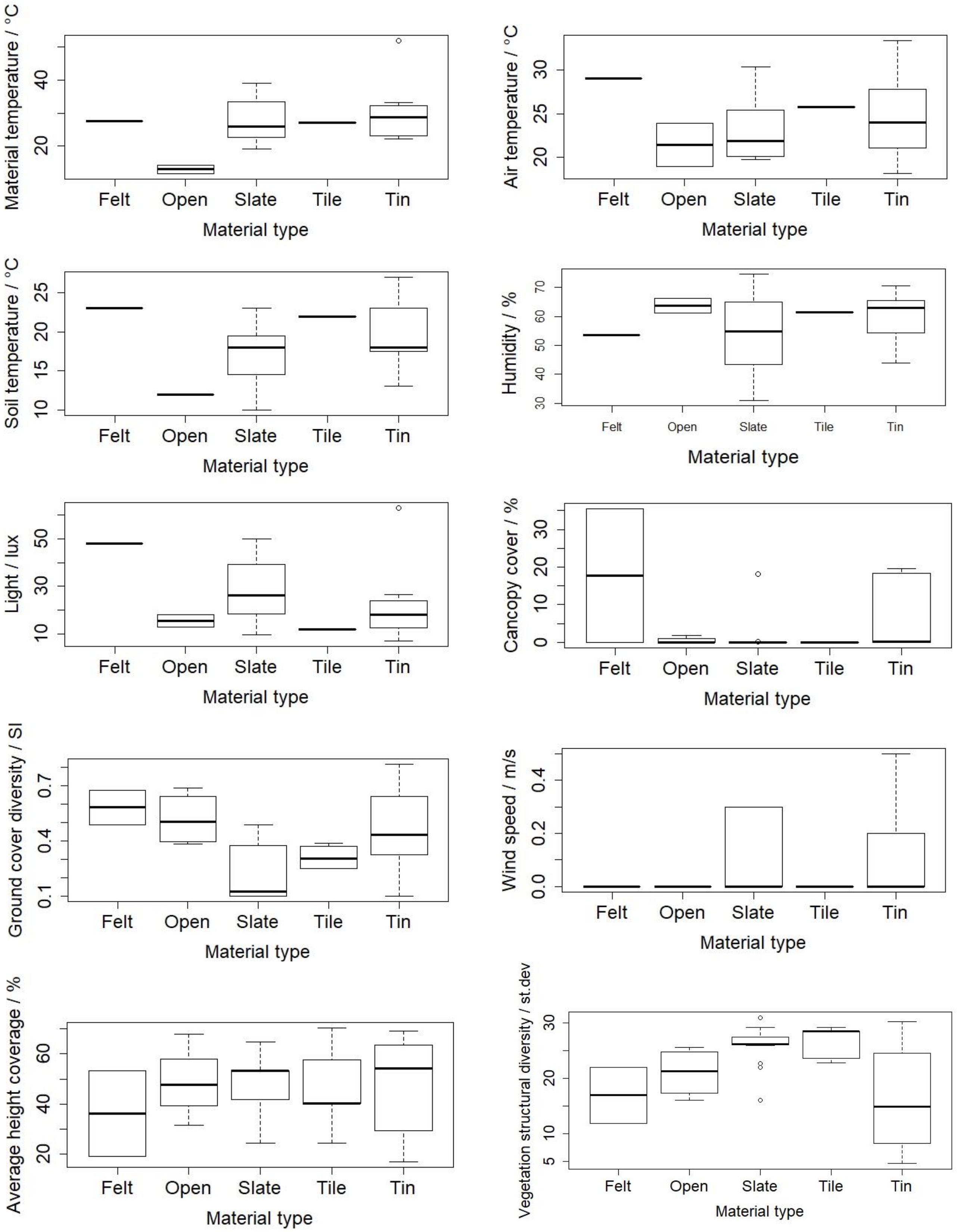
Coefficients	Estimate	Std. Err.	z value	*P*
Intercept	-8.950	1.260	-7.105	**<0.001**
Slate: structural diversity	0.246	0.079	3.133	**0.002**
Tile: structural diversity	0.285	0.125	2.275	**0.023**
Slate	-5.495	1.894	-2.902	**0.004**
Tile	-7.614	3.240	-2.350	**0.019**
Structural diversity	0.016	0.053	0.295	0.768
Felt	-2.158	1.815	-1.189	0.235
Open	-2.991	1.742	-1.717	0.086
Felt: structural diversity	0.005	0.098	0.054	0.957
Open: structural diversity	0.086	0.081	1.050	0.294
AIC	**646.8**			

Environmental factors associated with **grass snake** material type use in dry heath habitat, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are ***bold italicised*** and any significantly different material pairs following post-hoc testing are highlighted.

Grass snake

	Felt					**Open**					**Slate**					**Tile**					**Tin**					**Main model (ANOVA/Kruskal-Wallis)**					
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	**F value**	**Kruskal-W χ^2**	**df**	**P value**	**Sig. pairs**	**Transform.**
MICROCLIMATE																															
Mat.temp °C	27.50	27.50	27.50	27.50	1	13.00	13.00	11.70	14.30	2	28.03	26.00	22.65	33.30	11	27.00	27.00	27.00	27.00	1	30.33	28.80	23.15	32.30	8		5.618	4	0.230	NA	NA
Air temp °C	29.10	29.10	29.10	29.10	1	21.45	21.45	19.00	23.90	2	23.12	21.90	20.10	25.40	11	25.80	25.80	25.80	25.80	1	24.69	24.05	21.10	27.80	8	0.795		4,18	0.544	NA	NA
Soil temp °C	23.00	23.00	23.00	23.00	1	12.00	12.00	12.00	12.00	1	17.00	18.00	14.50	19.50	11	22.00	22.00	22.00	22.00	1	19.63	18.00	17.50	23.00	8	1.701		4,17	0.196	NA	NA
Rel hum %	53.60	53.60	53.60	53.60	1	63.75	63.75	61.20	66.30	2	53.83	54.90	43.30	64.90	11	61.50	61.50	61.50	61.50	1	59.98	63.00	54.30	65.40	8	0.526		4,18	0.718	NA	NA
Light Lux	47.90	47.90	47.90	47.90	1	15.51	15.51	12.99	18.03	2	29.64	26.25	18.52	39.26	11	11.95	11.95	11.95	11.95	1	22.46	18.21	12.49	24.08	8	1.431		4,18	0.264	NA	log
Wind sp m/s	0.00	0.00	0.00	0.00	1	0.00	0.00	0.00	0.00	2	0.12	0.00	0.00	0.30	11	0.00	0.00	0.00	0.00	1	0.11	0.00	0.00	0.20	8		1.634	4	0.803	NA	NA
MICROHABITAT																															
Cover div SI	0.58	0.58	0.49	0.67	2	0.52	0.50	0.39	0.64	4	0.22	0.12	0.10	0.38	16	0.31	0.31	0.25	0.37	5	0.47	0.43	0.33	0.64	16	6.069		4,38	<0.001	Sl/Op, Sl/Tn	NA
Struct st.dev	16.95	16.95	11.93	21.97	2	21.08	21.30	17.34	24.82	4	25.91	26.21	26.07	27.47	16	26.54	28.51	23.68	28.51	5	16.47	14.91	8.33	24.61	16	5.076		4,38	0.002	Tn/Sl, Tn/Tl	NA
Height cover %	36.14	36.14	19.09	53.19	2	48.64	47.63	39.47	57.81	4	47.79	53.28	41.69	53.28	16	46.55	40.13	40.13	57.75	5	48.78	54.16	29.56	63.50	16		2.431	4	0.657	NA	NA
Can cov %	17.75	17.75	0.00	35.49	2	0.46	0.00	0.00	0.91	4	1.15	0.00	0.00	0.00	16	0.00	0.00	0.00	0.00	5	6.54	0.13	0.00	18.33	16		13.975	4	0.007	Tn/Sl	NA

Microclimate and microhabitat conditions associated with material use by grass snake in dry heath. Plots highlight the median, interquartile range, extreme values and outliers.



C.14 The effect of environmental conditions on refugium selection by slow worms in dry heath

Minimum adequate negative binomial GLMM considering microhabitat factors and material type selected by slow worms. ‘Tin’ was the reference level for material type; data were rescaled prior to running model.

Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
Intercept	-3.928	0.143	-27.552	**<0.0001**
Tile: humidity	-0.295	0.115	-2.556	**0.011**
Felt: humidity	-0.289	0.127	-2.274	**0.023**
Wind speed	0.122	0.046	2.675	**0.007**
Light	-0.155	0.060	-2.577	**0.010**
Air temp	0.236	0.114	2.068	**0.039**
Felt	-0.455	0.125	-3.649	**<0.001**
Slate	0.266	0.104	2.560	**0.010**
Soil temp	0.068	0.099	0.689	0.491
Humidity	0.086	0.088	0.969	0.332
Mat temp	-0.085	0.082	-1.042	0.297
Open	-12.668	13.434	-0.943	0.346
Tile	-0.126	0.114	-1.102	0.271
Open: humidity	0.031	11.797	0.003	0.998
Slate: humidity	0.161	0.104	1.550	0.121
AIC	**6340.20**			

Minimum adequate negative binomial GLMM considering microhabitat factors and material type selected by slow worms. 'Tin' was the reference level for material type.

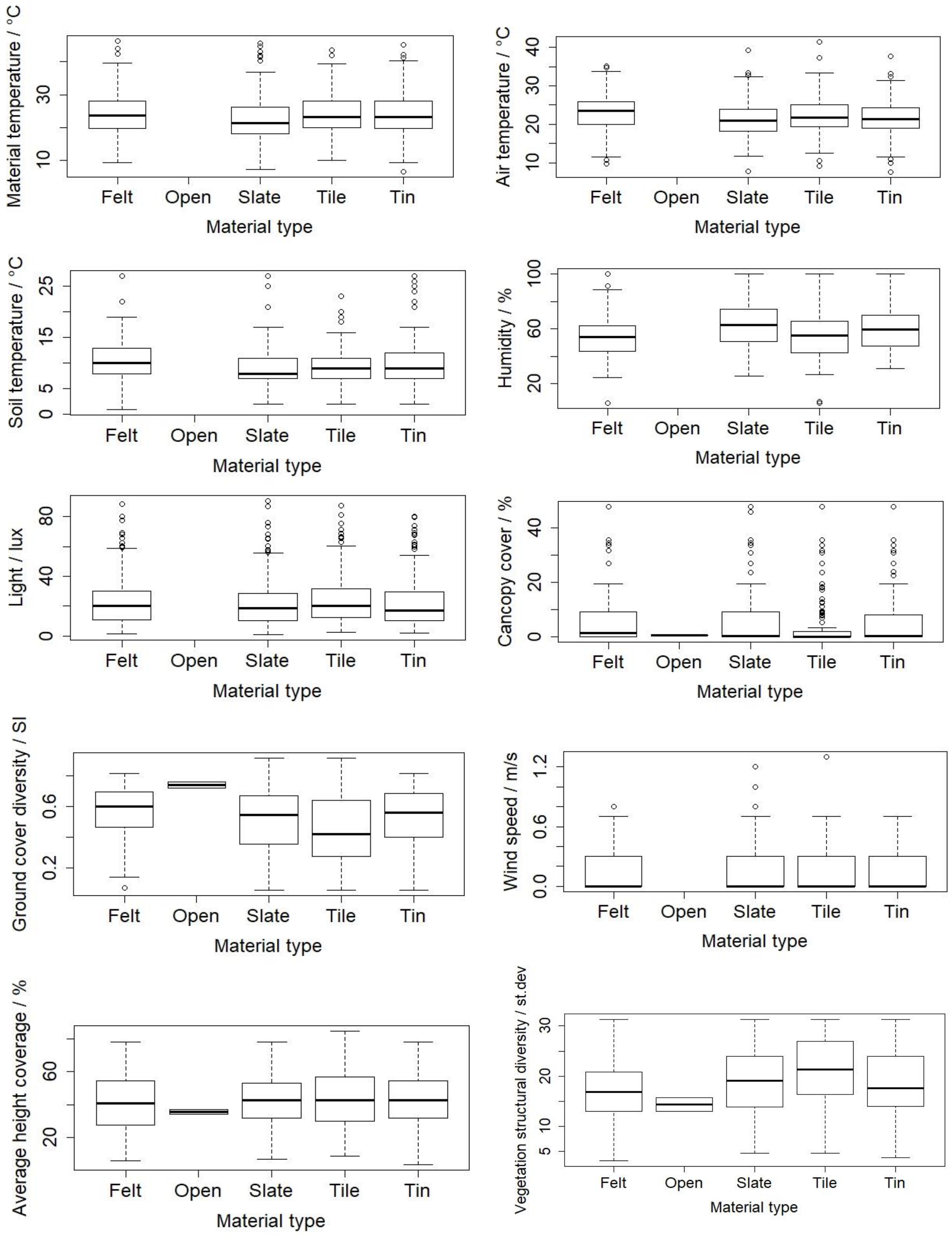
Coefficients	**Estimate**	**Std. Err.**	**z value**	***P***
Intercept	-5.358	0.537	-9.974	**<0.001**
Open: cover diversity	11.803	1.907	6.189	**<0.001**
Tile: cover diversity	-1.930	0.365	-5.290	**<0.001**
Tile: structural diversity	0.027	0.012	2.251	**0.024**
Canopy cover	0.026	0.010	2.537	**0.011**
Height cover	0.011	0.005	2.336	**0.019**
Structural diversity	0.046	0.016	2.931	**0.003**
Open	-9.370	1.494	-6.270	**<0.001**
Slate	0.765	0.334	2.288	**0.022**
Tile	0.744	0.354	2.103	**0.035**
Cover diversity	-0.364	0.519	-0.700	0.484
Felt	-0.614	0.432	-1.420	0.155
Felt: cover diversity	0.790	0.464	1.700	0.089
Slate: cover diversity	-0.656	0.348	-1.886	0.059
Felt: canopy cover	0.008	0.008	0.910	0.363
Open: canopy cover	-0.446	0.467	-0.955	0.340
Slate: canopy cover	0.003	0.007	0.366	0.714
Tile: canopy cover	-0.016	0.009	-1.843	0.065
Felt: structural diversity	-0.021	0.015	-1.450	0.147
Open: structural diversity	-0.191	0.130	-1.465	0.143
Slate: structural diversity	0.006	0.011	0.512	0.609
AIC	**15674.6**			

(Next page) Environmental factors associated with **slow worm** material type use in dry heath habitat, including mean, median, lower and upper quartiles of each factor and number of observations included in each test per material type. Included are ANOVA / Kruskal-Wallis results for microclimate factors (material temperature / °C, air temperature / °C, soil temperature / °C, relative humidity / %, light / lux, wind / m/s) and microhabitat factors (vegetation cover diversity / Simpson's Index, vegetation structural diversity / standard deviation, vertical/height cover / %, canopy cover / %) associated with material use. Significant factors are ***bold italicised*** and any significantly different material pairs following post-hoc testing are highlighted.

Slow worm

	Felt					**Open**					**Slate**					**Tile**					**Tin**					**Main model (ANOVA/Kruskal-Wallis)**					
	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	Mean	Median	Lower Q	Upper Q	n	**F value**	**Kruskal-W**	**df**	**P value**	**Sig. pairs**	**Transform.**
MICROCLIMATE																															
Mat.temp °C	24.44	23.70	19.80	28.20	125	NA	NA	NA	NA	0	22.59	21.50	18.20	26.20	245	24.03	23.20	20.00	28.20	177	24.14	23.35	19.70	28.10	190	3.300		3,733	**0.020**	**NA**	NA
Air temp °C	23.14	23.40	20.00	25.85	127	NA	NA	NA	NA	0	21.17	21.00	18.20	23.95	256	22.27	21.70	19.40	25.15	183	21.59	21.25	19.00	24.30	190	5.845		3,752	**<0.001**	Fe/Sl, Fe/Tn	NA
Soil temp °C	10.72	10.00	8.00	13.00	125	NA	NA	NA	NA	0	8.70	8.00	7.00	11.00	253	9.17	9.00	7.00	11.00	180	9.60	9.00	7.00	12.00	189	7.272		3,743	**<0.001**	Fe/Sl, Fe/Tl	Sq.rt.
Rel hum %	54.11	54.00	43.60	62.25	127	NA	NA	NA	NA	0	62.72	62.65	50.90	74.05	256	54.52	55.25	42.30	65.70	184	59.51	59.45	47.30	70.00	190	14.040		3,753	**<0.001**	Sl/Fe, Sl/Tl, Tn/Fe, Tn/Tl	NA
Light Lux	24.56	20.12	10.78	30.09	127	NA	NA	NA	NA	0	22.20	18.45	10.17	28.46	256	24.61	20.23	12.34	31.83	184	23.11	17.06	10.57	29.40	190	1.360		3,753	0.254	NA	log10
Wind sp m/s	0.18	0.00	0.00	0.30	127	NA	NA	NA	NA	0	0.12	0.00	0.00	0.30	256	0.14	0.00	0.00	0.30	184	0.15	0.00	0.00	0.30	190		10.042	3	**0.018**	Sl/Fe	NA
MICROHABITAT																															
Cover div Sl	0.57	0.60	0.47	0.70	232	0.74	0.74	0.72	0.76	2	0.50	0.55	0.35	0.67	686	0.43	0.42	0.27	0.64	529	0.53	0.56	0.40	0.68	393	24.850		4,1837	**<0.001**	Fel/Sl, Tl/Fe, Tl/Sl, Tl/Tn	NA
Struct st.dev	17.44	16.82	13.06	20.80	232	14.41	14.41	13.02	15.80	2	19.23	19.10	13.92	24.02	686	21.20	21.34	16.34	26.90	529	18.85	17.66	14.04	24.02	393	16.990		4,1837	**<0.001**	Fe/Sl, Tl/Fe, Tl/Sl, Tl/Tn	NA
Height cover %	40.54	40.70	27.83	54.34	232	35.84	35.84	34.41	37.28	2	42.69	42.88	32.06	53.19	686	43.42	42.88	30.19	56.59	529	42.05	42.84	31.72	54.28	393	1.532		4,1837	0.190	NA	NA
Can cov %	7.22	1.30	0.00	9.10	232	0.39	0.39	0.39	0.39	2	5.62	0.13	0.00	9.10	686	3.28	0.00	0.00	1.82	529	5.58	0.13	0.00	7.93	393		55.434	4	**<0.001**	Fe/Sl, Fe/Tin, Tl/Fe, Tl/Sl, Tl/in	NA

Microclimate and microhabitat conditions associated with material use by slow worm in dry heath. Plots highlight the median, interquartile range, extreme values and outliers



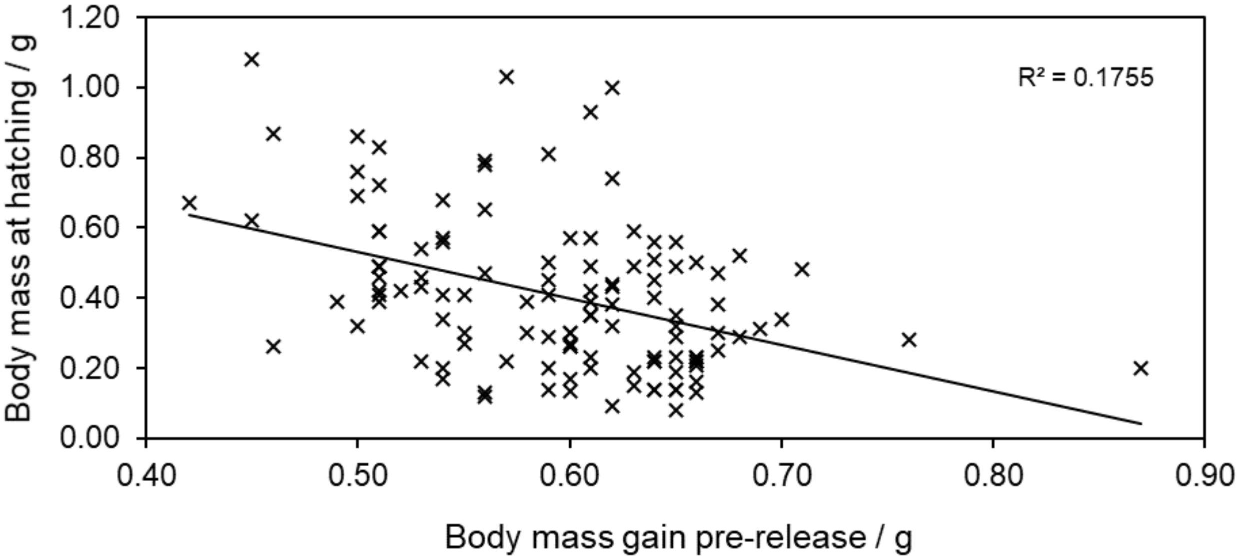
Appendix D Chapter 5

D.1 Observations of individual sand lizards (total n = 307) from post-release September 2017 to end of August 2019.

(Overleaf) Each column is a survey date / date of observation. Each row of black-text table represents individuals released. Lower grey-text table shows observations where individuals were not identified; unidentified observations following 2018 release could be lizards from either release but have been listed by 2018. Lizards are grouped by age at time of release. Solid dark grey blocks are observations on surveys (n = 300), hashed blocks are observations out of survey time (n = 5), black-bordered blocks are sloughed skins (n = 2), solid black blocks are deceased lizards (not included in observation count).


Age group
Sept-Nov 2017 (survey n = 27)
Mar-Sept 2018 (survey n = 29)
Sept-Nov 2018 (survey n = 24)
Feb-Aug 2019 (survey n = 38)
2017 release
Yearling (n = 26)
Hatchling (n = 54)
2018 release
2-year old (n = 10)
Yearling (n = 25)
Hatchling (n = 51)
2017
Hatchling
Yearling
2018
Hatchling
2 Y / Yearling
1

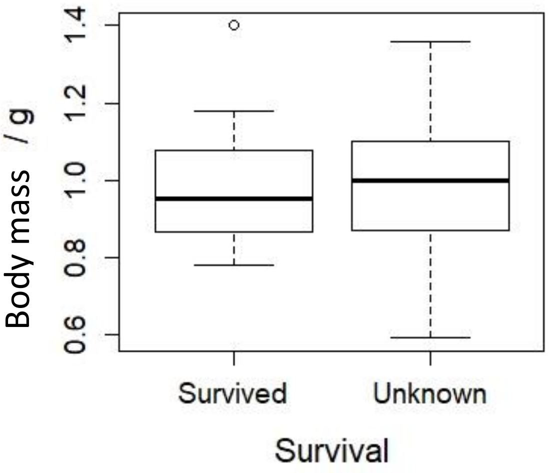
D.2 Hatchling sand lizard body mass gain between hatching and release

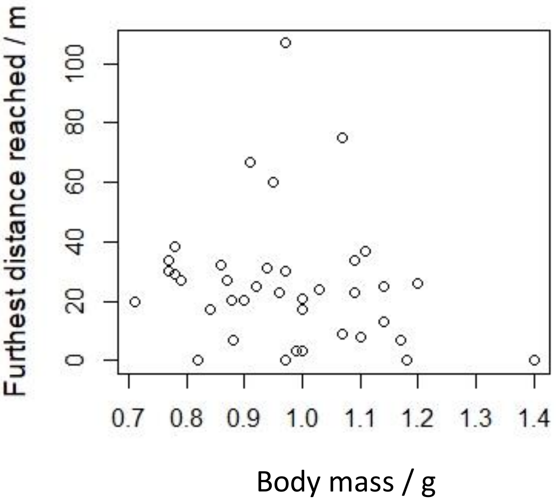


Appendix E Chapter 6

E.1 Effect of morphology on survival and movement of hatchling sand lizards

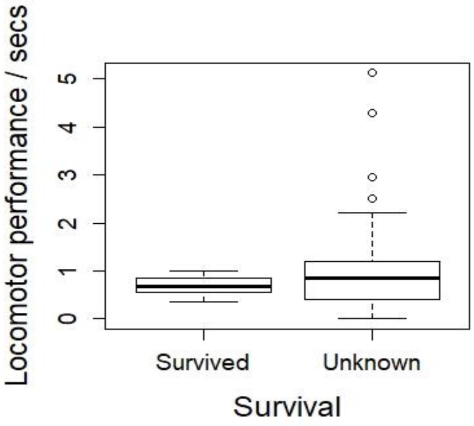
There was no difference in body mass of hatchlings known to have survived the winter (mean: 1.001, 95% CI[0.825, 1.075]) and those that were not seen after it (mean: 0.986, 95% CI[0.963, 1.037]) (t_{102} = 0.186, p = 0.858)). There was no correlation between hatchling lizard body mass and furthest distance reached when fitting a polynomial regression ($F_{2,35}$ = 2.291, p = 0.116, R^2 = 0.116). The mean and median body mass were both 0.97 g (with a minimum of 0.71 and maximum of 1.4). (Known survival post-winter n = 7, unknown survival n = 97; hatchlings observed post-release n = 38; NB numbers represent lizards for which relevant data also exist).

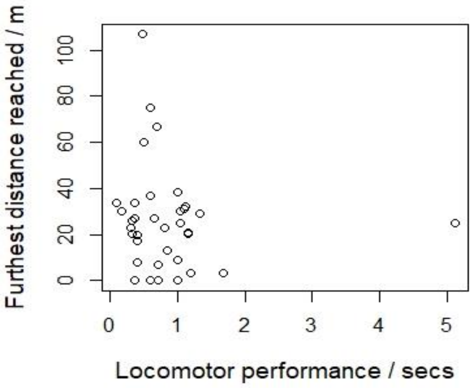




E.2 Effect of locomotor performance on survival and movement of hatchling sand lizards

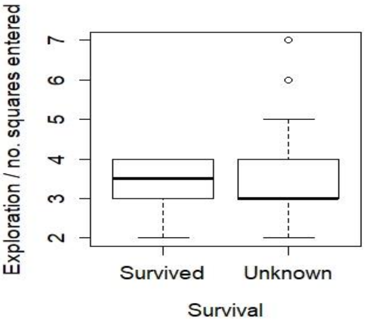
There was no difference between the locomotor performance of hatchlings that were known to have survived winter and those that were not observed post-winter ($U_{7,93}$ = 277.50, p = 0.521). The individual that died performed the locomotion test in 0.36 seconds. All locomotion values for the lizards that definitely survived were $\leq$ 1 second. There was no correlation between locomotor performance and furthest distance reached by hatchling lizards ($F_{1,33}$ = 0.348, p = 0.559, R^2 = 0.010). (Known survival post-winter n = 7, unknown survival n = 93; hatchlings observed post-release n = 35; NB numbers represent lizards for which relevant data also exist).

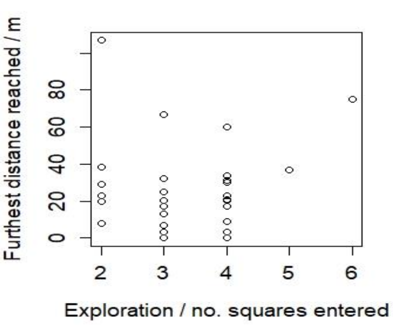


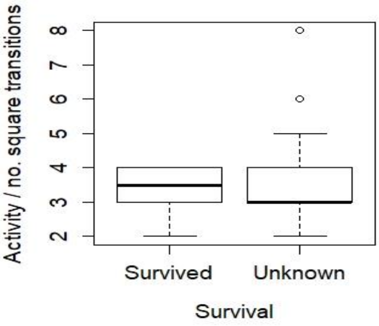


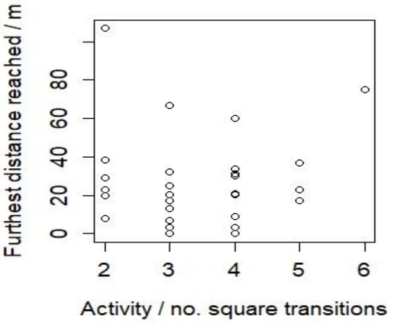
E.3 Effects of release response on survival and movement of hatchling sand lizards

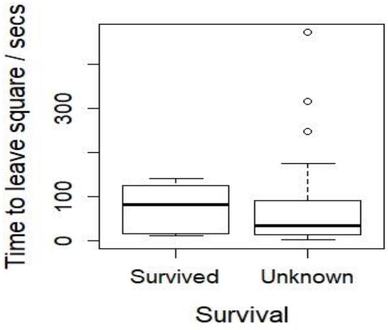
There was no difference between response to release in terms of exploration ($U_{6,74}$ = 232.00, p = 0.855), activity ($U_{6,74}$ = 227.50, p = 0.924) or time taken to leave the release square (t_{78} = 0.987, p = 0.362) and survival of hatchlings. The median value for time taken to leave the release square was almost as great for lizards known to survive the winter (81.5 seconds), as the upper quartile of those whose survival is unknown (90.7 seconds). The deceased hatchling pre-winter had exploration and activity scores of 4 and took 80.9 seconds to leave the release square. There was no difference between response to release in terms of exploration (rs = 0.067, p = 0.727, n = 30), activity (rs = 0.055, p = 0.774, n = 30) or time taken to leave the release square ($F_{1,28}$ = 0.637, p = 0.431) and furthest distance that those hatchlings observed post-release were found at, pre-winter. (Known survival post-winter n = 6, unknown survival n =74; hatchlings observed post-release n = 30; NB numbers represent lizards for which relevant data also exist).

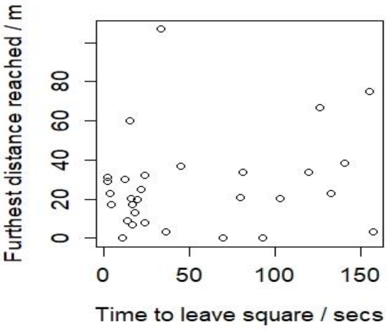












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