# Conservation of U.K. dune system native Lacertidae species.

## A thesis submitted to Bangor University for the degree of Doctor of Philosophy

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# Summary

After years of decline, resulting in the extinction of the sand lizard *Lacerta agilis* in Wales, the species has now been returned to several dune system sites which also contain the viviparous lizard *Zootoca vivipara*. As dune system habitats are becoming an increasingly important habitat type in the UK for *L. agilis*, providing a relatively safe haven for these and *Z. vivipara* populations from anthropogenic change, it is time to ensure the long-term survival of these populations. In this study, environmentally advantageous conditions for detection of both species were established for three dune system sites in North-West Wales. Surface temperature, UV, cloud cover and wind speed were identified as being the most influential detection parameters, with cloud cover and wind speed noted as having a negative influence on detection. Furthermore, favoured habitat was identified and 'suitable habitat' modelled for both species. Across the research sites both species were found to utilise habitats of a similar composition with reintroduced *L. agilis* favouring specific habitat features such as basking direction and degree of angle of their basking position. Habitat and site utilisation models indicate that for highly mobile dune systems *L. agilis* dispersal across a site is not of concern. For static or eroding dune systems, large, connected areas of favoured habitats are absent. In this situation, *L. agilis* dispersal (from introduction locations) is limited. Mean yearly *L. agilis* dispersal distances of 19.64 m – 28 m represents an approximate trend across the three research sites. The physical presence of *L. agilis* alone, however, does not constitute a successful reintroduction. Genetic diversity of the reintroduced populations was observed to be lower than those calculated in naturally occurring British populations. This is a matter of conservation concern, with a number of targeted mitigatory measures proposed to improve the genetic integrity, reducing the chance of population extinction(s). Mean time to extinction estimates indicate that for populations on highly mobile dune systems population survival times of between 11.4 yrs and 63.1 yrs could be expected. This is reduced for static or eroding dune system sites. The implications of these findings for the conservation of *L. agilis* and *Z. vivipara* are discussed in the context of current challenges and future management requirements. It is hoped that this thesis will help guide the future development of the reintroduction program, and in addition, provide a sound scientific basis for the future management of reintroduction sites and species monitoring.

# Acknowledgments

I have been fortunate over the last few years to have experienced working with species that many do not realise exist, and to have done so in region of the U.K. close to where I spent most of my childhood. Many deserve my deepest appreciation and gratitude. First, I would like to thank Dr. Anita Malhotra for her assistance with this project, and for her support and guidance throughout. Without it, this project would not have been possible and it has been a privilege to be her student. Academic and moral support was further provided by my guiding committee, Dr. Wolfgang Wüster and Prof. George Francis Turner, and my deepest gratitude also goes out to them.

Collecting data and genetic material for this study was a monumental task over a long timescale and there are many people who helped to make the countless hours bothering the lizards of North Wales considerably easier. Special thanks is given to James Hicks (soon to be Dr. Hicks), Mark Barber, Peter Hill (both of ARC Trust) and Helen Abel for the many hours they spent sunning themselves on the dune systems, helping me search for lizards. Nick Moulton and Dr. John Wilkinson (both of ARC Trust) provided invaluable advice, historical data and words of wisdom during times of crisis. Paul Hudson, Raymond Lynch and the team at Chester Zoo (captive breeders) provided advice and sampling of their collections.

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Chris Davis deserves special recognition, however, for introducing me to these magnificent species and showing me how to noose my first lizard. All this (the hours, days and years working on this thesis) is your fault and I will never forgive you. Thank you for your inspiration!!

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# Glossary

| | |
|---|---|
| AIC | Akaike Information Criterion |
| AD | Anno Domini |
| ARC | Amphibian and Reptile Conservation |
| BIC | Bayesian Information Criterion |
| BC | Before Christ |
| BP | Before Present |
| CCW | Countryside Council for Wales |
| CITES | Convention on International Trade in Endangered Species |
| DNA | Deoxyribonucleic Acid |
| EU | European Union |
| GAT | Gwynedd Archaeological Trust |
| GIS | Geographic Information System |
| GPS | Global Positioning System |
| HCT | Herpetological Conservation Trust |
| IUCN | International Union for Conservation of Nature |
| JNCC | Joint Nature Conservation Committee |
| KMO | Kaiser-Meyer-Olkin |
| LiDAR | Light Detection and Ranging |
| MYBP | Million Years Before Present |
| NARRS | National Amphibian and Reptile Recording Scheme |
| NCC | Nature Concervation Council |
| NMARG | North Merseyside Amphibian and Reptile group |
| NNR | National Nature Reserve |
| NRW | Natural Resources Wales |

# Glossary Cont.

| | |
|---|---|
| NVC | National Vegetation Classification |
| NWW | North-West Wales |
| OS | Ordnance Survey |
| PCA | Principal Component Analysis |
| PIT | Passive Integrated Transponder |
| RPAS | Remote Piloted Aerial System |
| SAC | Special Area of Conservation |
| SMP | Shoreline Management Plan |
| SPSS | Statistical Package for the Social Sciences |
| SSAR | Society for the Study of Amphibians and Reptiles |
| SSSI | Sites of Special Scientific Interest |
| SVL | Snout to Vent Length |
| UAVs | Unmanned Aerial Vehicle |
| UHF RFID | Ultra-High Frequency Radio-frequency Identification |
| UK | United Kingdom |
| UKBAP | United Kingdom Biodiversity Action Plan |
| UPW | Ultrapure Water |
| UV | Ultra Violet |
| UVA | Long Wave Ultra Violet - A |
| VTL | Vent to Tail length |
| WWF | World Wildlife Fund |

# Chapter 1

## General Introduction

# 1 Introduction

The sand lizard *Lacerta agilis* was first described in 1758 AD by Linnaeus from a specimen found in southern Sweden, being one of the first formally described reptiles. Nearly 20 years later the viviparous lizard *Zootoca vivipara,* (formerly *Lacerta vivipara*) was described by von Jacquin in 1787 AD. It is, however, difficult to pin down exactly when the first literature dealing specifically with reptiles appeared, however, Beebee & Griffiths (2000) have indicated that Pliny's *Naturalis Historiae* of 77 -79 AD and Aristotle's *Historia de Animalium* of 350 BC are often cited as being among the earliest of writings on natural history. Beebee & Griffiths (2000) also go on to suggest that Edward Topsell's renowned '*The History of Four Footed Beasts and Serpents'* of 1658 AD and its subsequent modifications are regarded as the first books to be published on natural history in the English language.

In 1839 AD Thomas Bell produced *A History of British Reptiles,* which also included a description of amphibians. Bell (1839) described each reptile and amphibian species, including many hand-drawn detailed plates of the individuals. Since Bell's (1839) original description of native reptiles, further published works include W. S. Berridge's (1935) *All about Reptiles and Batrachians* and Richard Ford's (1954) *British Reptiles and Amphibians*.

Since these earlier publications, the number of reptile species which are known to science has significantly increased to 10,400 (Tingley *et al*., 2016) and is expected to continue to do so, with accelerated advances in molecular genetics and the ability to access more remote habitats in isolated corners of the world (Böhm *et al*., 2013; Tingley *et al*., 2016).

The disappearance of reptiles from the natural world is genuine and should be a matter of concern, not simply because of reptile's perceived associations with amphibians, but their loss is serious in its own right. Current evidence suggests that amphibian and reptile declines, which are exacerbated by burgeoning human populations and anthropogenic changes, constitute a worldwide crisis (Gibbons *et al*., 2000; Tingley *et al*., 2016). Although historically reptiles were considered by many to be 'interesting and unusual, although of minor importance' and should all of them suddenly disappear, 'it would not make much difference one way or the other' (Zim & Smith 1953; Gibbons *et al*., 2000), people have come to recognize and accept the value of reptiles as an integral part of natural ecosystems and as heralds of environmental quality (Gibbons *et al*.,

2000) playing an important role in natural systems as predators, prey, grazers and seed dispersers (Böhm *et al*., 2013).

It has been highlighted through numerous publications, including that by Sewell *et al.* (2012) that amphibians are experiencing a serious decline in numbers, however Gibbons *et al*. (2000), Böhm *et al.* (2013), Bland & Böhm (2016) and Tingley *et al*. (2016) goes on to state that reptiles appear to be in even greater danger of extinction worldwide; particularly marine reptiles, with 59% of all assessed turtles at risk of extinction (Koh, 2013; van Dijk *et al*., 2014; Tingley *et al*., 2016). With the world currently experiencing a period of "biodiversity crisis" as a result of increased extinction, primarily due to human activity (Pough *et al*., 2004; IUCN, 2010) it has been suggested that every day species' extinctions are continuing at up to 1,000 to 10,000 times or more the natural rate (IUCN, 2010; WWF, 2015) of one species per million years (Whitty, 2008), taking into account plants, fungi, vertebrates and invertebrates. Of course, decline of a species may often be a cumulative effect of numerous potential causes such as habitat loss and degradation, introduced invasive species, environmental pollution, disease, parasitism and global climate change (Böhm *et al*., 2013). There is little published research that has investigated how certain reptile species respond to different environmental variables throughout their seasonal cycle, especially concerned with when and in what conditions these species are visible. Assessing species' responses to climate change is one of the greatest challenges for ecologists because global warming is expected to be a major threat for biodiversity in coming years (Hughes, 2000; McCarty, 2001; Walther *et al*., 2002; Parmesan & Yohe, 2003; Thomas *et al*., 2004 and Böhm *et al*., 2013; Böhm *et al*., 2016). Present-day climate change has altered the phenology (the timing of periodic life cycle events) of many plant and animal populations' worldwide (Ljungström *et al*., 2015). A recent study showed that up to 37% of species on Earth (1 in 5 reptiles species, Böhm *et al*., 2016) might be threatened by extinction because of the recent rise in temperature (Thomas *et al*., 2004; Pounds *et al*., 2006; Araújo *et al*., 2006). Further numbers suggested by the WWF (2015) indicate that between 0.01% and 0.1% of all species will become extinct each year. Understanding how and which environmental variables influence the behaviour of the lizards can assist us with identifying the appropriate times of year and weather conditions in which to conduct surveys (Böhm *et al*., 2016).

With a near catastrophic decline of some reptile species native to the United Kingdom (UK) during the late 19th century and 20th century, such as *Lacerta agilis,* and a further decline in the remaining species as yet there has not been a study on native dune system lizards which has successfully estimated the size or viability of a wild population (House, 1980; Nicholson, 1980; House & Spellerberg, 1982; NCC, 1983; Fearnley, 2009). This has been partly accredited to the variability encountered in census counts (House, 1980; Nicholson, 1980; House & Spellerberg, 1982; NCC, 1983; Foster & Gent, 1996; Fearnley, 2009). Long-term monitoring of both *Zootoca vivipara* and *Lacerta agilis* populations is essential and must be aided by the establishment of standard monitoring, methods and techniques (Gibbons *et al.*, 2000; Sewell *et al.*, 2012; Blanke and Fearnley, 2015).

The remainder of this Chapter provides context for the research questions addressed in this thesis. Geographical, physical, biological and ecological characteristics of the Lacertidae species and populations are discussed. The aims of this thesis are described at the end of the chapter.

## 1.1 Declining Reptile Numbers

As a result of ever-increasing anthropogenic pressures worldwide herpetofauna populations have experienced a drastic decline, with a total of 61 (known) species having become classified as extinct (IUCN, 2016). Alroy (2015) indicates that this number may be as high as 120. A further 196 classified as ‘critically endangered’, 838 ‘endangered’ and 411 as ‘vulnerable’ on the 2016 IUCN’s Red List of Species (IUCN, 2016). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), has a total of 607 reptile species listed within its three Appendices: 94 within Appendix I (most endangered species), 473 within Appendix II (not necessarily now threatened with extinction but that may become so unless trade is closely controlled) and 40 within Appendix III (species included at the request of a Party that already regulates trade in the species and that needs the cooperation of other countries to prevent unsustainable or illegal exploitation) (CITES, 2017). Uetz & Hosek (2015) and Tingley *et al*. (2016) indicate that only 45% of the 10,400 reptile species have been assed by the IUCN, however, and species have been assigned a Red List status on basis of geographical range size due to insufficient data on population trends (Tingley *et al.*, 2016).

Considering an absence in complete analysis, across the European Union (EU), although there have been no extinctions six species are classified as ‘critically endangered’, with a further 11 species classified as ‘endangered’ (Cox & Temple, 2009). The 2009 European Red List of Reptiles has gone further and identified that 20% of reptiles are considered threatened in Europe (Cox & Temple, 2009). Böhm *et al*, (2013) predicts that one fifth of species threatened with extinction through over-harvesting, invasive species, habitat loss and fragmentation and global climate change (Auliyai *et al*., 2016; Tingley *et al*., 2016), increasing the need for extinction risk analysis, addressing taxonomic and regional knowledge gaps and conservation intervention. A need for new 'Global Reptile' Assessments to validate and refine models of extinction risk has been highlighted by Böhm *et al*., 2013; Bland & Böhm 2016 and Tingley *et al*., 2016, although to-date this has not been implemented. Knowing that a decline has occurred is not enough, effective conservation requires that we understand what the causes of the decline were (Beebee & Griffiths, 2000), as it has already been highlighted that reptiles are especially susceptible to environmental degradation, thus making them potentially important indicators of overall environmental health.

## 1.2 Current Population Status

*Sand lizard*

More than twenty 'potential' subspecies of sand lizard have been described across the species range (Blanke & Fearnley, 2015). Currently there are nine recognised subspecies (Bischoff, 1988; Kalyabina-Hauf *et al*., 2001; Edgar & Bird, 2005; Andres *et al*., 2014 and Blanke & Fearnley, 2015), although the number of accepted subspecies varies amongst authors (Blanke & Fearnley, 2015).

These are:

- ***Lacerta agilis agilis*** (western, north-western and western central Europe including the UK)
- ***Lacerta agilis argus*** (eastern central Europe)
- ***Lacerta agilis boemica*** (north-eastern Caucasus)
- ***Lacerta agilis bosnica*** (mountains of the Balkans, as far south as the Pindos in central Greece)
- ***Lacerta agilis brevicaudata*** (Transcaucasia, Armenia and adjacent Turkey)
- ***Lacerta agilis chersonensis*** (eastern Europe and western Russia)
- ***Lacerta agilis exigua*** (Russia, east of the Dniepr river, to Mongolia and northwest China)

- ***Lacerta agilis grusnica*** (eastern and southern coasts of the Black Sea)
- ***Lacerta agilis ioriensis*** (upper valleys of the Iori River in eastern Georgia)

A member of the most species-rich reptilian family in Europe, the Lacertidae (Cox & Temple, 2009), it was estimated that in the 'north-west' of Britain, the sand lizard population declined from ca. 8000 individuals in the 1930s to ca. 240 individuals in 1974 (Corbett, 1974; Prestt *et al*., 1974; Jackson, 1978). Following such a catastrophic decline, the most recent estimates propose that sand lizard populations in the UK could now only cover a surface area of 8,850km$^2$ (JNCC, 2007), only 0.036% of total UK surface area, despite reintroductions, due to a ca. 80% decline in its range over the last 200 years (Blanke & Fearnley, 2015).

Found only within two specific habitats across the British Isles (heathland and dune systems) (Corbett, 1988; Moulton & Corbett, 1999; Blanke & Fearnley, 2015), declines are directly attributed to anthropogenic changes explained further in this Chapter. The anthropogenic changes finally resulted in the species being registered as extinct within Wales (Blanke & Fearnley, 2015) in the 1960s. As a result of its extinction, the sand lizard has been reintroduced into a number of sites across north Wales since the mid 1990s, through the 'sand lizard (*L. agilis*) Biodiversity Action Plan Translocation Programme for England and Wales'. Sand lizards have not only been introducted throughout north Wales and southern England Keith Corbettalso established a new population on the Hebridean island of Coll off the north-western coast of Scotland, through the introduction of 51 lizards in 1970 (although there is no evidence of sand lizards having ever been found in Scotland (Arnold, 1995; Moulton, 2011; Blanke & Fearnley, 2015; McInerny & Minting, 2016). Regardless of these introductions, the conservation status assessment of the UK's reptiles and amphibians, undertaken in 2008, indicated that the status of the sand lizard (see photographic plates 1.1 & 1.2) was 'inadequate but improving' with the range of the sand lizards deemed as inadequate but stable at 8,850 km$^2$, and a stable number of 580 populations. In contrast, it is estimated that for the status of the species to be favourable, it would have to have a range of at least 9,833 km$^2$ with a minimum of 645 populations (JNCC, 2007; Davis, 2009; Fearnley, 2009 and JNCC, 2010).

**Plates 1.1 & 1.2:** images of a male and female sand lizard (respectively).

*Viviparous or common lizard*

The viviparous lizard or common lizard (see photographic plates 1.3 & 1.4) is also a member of Lacertidae, as is the Sand lizard, however it has now been placed in a separate monotypic genus. The viviparous lizard has been listed by the IUCN (2010) as being of ‘least concern’, although the population trend is listed as ‘decreasing’. Although it is our most common reptile species, the UK species assessment (JNCC, 2010b) mirrors that of the IUCN in that it is known to be experiencing a significant decline, in both numbers and localised range as a result of brownfield site loss and continuing development pressure in the countryside. If rates of development and habitat sterilisation by mankind continue, it is more than likely that this conservation status will change in the future.

**Plates 1.3 & 1.4:** show images of a female and neonate viviparous lizard (respectively).

## 1.3 Distribution of the Sand and Viviparous Lizard

The distribution of animals in their natural environment is not random. Habitat selection can be regarded as an interaction between choices made by individuals and the action of agents that remove individuals from specific places (Orians, 2000). On the mesoscale level, reptile distribution (including lizards) is mostly explained by climate and topography (Guisan & Hofer, 2003; Korsós & Bischoff, 1997).

*Sand Lizard*

With a wide distribution range, only second to that of the viviparous lizard (Agasyan, 2010a; Blanke & Fearnley, 2015), sand lizards occur in 36 countries across Europe (Edgar & Bird, 2005), from the Pyrenean Mountains, Southern England and Southern Scandinavia to Central Asia and Mongolia (Arnold & Ovenden, 2002). Populations are large in southern and eastern parts of the range (Jablokov, 1976), but north-west European populations, belonging to subspecies *L .a. agilis*, and northern populations of *L. a. chersonensis* are generally rare and considered to be declining (Berglind, 2005; Ceirāns, 2006; Blanke & Fearnley, 2015).

In mainland Europe, where summers are warmer and adequate conditions for egg incubation are more widely met, sand lizards occupy a much greater variety of habitats. In Hungary, for example, they abound on relatively damp ground such as the herb-rich grasslands of the Danube flood plain and still further east are found on the steppes of Central Asia. It has been postulated (Yablokov *et al.*, 1980) that the present range of *L. agilis* in Europe is recent (Amat *et al*., 2000). The species had previously only been described as occupying habitats up to an altitude of approximately 1,000 meters by Beebee and Griffiths (2000). Records provided by Yakovleva (in Blanke & Fearnley, 2015) from 1964, however, indicate the historic presence of the species along the Kermenty pass in Kirgizia (formerly Kyrgyzstan), at an altitude of 3,512 meters. Animals similar to those found in Britain also occur in France, Belgium, the Netherlands, Germany, Denmark and Sweden (Olsson, 1994; Ryberg *et al*., 2004), Slovakia, Poland and Romania (Majláthová *et al*., 2008; Borczyk, 2001) and a few isolated populations have been discovered in northern Italy (Blanke & Fearnley, 2015). Recently, it has been suggested that lizards east of the river Rhine, including a red-backed variety, are sufficiently different as to warrant subspecies status (*L. agilis argus*) (Beebee & Griffiths, 2000).

The estimate for the time of separation between British and continental European *L. agilis* populations (8,035 years BP) approximately coincides with the presence of a land bridge across the North Sea 8,300 – 7,800 years BP (Russell, 2012). In Britain, the sand lizard is most frequently associated with localised, topographical features that receive a lot of sunshine in mature areas of dry sandy heaths and occasionally of coastal sand dunes (Beebee & Griffiths, 2000; Blanke & Fearnley, 2015): this is identified further in Chapter 3. It is much more fastidious in its requirements than the viviparous lizard and thrives only where there is a dense dwarf-shrub or similar vegetation structure at ground level. Due to vast habitat loss, primarily during the twentieth century, they persist as native populations only in Surrey, Hampshire, Dorset and Merseyside (Corbett, 1988; Blanke & Fearnley, 2015). Natural populations were lost in Kent, Sussex, Hampshire, Wiltshire, Berkshire, Cheshire and North and West Wales, along with further substantial colony losses of 97%, 95% and 90% observed in parts of Merseyside, the Weald & North Surrey and Dorset (respectively) as indicated in a modern interpretation of Arnold's (1973) sand lizard distribution map (Figure 1.1). Remaining colonies are mostly on areas of heath or dune that are often fragmented and surrounded by coniferous forests or urban development (Beebee & Griffiths, 2000), although in 1839 AD Bell mentioned sightings of sand lizards in marshes and on green banks in the Poole Basin around the small village of Hamworthy (Blanke & Fearnley, 2015). More recently sand lizards are also found in secondary habitats which have been derived from either dune or lowland heathlands, such as private gardens, rough grassland, hedgerows and field banks, disused mineral extraction sites, forest ride edges, rubble piles, motorway and railway embankments (Corbett, 1988; Moulton & Corbett, 1999; Blanke & Fearnley, 2015).

Recent years have seen major efforts by conservationists to establish new populations in the West Country, West Sussex, North and West Wales and even Scotland as well as at sites within the existing range. Most of these, including the attempt in Scotland (1970, Isle of Coll - Moulton, 2011; McInerny & Minting, 2016), well north of the natural range limits, have proved reassuringly successful (Beebee & Griffiths, 2000) as seen in Figure 1.2.

To initiate the species translocation programme, it was decided that wild animals from robust populations should be caught and captive bred (Edgar, 2007). Currently there are ten captive breeding centres for sand lizards, including Chester Zoo, Marwell Wildlife Park, New Forest Reptile Centre, Avon Heath Country Park and a number of privately run vivariums. It was decided that any captive breeding programme should use animals from the nearest known populations for genetic integrity (Edgar, 2007). These breeding centres have outdoor enclosures that mimic the sand lizard's natural environment. So far, the partnership has instigated 74 reintroductions to both dune and heathland sites in

12 vice-counties and restored the species to seven of these. 80% of these have been successful or going well and more are planned for the future (Webster, 2011).

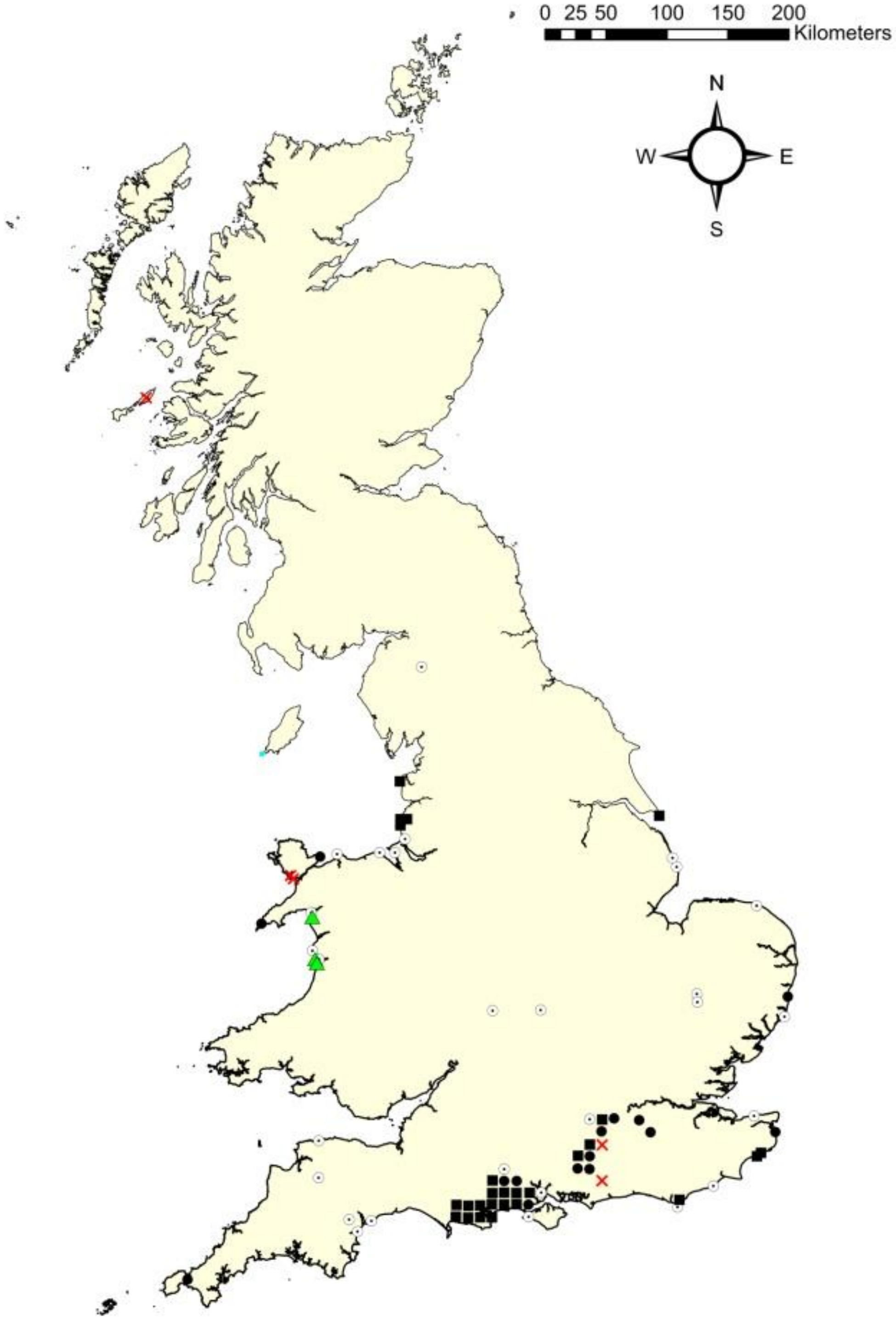


**Figure 1.1:** Interpretation of Arnolds (1973) Sand Lizard distribution map. (■ 1970 onwards, • 1960 – 1969 inclusive, ⊙ Pre 1960, X Introductions, ▲ Research locations).

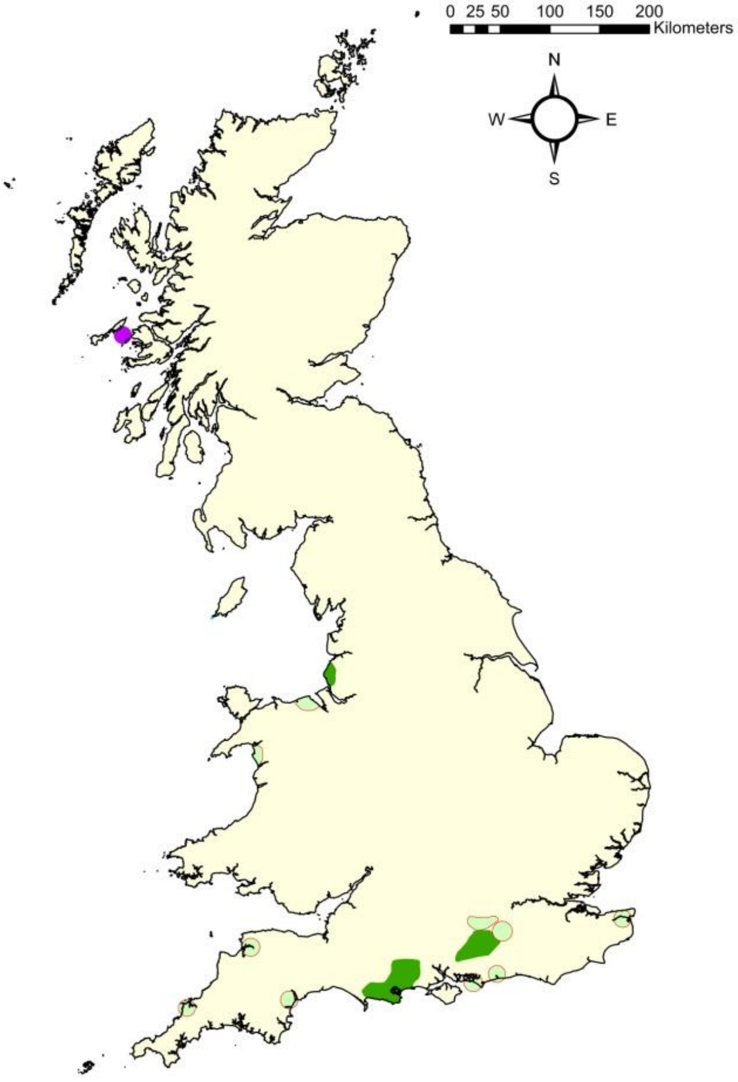


**Figure 1.2:** Current and known ranges of the sand lizard in Britain: known, past range includes areas with validated records and is highlighted in green. Pink dot indicates reintroduction outside of known geographic range (adapted from ARC, 2015a).

A reintroduced population of *L. agilis* was established at three localities across Morfa Harlech, Gwynedd between 1995 AD and 2002 AD. The reintroductions at Harlech have already proven to be successful with the animals surviving hibernation, breeding and colonising new areas (Edgar, 2007). Further releases at Aberdyfi-Tywyn Burrows (Aberdyfi), Gwynedd and Ynyslas, Ceredigion between 2006 AD – 2009 AD and 2009 AD – 2012 AD (respectively) have also proven to be successful with the animals also having survived hibernation, breeding and have colonising new areas.

A further population at Aberffraw on the Isle of Anglesey, Wales was bought to the attention of ARC in 2011 AD. Initially it was thought that this population may be a remnant population of a 'relict' Welsh population, although upon further investigation by Liam Russell and myself in 2012 AD (which involved the collection of DNA samples) analysis concluded that the population had been an 'un-condoned and unofficial' release made at some point during the 21st Century (Russell, 2012). In more recent years a further population of sand lizards has been identified at Newborough Warren Nature Reserve on the Isle of Anglesey. No analysis has been undertaken to establish the origins of this additional population, although it is anticipated that this too is an ‘un-condoned and unofficial’ release made at some point during the 21st Century.

*Viviparous lizard*

Ecologically it must be seen as the most successful and adaptable native lizard species (Beebee & Griffiths, 20000) as it has one of the widest distributions of any vertebrate in the world (Roitberg *et al*., 2013) as shown in Figure 1.3. Currently there are five known subspecies recognised across their European range (Uetz *et al*., 2015; McInerny & Minting, 2016). These are:

- ***Zootoca vivipara louislantzi*** (Northern Spain);
- ***Zootoca vivipara pannonica*** (West and South Balkan peninsula);
- ***Zootoca vivipara sachalinensis*** (Poland, Belarus, Russia east through (Sakhalin island), Japan (in Hokkaido);
- ***Zootoca vivipara vivipara*** (In the north beyond the Arctic Circle, in the south up to N Italy, in the west over to Spain and the east over to Japan); and
- ***Zootoca vivipara carniolica*** (Possibly larger parts of Slovenia, NE Italy, S Austria, NW Croatia).

The viviparous lizard has adapted to living in relatively cool regions for a reptile, and can survive in damper, marshier areas than other European species of lizard such as the sand lizard (Beebee & Griffiths, 2000; Wycherley & Anstis, 2001). It is also the most common and familiar lizard of mainland Britain, occurring over the majority of England and Wales, also being widespread over much of Scotland with the exception of the Outer Hebrides and a few Western Isles (Beebee & Griffiths, 2000; Wycherley & Anstis, 2001; McInerny & Minting, 2016). Surprisingly, they are also the only reptiles found in Ireland and the Isle of Man, and debate exists over how they colonized these islands. The most obvious route would see them migrate via a land bridge from either England or further south in Europe. However, the identity of this founder population is not known and details regarding the mechanism and time of colonization have yet to be determined (Simms, 1970; Massot *et al*., 1992; Wycherley & Anstis, 2001; Inns, 2009). It is believed by some, however, to have been deliberately introduced to Ireland in the 1970s (McGuire & Marnell, 2000), although this had not been proven.

**Figure 1.3:** Indicated global distribution of *Zootoca vivipara* adapted from Agasyan (2010b).

Viviparous lizards occupy a very tiny proportion of what would be perceived as suitable habitat in the UK. In many parts of the countryside, lizards cannot be found at all, a point made long ago by Simpson (1913): in other words, strictly speaking, viviparous lizards are not common. They are more common in some areas than others, and hence might be more correctly spoken of as locally common, as indicated in Figure 1.4 (Beebee & Griffiths, 2000).

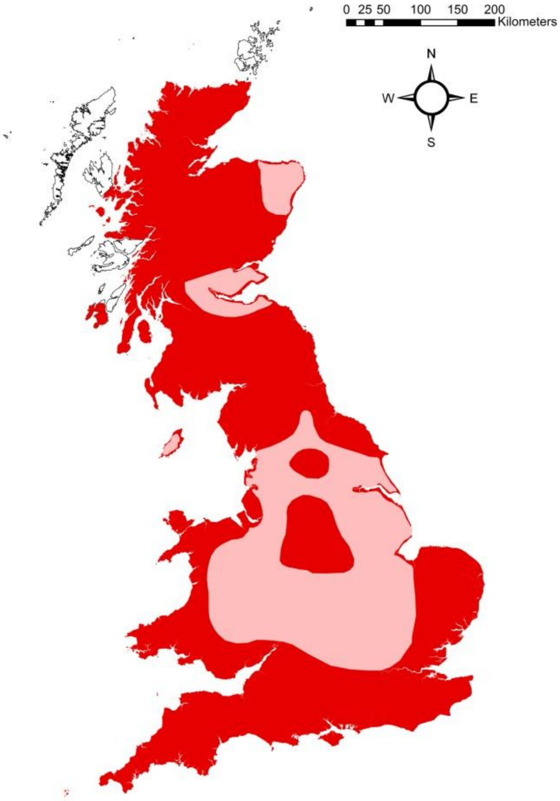


**Figure 1.4:** Map indicating the current (red) and historic (pink) distribution of the viviparous lizard across England, Scotland and Wales (adapted from ARC, 2015b).

It is well distributed in the north and east of Scotland where there is some evidence that populations are thriving. This, however, is in contrast to the Scottish Lowlands and more southern areas, where there is an increasing concern that the species is being lost, or suffering significant population declines at sites where it was once common (Inns, 2009). This observation shows that the species could potentially be experiencing a significant decline, in both numbers and range as a result of increased agricultural practices and residential & industrial development (Beebee & Griffiths, 2000; Inns, 2009).

## 1.4 Ecology

*Sand lizard*

Sand lizards are described as being slightly stocky and short legged, ground dwelling lizards, growing to between 160-240 mm in length (Beebee & Griffiths, 2000; Wycherley & Anstis, 2001; Wareham, 2008; Inns, 2009; Fearnley, 2009), of which 110-150 mm can be made up by the tail (NCC, 1983; Fearnley, 2009). They weigh approximately 20 g as adults (Olsson, 1994; Nemes, 2002). By ‘lizard’ standards, sand lizards are not especially nimble or fast moving, though they are still quick and intelligent. They rarely climb, except perhaps a little way up in a stand of heather (Wycherley & Anstis, 2001) or marram, and only jump when under threat. Males overall have a less stocky build than females, except for the head of the male, which is noticeably larger than that of the female (Wycherley & Anstis, 2001). The head of the sand lizard is however shorter and heavier than that of the viviparous lizard, especially in the male, and the snout blunter (Beebee & Griffiths, 2000; Wareham, 2008). As with the viviparous lizard, the separate scales can be clearly seen to give a ‘beaded’ appearance to the body, and the overlap of the scales on the tail can give it a ragged-looking or serrated edge (Wycherley & Anstis, 2001). The sand lizard also has a pronounced band of narrow scales along the centre of the back and a greater number of dorsal scales (32-42) at mid-body compared to the viviparous lizard (25-37) (Wareham, 2008).

Variations in colouration between the lowland heath (Surrey and Dorset) and coastal sand dune system populations (North Wales and Merseyside populations) are frequently observed, with lowland heath populations experiencing darker colouring than those of the light-grey costal sand dune populations, sometimes making them difficult to distinguish from the viviparous lizard (Wycherley & Anstis, 2001). The colouration may however vary considerably according to age, sex and habitat (Wareham, 2008). Background colouration of the upper body of both the male and female sand lizard varies from grey to dark brown with three longitudinal lines (one vertebral and two lateral) of irregular ocellated spots, which run for the full length of the individual’s body (NCC, 1983; Dent, 1986; Beebee & Griffiths, 2000; Fearnley, 2009). In the breeding season only, during April and May, the brightness of the nuptial green flanks of the sexually mature males change to a metallic green or yellowish green (Wycherley & Anstis, 2001; Wareham, 2008), a colouration which is most prominent on their flanks. This makes them easily distinguishable and easier to observe in comparison to females (NCC, 1983; Dent, 1986; Beebee & Griffiths, 2000), which are normally brown to light-grey in colouration. The underside of both sexes is either creamy white or pale yellow with black spots present in males, but absent from females (Beebee & Griffiths, 2000; Wareham, 2008). The bright green colour fades to a dull greenish-yellow after the breeding season (Fearnley, 2009). Unlike the viviparous lizard, melanistic individuals have not been observed within the UK, although there are examples from mainland Europe (Beebee & Griffiths, 2000).

Sand lizards usually emerge from hibernation in March or April (as shown in Figure 1.5), depending on the spring weather, with the males emerging first and females around two weeks later (Beebee & Griffiths, 2000; Wycherley & Anstis, 2001; Wareham, 2008; Fearnley, 2009).

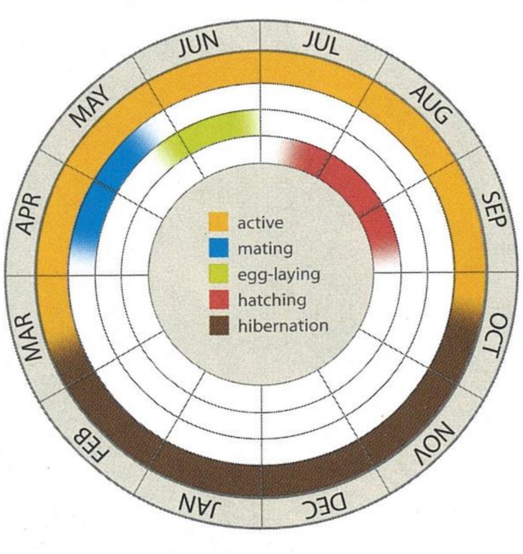


**Figure 1.5:** Seasonal activity of the sand lizard (adapted from Inns, 2009).

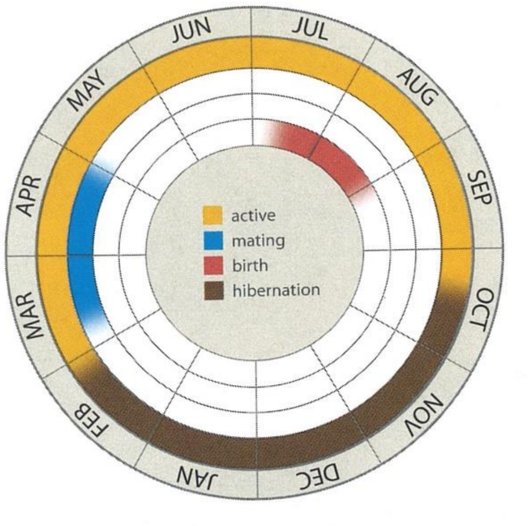


**Figure 1.6:** Seasonal activity of the viviparous lizard (adapted from Inns, 2009).

Males appear before females being both drab and lethargic for the first few days, basking near their burrow entrances in the weak early spring sunshine (Beebee & Griffiths, 2000). Olsson and Madsen (1996) have stated that females probably delay their emergence to avoid being mated by infertile males as spermatogenesis in males is incomplete until sufficient basking time has elapsed. The females also bask at this time of year to regain condition lost through the duration of their winter hibernation (Fearnley, 2009).

After spring moult courtship and mating take place around May (Beebee & Griffiths, 2000; Wycherley & Anstis, 2001; Fearnley, 2009) (Figure 1.5) and, less frequently, in June (Wareham, 2008). There is significant territorial rivalry between males, with confrontations involving a great deal of posturing and displaying of their bright colours. Fighting may ensue if posturing does not resolve the dispute (Fearnley, 2009), during which males target direct bites against each other's heads, bodies and extremities (Olsson, 1992). It has been suggested that males do not have geographically defined territories, but rather, a non-site related territory around himself and his mate (Olsson, 1994). A willing female accepts her mate who then proceeds through a series of courtship routines involving bites to the tail and lower body before coitus is initiated, with coitus between the same male and female repeatedly undertaken (Wareham, 2008). Mating can take up to half an hour and is generally performed in the open although observations of 2 – 10 minutes (Bischoff, 1984), with an average of 3.6 minutes from 176 mating incidences was recorded by Weyrauch (in Blanke & Fearnley, 2015). If disturbed, however, the females make off, often with the male still attached and unable to disengage his hemipenis (Beebee & Griffiths, 2000). It is rare, however for a clutch of sand lizard eggs all to have the same father (Beebee & Griffiths, 2000). The females incubate their eggs internally by basking extensively (House, 1980; NCC, 1983; Beebee & Griffiths, 2000) for 39 – 45 days (Rudeberg, 1956), although there is much variation between incubation periods within and between years (Fearnley, 2009). A month or so later (Figure 1.5), the oviparous females dig holes in areas of warm bare sand, within ca. 40 cm of adjacent vegetation (Beebee & Griffiths, 2000). Clutches consist of between 2 to 16 (House & Spellerberg, 1983; Olsson & Shine, 1997; Wycherley & Anstis, 2001; Wareham, 2008; McInerny & Minting, 2016) tough, parchment-like light pink shelled eggs laid between 3 to 10 cm deep within a large cavity (Beebee & Griffiths, 2000; Rashid, 2007; Wareham, 2008). The oblong eggs, which measure 10 to 14 mm (Inns, 2009) by 8 to 9 mm at first, hatch from two to three months later in August or September (Wareham, 2008; McInerny & Minting, 2016). The eggs are incubated by the warmth of the sun, and so the length of time until they hatch out depends to some extent on the weather conditions (Beebee & Griffiths, 2000) (Figure 1.5). It has, however, been indicated by Beebee and Griffiths (2000) that in-situ incubation periods of between 53 - 73 days or 7 – 12 weeks at a median nest temperature of 16 $^{o}$C – 20 $^{o}$C are usually observed. Extreme weather conditions can however be catastrophic, with hot dry summers being as problematic as cold wet ones. The former increase mortality from desiccation while the later delays hatching and probably increases the chance of fungal infections. Beebee & Griffiths, (2000) state that the ideal sand humidity should be in the range 15-18%, with an average temperature between 16.5 $^{o}$C and 20 $^{o}$C.

Hatchling sand lizards usually measure around 50 - 65 mm in length (Beebee & Griffiths, 2000; Wareham, 2008; Inns, 2009; Hudson, 2011) and emerge with the same ocellate spots and blotches as adults, with immature sand lizards generally resembling the females in colouration until the males reach maturity (Beebee & Griffiths, 2000). Although juveniles may remain in the vicinity of one another for a few days following their emergence from their eggs, they soon disperse into the surrounding undergrowth. At the end of the autumn, they average 80 mm in total length and by the following year they average 130 mm in total (Wareham, 2008), with sexual dimorphism often detectable by the end of the second year of life (Beebee & Griffiths, 2000). The young are wholly independent almost immediately, and bask and feed vigorously for the remaining few weeks before they need to dig their own burrows in which to hibernate (Wycherley & Anstis, 2001).

Hibernation begins in late September or early October (Figure 1.5) depending upon temperatures at the time (House & Spellerberg, 1983; Beebee & Griffiths, 2000; Wycherley & Anstis, 2001; Wareham, 2008; Fearnley, 2009). An excellent burrower, the holes and burrows which were excavated during the summer, as shelter from heat and predators, are used as hibernating quarters and the disused burrows of rodents, holes in walls, and holes amongst the roots of gorse *Ulex gallii* and rhododendron *Rhododendron occidentale* are also utilised. Males seem to prefer to hibernate alone but females may spend the winter in small groups (Wareham, 2008).

Studies in the Netherlands show that males can live up to 12 years, with females estimated to live for longer, on average, with an estimated ‘population turnover’ time (life expectancy) of any generation calculated at 4.83 years (Strijbosch & Creemers, 1988). It was however shown that the highest mortality arises during the juvenile stage and at first reproduction (Strijbosch & Creemers, 1988; Beebee & Griffiths, 2000).

*Viviparous lizard*

The smaller of our two Lacertidae species, viviparous lizards are surprisingly variable in both size and colour (Beebee & Griffiths, 2000) with the only other lizard species with which it might be confused being the sand lizard and the geographically limited non-native wall lizard *Podarcis mauralis* (Wycherley & Anstis, 2001). With viviparous lizards often found on the same sites as sand lizards, they can be distinguished by their smaller size, slimmer build and lack of the prominent 'eye-spot' dorsal markings of the sand lizard. In addition, male viviparous lizards in the breeding season lack the sand lizard's bright green colouration (Inns, 2009).

Viviparous lizards are very agile, fast-moving animals, with male viviparous lizards growing to between 85 to 200 mm in length from snout to tip of tail (between 50 and 70 mm SVL, Massot *et al*., 1992; Le Galliard *et al*., 2005a, b; Wareham, 2008), but usually only reaching 150 mm. Legs are relatively short and stocky, with five tapering toes on each foot, and (when intact) a long tapering tail that makes up about two-thirds of the total length of an individual (Wycherley & Anstis, 2001). Many observed individuals, however, have a tail that has been lost and has re-grown. This re-growth is usually shorter and darker.

Viviparous lizards occur in a range of colours, with shades of grey, ginger/reddish brown and olive green frequent observed (McInerny & Minting, 2016) and unlike the sand lizard, melanistic individuals can frequently be observed in Britain. Colours observed are produced from iridescence of the scales and does not appear to be caused by pigment as in the case of the sand lizard (Wareham, 2008). Adult males usually have an overall dark brown colour, with complex patterns of lighter or darker brown stripes and broken lines running the length of the body, which have scattered but well-defined pale-centred spots. On the contrast, adult females are usually paler, frequently having a dark stripe (continuous or broken) down the centre of the back from head to the base of the tail with a broader stripe on each side edged with yellow or white streaks above and below, turning to spots on the tail (Beebee & Griffiths, 2000; Wycherley & Anstis, 2001; Wareham, 2008; Inns, 2009). If in the hand, viviparous lizards can be sexed more effectively with the underside of males generally accepted as being brightly coloured, usually yellow or orange, but sometimes vermillion and densely covered in black spots, whereas the belly of the female is normally much paler and can be greyish, bluish, yellow or orange generally with few or no spots (Beebee & Griffiths, 2000). It must be noted, however, that orange ventral surfaces in females are found in a single population in Dorset (Wareham, 2008).

Emerging from their winter hibernation in March or April (sometimes as early as February, depending on the winter/spring weather), as shown in Figure 1.6, viviparous lizards bask in full sunshine at any time of day wherever there are open spots within easy reach of cover (Beebee & Griffiths, 2000). At this time of year, basking is necessary for males to complete spermatogenesis before mating can take place and as a result, females usually delay their appearance until a week or two later to ensure this has taken place (Beebee & Griffiths, 2000). Breeding activity (as shown in Figure 1.6) starts just after and involves threat-posturing and fighting between males, with chasing and biting, but aggression is rarely observed to the same extent as that displayed by the sand lizard (Beebee & Griffiths, 2000; Inns, 2009). Unlike the sand lizard, however, females take the initiative when ready to mate, seeking out males which then follow the females and begin courtship (Beebee & Griffiths, 2000; Inns, 2009). Breeding behaviour of the viviparous lizard has been described by both Simms (1970) and Beebee & Griffiths (2000) as less elaborate than that of the sand lizard, with males usually seizing females in their jaws before copulation takes place. Like the sand lizard, mating by the viviparous lizard only last a few minutes (30 min at the most), during which time the animals are often exposed and vulnerable on account of their immobility. Nevertheless, females usually mate more than once with either the same male or, quite commonly, with several (Beebee & Griffiths, 2000). After mating, females become noticeably swollen with development of the eggs taking about three months.

Females usually give birth between July or August (Figure 1.6), although in cold summers this event can be considerably delayed and dates as late as early September have been recorded in Britain (Beebee & Griffiths, 2000; Inns, 2009). Average clutch size for a viviparous lizard is about seven or eight (Avery, 1975), although clutch sizes can vary between three to thwelve (Beebee & Griffiths, 2000; Wareham, 2008; Inns, 2009). As the scientific name of the lizard suggests (viviparous), it gives birth to live young, delivered within transparent egg membranes, which are quickly (from a few minutes to one day, Massot *et al*., 1992; Le Galliard *et al*., 2005b) ruptured by the struggling lizard inside. Elsewhere in its range, this species is oviparous. Populations within the Pyrenees and northern Spain lay eggs in the standard fashion of other lacertids, depositing clutches beneath stones (Beebee & Griffiths, 2000; Vences *et al*., 2006; Lindtke *et al*., 2010). It has been suggested that viviparity within this species was only selected during a period of cold climate conditions: the Pleistocene (between 2 and 0.5 mybp, Guillaume *et al*., 1997; Heulin *et al*., 1999; Surget-Groba *et al*., 2001), suggesting that originally oviparity was the ancestrial reproductive mode (Surget-Groba *et al*., 2002).

A secluded place is usually chosen for birthing, often a cavity the female makes herself in slightly damp vegetation, with parturition of the entire clutch accomplished in a single day or extended over a week (Beebee & Griffiths, 2000). Measuring between 25 – 44 mm at birth (Beebee & Griffiths, 2000; Wycherley & Anstis, 2001; Wareham, 2008; Inns, 2009), young lizards are usually uniform black in colour and are fully independent of their parents (Le Galliard *et al*., 2005; Wareham, 2008). Within weeks this colour changes, becoming dark bronze-brown above, often speckled with gold together with initial signs of the adult colour pattern (Beebee & Griffiths, 2000).

The winter months are spent in torpor below ground amongst the roosts of vegetation, in old rabbit warrens or other suitable (frost-free and low-flood risk) holes and cavities (Wareham, 2008; Inns, 2009). Adult viviparous lizards usually retreat to their hibernation site sometime in October or November (as shown in Figure 1.6), juveniles, however have been observed to be active for a further month or so (Beebee & Griffiths, 2000). Females have additional hibernation requirements, requiring a period of hibernation at temperatures below 8 $^{o}$C – 10 $^{o}$C. Failure to achieve this results in a lack of ovarian growth, resulting in lack of breeding success during the following breeding season (Gavaud, 1983; Beebee & Griffiths, 2000). Adult viviparous lizards entering hibernation take with them substantial reserves of fat and glycogen built up through the summer months, upon which they rely for survival until the following year (Beebee & Griffiths, 2000).

## 1.5 Constraints to Conservation

Mankind is quick to exploit the earth’s resources for its own gain however this often has a devastating effect on the species which inhabit it. Identifying that species were being affected and becoming either locally or fully extinct by mankind’s efforts, conservation efforts were originally undertaken by a very small number of individuals.

In the early 20th century, a conservationist approach (the protection and preservation of the environment and wildlife) was being applied to the loss of many (more glamorous) large game species in Africa (e.g. lion, elephant and rhino). Lost through years of man’s exploitation for curiosity and research little or no effort had been applied to species, such as snakes and lizards, which were considered to be evil, a pest species or a threat to locals and/or their livestock. However, this changed on 22 June 1958 with the first annual meeting of the Ohio Herpetological Society at the Toledo Zoological Park and Museum of Science. Originally comprising of a small group of amateur herpetologists, its aims included expanding scientific knowledge, amongst others: including conservation. Re-named in 1967, it now forms the 'Society for the Study of Amphibians and Reptiles' (SSAR): now with over 2,267 members from 55 countries on six continents (Moriarty, 2007).

Reptile research has historically lagged behind some trends in conservation, although numerous papers and publications having been produced on topics such as distribution (Sillero, 2014) captive breeding, headstarting and species reintroduction, such as those wrote by Dodd & Seigel (1991), Germano & Bishop (2008), Sacerdote-Valet *et al.* (2014) and Dolman *et al.* (2015) on amphibians and reptiles, Griffith *et al.* (1989) and Towns & Ferreira (2001), Germano & Bishop (2008) and Carter *et al.* (2017) on translocations, Smith *et al.* (2006), Germano *et al.* (2014), on conservation planning, Connolly & Cree (2008) and McFadden *et al.* (2013), on captive management, Sarrazin & Barbault (1996), Jacobson (1999), Cogger *et al.* (2005), Wisely *et al.* (2007), Kock *et al.* (2007), Gibbs *et al.* (2008) and Dunwiddie & Martin (2016) on species reintroductions, Towns *et al.* (2001) on a review of conservation, Tear *et al.* (2005), on measurable objectives in conservation and Enge *et al.* (2004), Allan *et al.* (2006) on species introductions.

In cool, temperate, climates such as those found in the UK, reptiles face significant physiological and behavioural challenges and are often regarded as being confined to 'warm' habitats such as heathlands, dune systems and south-facing slopes. Widespread species (i.e. the viviparous lizard) are actually found in a diversity of habitats, whereas species such as the sand lizard are now solely confined to isolated patches of specific habitat. Since the mid 1970s, there has been an increased interest in the UK into native reptile species with research having been conducted into all of our native species by a number of interested individuals, specialist non-scientific groups, academic institutes and government agencies. Autecological research undertaken by Corbett (1988, 1988a & 1994) and Gent & Gibson (1998) into sand lizard populations, conservation and survey techniques resulted in the production of a sand lizard conservation handbook (Moulton & Corbett, 1999) addressing the UK's obligation to comply/enforce international legislation. More recently, the work that has been undertaken with sand lizards by Fearnley (2009) and Russell (2010) has concentrated on populations occurring within heathland habitats in Hampshire and Dorset (respectively) and has only very briefly touched on their conservation. In comparison, the viviparous lizard has historically received very little research although more recent research has been produced investigating dispersal (Meylan *et al*., 2002; Bestion *et al*., 2015), density (Massot *et al*., 1992; Le Galliard *et al*., 2005a; Lena *et al*., 1998), feeding (Van Damme *et al*., 2002), reproductive traits (Le Galliard *et al*., 2005b; Uller & Ollsen, 2003; Fitze *et al*., 2005; Cornetti *et al*., 2015; Rutschmann *et al*., 2016), freezing tolerance (Voituron *et al*., 2002; Grenot *et al*., 2000), thermoregulation (Herczeg *et al*., 2003; Goller *et al*., 2014) and the effect of global warming (Chamaille-Jamme *et al*., 2006).

Little or no consideration has been given to possible future conservation measures and strategies and their effect on dune system lizard populations. Examples of such conservation measures which have received little or no attention in respect are described further:

### *Species Detection*

Few species are likely to be so evident that they will always be detected at a site where they are present (MacKenzie & Bailey, 2004; Wintle *et al.*, 2012; McCarthy *et al.*, 2013). Therefore, biodiversity monitoring is now an important factor in identifying conservation needs and testing the efficiency of species management (Kèry & Schmidt, 2008; Lindenmayer *et al*., 2013), with the estimation of the abundance and distribution of a species becoming a fundamental cornerstone of conservation within the last decade. As such, the scientific community have invested much time and effort into improving 'detection probability' and other biologically meaningful parameters (Thompson, 2004; McCarthy *et al*., 2013). Driven by the practical need to estimate population abundance and to monitor changes over time, numerous models and technologies have been developed that have played a key role in the advances such as radio (UHF RFID) and satellite telemetry, passive integrated transponder (PIT) tags, coded wire tags, global positioning systems (GPS), geographic information systems (GIS) and comprehensive free software for the specialised analysis of the data. These have arisen from a plethora of new statistical models and applications (Thompson, 2004; Fearnley, 2009; Luca *et al*., 2013; Catarinucci *et al*., 2013; Outerhout & Semlitsch, 2014; Gerber *et al*., 2014; McClintock *et al*., 2015). There are many studies in which these approaches and technologies cannot be applied, such as those undertaken by Royle & Young (2008), Blanc *et al.* (2013) on the Eurasian Lynx (*Lynx lynx*), Faulkner *et al.* (2015) Erb *et al.* (2015) on eastern box turtles (*Terrapene carolina carolina*) and Gour & Reddy (2016) on the Indian tiger (*Panthera tigris*), due to the size of the individuals, populations or both.

In studying wild animal populations to estimate population size and other parameters for preservation or conservation, typically not all individuals are detected and/or not all of the home-range of a species is sampled. In many instances a census or complete count of the population is impossible (Thompson, 2004; Gerber *et al*., 2014). Without animals being "available" to a sampling method (to be observed) or the home-range of the species being unknown (Thompson, 2004), as is often the case with rare or elusive species, scientists, conservationists and in particular herpetologists have endeavoured to develop survey field census methodologies, leading to more accurate population estimates (Nicholson, 1980; Thompson, 2004; MacKenzie *et. al*., 2006) and demarcation of home-ranges. Modifications to traditional sampling methods have tried to address the most frequently encountered problems in sampling natural populations (Thompson, 1990, 1991, 2002; Thompson *et al*., 1992; Thompson & Seber, 1996; Foster & Gent, 1996; Fearnley, 2009). The further development of these survey methods, collectively referred to as 'adaptive sampling', however have the potential to greatly increase the efficiency and return of useful information (measured in terms of animals detected per unit effort) and information on the ecology of the target species, as well as increase in the precision and decrease in the bias associated with estimates of population parameters (Noon, 2006; Sewell *et al*., 2012).

### *Habitat Degredation*

Howe & Rhind (2011) reported that in the latter part of the 20th century sand dunes in Wales have significantly changed with mobile dunes declining from about 75% in the 1950s to just 6% today. They now estimate that bare sand now accounts for just 1.7% (135ha) of the Welsh sand dune resource. Factors such as depletion of sand supply, nutrient enrichment, enhanced $CO_2$ levels, soil development, artificial stabilization, and possibly natural climatic variation have been implicated by Rhind *et al.* (2008) and Howe & Rhind (2011). This lack of mobility has now been described as 'critical' since many dune species are totally dependent on the open sandy areas of early successional habitats. These species include the now critically endangered fen orchid *Liparis loeselii* (Howe & Rhind, 2011) and reintroduced sand lizards.

Management practices employed across a number of dune system reserves by CCW (now NRW) indicates that traditional grazing practices have had little impact in terms of reversing stabilization and although mob grazing can be effective (Erlandson *et al*., 2005) this has been rejected on animal welfare grounds (Howe & Rhind, 2011). NRW have further utilised scrub control, topsoil stripping and even deep ploughing across a number of dune system reserves to control stabilisation. Howe & Rhind (2011) noted that deep ploughing exposes low fertility subsoil, however, leading to tall ruderal plants blanketing these areas. Radical intervention is now urgently required if we are to reverse dune stabilisation, reinstate an element of dynamism to the dune systems and restore dune habitats and species to favourable condition at dune system sites, with the aim of restoring Welsh dune systems to support >30% early-successional habitats with >10% bare sand (Howe & Rhind, 2011).

One option employed at a number of dune systems in the Netherlands: Bride of Haarlem and Den Hoorn (Heslop, 2009), Kenemmerland (Durkin, 2010) and is now being implemented in Wales (Howe *et al*., 2012) is to reactivate stabilized blowouts. This re-mobilisation is undertaken by excavating areas of frontal dune, dune slack and parabolic ridges to bare sand to provide pioneer conditions for rare invertebrates and bryophytes (Howe *et al*., 2014), although this would provide more valuable habitat dynamics for both sand, and to a lesser extent, viviparous lizards. For these areas of re-mobilised dune to remain active, Boxel *et al*. (1997) details that the area of reactivation needs to be in excess of 500 m$^2$, however. Reactivations smaller than this tend to rapidly re-stabilize (Howe & Rhind, 2011).

Trialled at Kenfig Burrows (Carrington, 2010; Howe & Rhind, 2011; Howe *et al*., 2014), across the Sefton Coast (Smith, 2012) and South Milton Sands (Hill, 2009), dune re-mobilisation has also been employed as a management practice at Newborough Warren (Howe & Rhind, 2011; Howe *et al*., 2012; Howe *et al*., 2014; Hill *et al*., 2016). Estimated to have lost 94% of bare sand cover since the 1940s (Hill *et al*., 2016), Newborough Warren is the known location of an unofficial sand lizard population (identified in 2010, it is thought to originate from an unauthorised release because no releases from the formal reintroduction programme have occurred, Hill *et al*., 2016). The re-mobilisation works at Newborough Warren were subject to a European Protected Species licence (issued by NRW), however, concern has been raised due to the re-mobilisation entailing substantial ground works on areas known to support sand lizards and viviparous lizards (Hill *et al*., 2016). The main issue raised was that the re-mobilisation works could have harmed lizards and reduced the quality and extent of their habitat (Hill *et al*., 2016). Re-mobilisation had taken places in what was known to be the core area of sand lizard presence. The main changes from the re-mobilisation works were a substantial increase in the amount of open sand, reduction in the amount of humic sand, reduction in the extent of fixed turf, reduction in marram and dune grasses cover and an altered topography (Hill *et al*., 2016). In terms of habitat available to sand lizards, Hill *et al.* (2016) reported a reduction in the extent of highly suitable habitat, being fragmented, with reduced dispersal potential between patches in the short term. Hill *et al.* (2016) have evaluated that in the medium to long-term, vegetation in the denuded areas will regrow and provide better quality habitat for sand lizards, creating a more dynamic dune system.

*Genetic Implications*

Genetic implications of habitat fragmentation and successional changes across a 'site' have been demonstrated in lizard species by Berry *et al.* (2005) and Levy *et al.* (2010) who investigated the fragmentation effects caused by agricultural land use in the grand skink (*Oligosoma grande*) from New Zealand and the agamid (*Ctenophorus ornatus*) in Australia. The studies showed that populations from fragmented landscapes were less genetically diverse and subsequently viable as a population and more highly structured than those from areas with natural vegetation. Surprisingly high levels of genetic diversity have, however, been observed in small European green lizards (*L. viridis*) (Böhme *et al*., 2007) and Iberian rock lizards (*Iberolacerta monticola*) (Remon *et al*., 2012) in Dorset, England (Russell, 2012), Bergherbos in the Netherlands and Hungarian populations of sand lizards (Gullberg *et al*., 1998, Schwartz & Olsson 2008).

With sand lizard reintroduction sites found across North West Wales being isolated units, there is an increased degree of population isolation, with no potential of natural immigration or emigration from an existing population. With smaller populations, such as these, the populations face a greater extinction risk than larger populations.

## 1.6 Thesis Aims

Both the viviparous and sand lizard are experiencing challenges to their long-term survival within the UK, to varying degrees, governed by external factors. Thus, the challenges facing both herpetologists and conservationists alike is to increase the knowledge and understanding of both species allowing for the formulation of more appropriate conservation strategies as ever increasing anthropogenic pressures are observed.

To date, NARRs and the UK sand lizard monitoring scheme, co-ordinated by ARC, are restricted to either presence or count surveys, which are limited by the influence of external variables on 'detectability' of these lizards. Such surveys have limited use as an indicator of likely species presence/absence, health of a population or even habitat suitability. The general aim of this research thesis is to further the knowledge of conservationists through identifying more accurate detection parameters, habitat utilisation and genetic health (*L. agilis* only) within three dune-systems at Morfa Harlech & Aberdyfi in Gwynedd and Ynyslas, Ceredigion, Wales on the two co-existing species, *Z. vivipara* and reintroduced *L. agilis* populations. Investigating the above will provide a greater understanding of reptile distribution across dune systems and sound scientific background for the development of future reptile conservation measures within the UK. The findings of this thesis will additionaly provide tools for more accurate recording of dune system sand lizard populations, thereby improving the quality and increasing the quantity of information relayed back to Europe as part of the UK's legal obligation to report on the conservation status of Schedule 5 species as part of article 17 of the Habitats Directive.

The general aims will be met by addressing the following objectives in subsequent chapters:

- **Chapter 2** External variables such as weather and seasonality, which play an important part in the detectability of both dune-system *Z. vivipara* and reintroduced *L. agilis* is explored in Chapter 2. The objective of this chapter is to investigate the influence of environmental conditions as a constraint to the detection of dune system lizards. To achieve the objective, three Welsh populations of dune-system *Z. vivipara* and reintroduced *L. agilis* was monitored over a combined period of five years. Visual observations of lizards were then considered with respect to measured micro-environmental variables and furthermore used to predict when it is environmentally advantageous for both species of lizards to be above ground and in the open, hence available for detection.
- **Chapter 3** investigates the identification and utilisation of dune system 'suitable habitat' by *Z. vivipara* and reintroduced *L. agilis* at the three dune system sites across Gwynedd and Ceredigion, Wales. The chapter objective is to identify and model 'suitable habitat' for both species. This identification of 'suitable habitat' will be used to evaluate the dune-system 'suitable habitat' currently occupied and available to both *L. vivipara* and *L. agilis* populations through GIS analysis compared to the 'suitable habitat' utilized by them. The identification and

utilisation of dune system ‘suitable habitat’ will provide a basis for further habitat conservation prescriptions.

- **Chapter 4** explores the genetic comparisons between the three reintroduced *L. agilis* populations across Gwynedd and Ceredigion, ARC’s captive breeding programme and original source population at Merseyside, England (now that the three reintroduction populations have been established). The chapter objective is to investigate the differentiation between the reintroduced populations through DNA analysis. It is predicted that a lower genetic diversity will be identified within the reintroduced populations to those of the original source stock at Merseyside, England.
- **Chapter 5** prescribes a scientific approach to *L. agailis* and *Z. vivipara* detection, the utilisation of dune system habitats by both reintroduced *L. agilis* and *Z. vivipara*. It also explores limitations in existing conservation strategies (related to reintroduced *L. agilis* populations) and details conservation strategies which may be utilised to increase the favourable conservation status of *L. agilis* and halt declining *Z. vivipara* numbers. Conservation practices employed for other reptile species, which could either be employed directly or adapted to improve the management of existing (and future) reintroduction *L. agilis* sites, have be identified.

# Chapter 2

## The Influence of Environmental Conditions on Detection of Dune System Lizards

# 1 Introduction

Previous studies have found that the activity of lizards is strongly associated with surface temperature (Angilletta, 2009), time, direct sunshine, solar radiation and humidity (Spellerberg, 1974; Jackson, 1978; Avery, 1979; House *et al*., 1980; Bennett, 1980; NCC, 1983; Dent, 1986; Korsos & Gyovai, 1988; Adolph & Porter, 1993; Ceirāns, 2006; Fearnley, 2009). Utilising solar radiation, through basking (heliothermic behaviour) or through direct contact with a heat source (thigmotheric behaviour) (Cowles & Bogerts, 1944; Spellerberg, 1975, 1982; Avery, 1979; Dent, 1986). Most lizards adjust their internal temperature to maintain their preferred body temperature. Each reptile species has a preferred body temperature which is optimum for the animal's physiological processes and enzyme production (Avery, 1979). A species' preferred body temperature is constant but may vary over its geographical range (Avery, 1979). There is a modest amount of published scientific literature regarding sand lizards in the UK (Corbett, 1988c; House, 1980; Nicholson, 1980; Dent, 1986; Foster & Gent, 1996; Henshaw, 1998; Fearnley, 2009; Hardaker, 2010; Russell, 2010, 2012; Blanke & Fearnley, 2015) although no recent direct studies could be found for the viviparous lizard other than those from 1971 AD – 1986 AD by Avery (1971, 1975), House *et al.* (1980) and Dent (1986). All sand lizard studies involved surveys to record presence and absence, but to date there is still no established standard survey methodology other than that recently proposed by Fearnley (2009) for heathland sand lizard populations. Both sand and viviparous lizards have seasonal and daily cycles, influenced by environmental variables (Spellerberg, 1974; Jackson, 1978; Avery, 1979; House *et al*., 1980; Bennett, 1980; NCC, 1983; Dent, 1986; Korsos & Gyovai, 1988; Adolph & Porter, 1993; Ceirans, 2006; Fearnley, 2009; Blanke & Fearnley, 2015). Active UK sand lizards have been shown to have a preferred body temperature of 31 $^{o}$C – 32 $^{o}$C with a recorded range of 12.5 $^{o}$C – 34 $^{o}$C (Spellerberg, 1982). The physiologically lethal critical minimum temperature is between 3.0 $^{o}$C - 5.9 $^{o}$C in British populations. Sand lizards are able to maintain themselves at 6.4 $^{o}$C -11.5 $^{o}$C higher than the shade temperatures and regularly operate in summer with body temperatures between 27.5 $^{o}$C and 32.5 $^{o}$C (NNC, 1983; Beebee & Griffiths, 2000). The viviparous lizard on the other hand is one of the most cold-tolerant reptiles in the world (Beebee & Griffiths, 2000). Viviparous lizards emerge from their refugia with body temperatures of around 15 $^{o}$C, but then soak up the sun until they reach an optimum operating temperature of about 30 $^{o}$C (Van Damme *et al*., 1986; Beebee & Griffiths, 2000). It is evident that until recently favourable parameters associated with detection such as temperature, time, cloud cover and wind speed were based on direct observations and experience of the surveyors. More recent studies, such as those undertaken by Bauwens *et al.* (1995), Amat *et al.* (2003), Fearnley (2009) and Kraft (2012) have investigated environmental variables that affect detection, either directly or indirectly. These have, however, been based on heathland populations or those inhabiting artificial habitats. Thus, none of these have taken into account possible changes in detectability associated with natural or even dune system habitats. As shown in Figure. 1.2, the reintroduced sand lizard populations within north Wales and relict populations at Merseyside, found predominantly on sand dune systems, now make up approximately 30% of known sand lizard sites in the UK (ARC, 2011a). As dune system habitats are becoming an increasingly important habitat type in the UK on which sand lizards are found or being reintroduced and proving a relatively safe haven for viviparous lizard populations against anthropogenic changes, it is now time to investigate lizard presence against combinations of climatic variables for this specific habitat type.

The findings can be used to better inform field surveyors on the optimum conditions in which to survey for both sand and viviparous lizards. Simple presence/absence data can then be gathered more efficiently and effectively and used to provide a standard survey methodology (Edgar & Bird, 2005), as well as a more accurate estimate of population condition under field conditions, therefore formulating more effective conservation prescriptions (MacKenzie *et al*., 2002, 2004). Even the establishment of the presence or absence of sand lizards means that surveys should be timed to conditions when the chance of encounter for this rare and elusive species is maximised.

The research presented investigates those environmental variables associated with the observation of dune system sand and viviparous lizards, contributing to our limited knowledge of the ecology of both species. By furthering our understanding of how the lizards respond to different environmental variables across dune system habitat structures we can identify optimal conditions to maximise the chance of encounters. This will formulate survey methodologies, making a direct contribution towards ARC’s reintroduction programme and contributing towards NARRS and the UK’s legal obligation regarding reporting on endangered species to Europe (under Article 17 of the Habitats Directive (Council Directive 92/43/EEC)). This chapter additionaly introduces the sites that were surveyed, reviews literature linked to the detectability of both species, presents the methods and statistics used to explore and quantify these environmental variables, and closes with a discussion of the findings.

## 2.1 Survey Sites

Arnold's (1973) maps gave an indication of the historic and current locations of both viviparous and sand lizards in Wales at the time. The interpretation of Arnold's (1973) map (Figure 1.1) indicates two sites (Tywyn and Aberdyfi), where sand lizards were found prior to 1973, and numerous sites where the viviparous lizards are found within the county of Gwynedd in northwest Wales.

Amphibian & Reptile Conservation's (ARC's) 'rare species database' (ARC, 2015c) identified three reintroduction sites within the counties of Gwynedd and Ceredigion: Aberdyfi, Morfa Harlech, and Ynyslas. At the final stage of ARC's captive breeding and reintroduction programme, the sites are currently perceived to represent a range of successional vegetation communities (between fore and aft dune). The sites chosen represent a range of sand and viviparous lizard population structures, with the sand lizard population at Morfa Harlech established in 1995 AD, Aberdyfi in 2006 AD and Ynyslas in 2009 AD (ARC, 2015a, b). An evaluation of the post release/reintroduction monitoring suggests that these sites (with the exception of Morfa Harlech, Ceredigion, Flintshire and Denbighshire) have historicaly received very little survey effort in general.

### *Morfa Harlech*

Part owned by the Harlech Estate (Jasset David Cody Ormsby-Gore, 7th Baron Harlech) and part in trust and managed by the 'Snowdonia National Park', Morfa Harlech is located within the shadow of the 13th century Harlech Castle. Forming a large triangular area of sand-flats, beaches and geomorphologically active and relic dunes (May & Hansom, 2003) it covers an area of 1,063 ha (JNCC, 2006) between an abandoned cliff north of Harlech and the estuary of the Afon Glaslyn and Afon Dwyryd (May & Hanson, 2003) (Figure 2.1).

Different sections of Morfa Harlech dune system are afforded protection under several National and International designations due (predominantly) to its embryonic sand dune vegetation. The site was designated as a combined biological and geomorphological SSSI with 883 hectares of the site also designated as a NNR (part-located with Baron Harlech's Estate and leased & managed by NRW and the 'Snowdonia National Park' Authority) in 1953 AD (GAT, 2005) and a Special Area of Conservation in 2004 AD (JNCC, 2006).

**Figure 2.1:** Map of the Morfa Harlech dune system and surrounding estuary adjacent to the town of Harlech in Gwynedd, Wales. ✦ indicates *L. agilis* introduction locations (Tele Atlas, 2010).

Receiving its first introduced sand lizards in 1995 AD Morfa Harlech has seen three phases of reintroduction. The first release area, located at approximately 257397E by 330168N (Harlech south) received a total of 58 individuals over a three-year period (1995 AD – 1997 AD) comprising of 38 unsexed juveniles and 20 1yr old unsexed individuals (ARC, 2015c). The second release area, located at 257102E by 331243N (Harlech central) received a total of 83 individuals over a three-year period (2000 AD – 2009 AD) comprising entirely of unsexed juveniles (ARC, 2015c). The third release area, located at approximately 256891E by 331748N (Harlech north) received a total of five unsexed juveniles in 2009 AD (ARC, 2015).

### *Aberdyfi*

Owned by Gwynedd County Council (GCC) and managed by both GCC & NRW, Aberdyfi dune system SSSI is part of a wide range of nationally important habitats and species covering an area of 3,792 ha (CCW, 2005). Encompassing the sand dunes and small harbour Bay resort of Aberdyfi (where the river Dyfi meets the waters of Cardigan Bay), (Figure 2.2), the dune system is set within Snowdonia National Park (Bailey, 1998).

The dunes at Aberdyfi are occupied by a golf course and are backed by a railway that runs along the eastern boundary of the site. With a number of camping areas, the combined visitor pressure is considerable during the summer with the associated trampling causing erosion to many areas of vegetation. The foredunes are also suffering an amount of marine erosion and a considerable amount of dune restoration and sea defence work has been undertaken, although the dune front at Aberdyfi is still cutting back (JNCC, 1991).

**Figure 2.2:** Map of the Aberdyfi dune system and adjacent small harbour and town of Aberdyfi, Gwynedd, Wales. ✦ indicates *L. agilis* introduction locations (Tele Atlas, 2010).

Receiving its first reintroduction of *L. agilis* in 2006 AD, Aberdyfi dune system (Tywyn burrows) has seen the completion of its single-phase reintroduction. The release area, located at 260057E by 295916N received a total of 216 individuals over a two-year period (2006 AD – 2007 AD) comprising of 216 unsexed juveniles (ARC, 2015c).

*Ynyslas*

Owned and managed by NRW, the dune system at Ynyslas is found within the County of Ceredigion. Displaying a succession of vegetation types ranging from newly formed embryo dunes to much older, mature dunes and neutral grassland communities and adjacent to the Borth & Ynyslas Golf Club, (Figure 2.3), the dune system forms part of the NNR covering an area of 97 ha (Bailey, 1998). Ynyslas is one of three dune systems in Gwynedd and Ceredigion that are actively growing (the other two being Morfa Harlech and Morfa Dyffryn) and in consequence have a significant expanse of young, mobile and semi-vegetated dunes with abundant marram grass (Bailey, 1998).

Lying at the distal end of a 6km shingle spit which stretches northwards from Borth to the mouth of the estuary, Ynyslas National Nature Reserve holds a special value for educational purposes (Bailey, 1998) receiving around 3,000 student visits per year (CCW, 2005). Having being incorporated into Dyfi National Nature Reserve, which is afforded protection under a number of national and international designations due to its outstanding physiographic interest, the site was designated as part of the Lleyn

Peninsular and Sarnau SAC, and the Cors Fochno-Dyfi RAMSAR site for its combined biological and geomorphological interest.

**Figure 2.3:** Map of the Ynyslas dune system and adjacent small town of Borth, Ceredigion, Wales. ✦ indicates *L. agilis* introduction locations (Tele Atlas, 2010).

Receiving its first reintroduction *L. agilis* in 2009 AD, Ynyslas dune system has seen the completion of its single-phase reintroduction. The release area, located at 260503E by 294074N received a total of 143 individuals over a five-year period (2009 AD – 2012 AD) comprising of 143 unsexed juveniles (ARC, 2015).

The captive-bred individuals released at Morfa Harlech, Aberdyfi and Ynyslas were originally sourced from the Ainsdale Sand Dune system. Viable populations of sand lizards have been recorded at Ainsdale Sand Dunes NNR continuously since before the 1970s, with known populations at adjacent sites pre-dating the 1960s (Arnold, 1973). Once part of the Formby Estate, Ainsdale Sand Dunes (at Merseyside, England) was purchased by English Nature (now Natural England) in 1965 AD to safeguard the estimated 1,660 species known to use the site (Simpson & Gee, 2001). The open dune habitats of the sand dunes have long been recognised for their importance to wildlife. In 1944 AD, the Nature Reserves Investigation Committee placed Ainsdale in its list of the top 22 British wildlife sites. It was ranked as ‘Category A’ – “outstanding merit” and “must be safeguarded” (Natural England, 2010).

Ainsdale Sand Dunes NNR, as seen in Figure 2.4, now forms part of the 21 mile long Sefton Coast SSSI. Designated for its intertidal mud and sandflats, embryonic shifting dunes, mobile dunes, dunes with creeping willow *Salix arenaria,* humid dune slacks, fixed dunes, dune grasslands and dune heath, the site is also of special interest for its populations of internationally important wintering waterfowl and it’s nationally and, in some cases, internationally important populations of individual waders. Its populations of sand lizard, natterjack toad *Bufo calamita* and great crested newt *Triturus cristatus* are also of special interest (Natural England, 2010). The site ia also internationally recognised through its incorporation within the Ribble & Alt Estuaries RAMSAR (The Convention on Wetlands) and the Sefton Coast SAC.

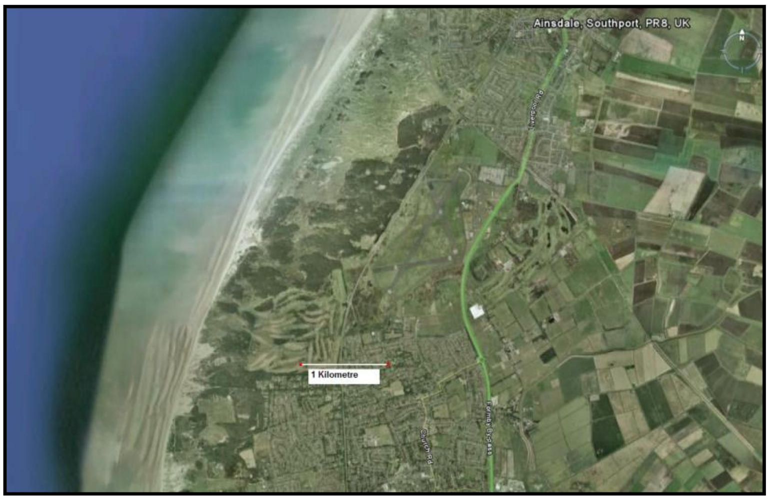


**Figure 2.4:** Map of a section of the 21 mile long Sefton Coast, showing Ainsdale Sand Dunes NNR (Tele Atlas, 2010).

## 2 Survey Methodology

The Morfa Harlech dune system was systematically walked to check for sand and viviparous lizards between March 2010 AD and October 2012 AD. Aberdyfi and Ynyslas were systematically walked to check for sand and viviparous lizards between March 2012 AD and October 2014 AD. All habitats across the three sites received reasonably equal surveyor effort during survey. Surveys predominantly followed the methodology detailed in Gent & Gibson (1998). Both ground and vegetation was carefully checked approximately 3 - 4 m ahead, with the sun kept behind the surveyor. All incidents where basking, burrowing or foraging lizards were observed were recorded, with care being taken to avoid disturbance prior to the visual sightings (Froglife, 1999; Moulton & Corbett, 1999).

Literature suggests that surveys should be undertaken in April, May and September, checking for lizards during optimum weather conditions, i.e. when the temperature is between 10 $^{o}$C and 17 $^{o}$C (Gent & Gibson, 1998; Froglife, 1999), typically between 09:00 - 11:00 Hrs and between 16:00 - 19:00 Hrs, during periods of ‘intermittent’ or hazy sunshine, with little or no wind (Gent & Gibson, 1998). These guidelines are based on years of surveyor experience, which should not be ignored, but has little or no scientific basis. Historically, where the findings of scientific studies have been included, these have been based on heathland or continental populations and not include UK dune system populations.

This survey further investigates and expands upon those environmental parameters suggested by Gent and Gibson (1998) and Froglife (1999). Environmental conditions outside of those suggested above were included within the survey strategy, such as surveying during all months between and including March to October, extending the time, temperature, wind and cloud cover ranges also. Further environmental parameters, which have received little attention from herpetologists studying UK native reptiles, were also investigated, including UVA radiation and ground temperature. These environmental parameters have been identified as being potentially utilised by dune system lizards, which have not been fully investigated by other surveys, which are concentrated on heathland sites.

Lizards were caught either by hand or by noosing of the individuals (depending upon the exact location of the individual in vegetation) following the methodology detailed within Gent & Gibson (1998) and Blomberg & Shine (2006). Noosing is preferable as it reduces risk to the lizard to a minimum while preventing disturbance to the microhabitat in the vicinity of the lizard (Gent & Gibson, 1998). In instances where it was anticipated that harm may come to an individual as a result of noosing (heavily gravid females who may abort eggs) or the individual was too small (hatchling/neonate), the individual was observed without being captured, or caught by hand.

Immediately upon observation/capture, morphological and environmental data was recorded (as detailed below) and the animal released again at point of capture/observation. In the case of sand lizards, a DNA sample (buccal swab) was taken (methodology and results detailed in Chapter 4).

- **Temperature** (using a non-intrusive infrared thermometer taken at close proximity);
- **Sex** (determined using guidance in Yablokow *et al*., 1980; Bischoff, 1984; Gent & Gibson, 1998; Arnold & Ovenden, 2004);
- **Life stage** (determined using guidance in Nicholson, 1980; Gent & Gibson, 1998; Arnold & Ovenden, 2004: juvenile, adult etc.);
- **Snout-vent length** (SVL) (using ‘wide jaw’ digital callipers, with the lizard held flat in the hand);

- **Tail length** (VTL) (using ‘wide jaw’ digital callipers, with the lizard held vertical in the hand with the tail allowed to fall freely to be measured);
- **Weight** (lizard secured in holding bag, of a known weight, and hung from microline scales);
- **Photographic image(s)** of individual (dorsal and lateral head shots); and
- Presence of scars/tail regeneration and other **visual aids** to individual identification.

Upon release, the following environmental data was recorded at point of capture/observation:

- **Location** (Garmin GPS position to 10 figures);
- **UV radiation** (taken 10 cm above substrate at point of capture/observation);
- **Surface Temperature** (taken 10 cm above substrate using an infrared thermometer at close proximity to point of capture/observation);
- **Wind speed** (taken 1.5 m above ground level at point of capture/observation);
- **Air Temperature** (taken 1.5 m above ground level at point of capture/ observation);
- **Microhabitat** (2 $m^2$ around location of *Z. vivipara*/*L. agilis*);
- **Slope** (using a clinometer at point of capture/observation); and
- **Aspect** (using compass at point of capture/observation)

A copy of the record sheet utilised during survey and list of field equipment including description and measurement accuracies (where appropriate) are shown in Appendix A, Table A.1. and Table A.2. (respectively).

The Ainsdale Sand dunes complex was visited a total of six times during the spring/summer of 2012/2013 AD. During the visits to the original capture locations for the captive-bred individuals in the areas known as ‘School Bank’ (330780E, 411930N), ‘Kenilworth Road’ (330750E, 411990N) and ‘Ainsdale Frontals’ (329100E, 411790N), individuals were caught either by hand or noose and a buccal swab taken (methodology and results detailed in Chapter 4).

The entire weather data set recorded at Capel Curig (N53.09 W3.94) has been received from the Meteorological Office. Capel Curig is the regional meteorological station covering the Cardigan Bay area, which includes Morfa Harlech, Aberdyfi and Ynyslas. Monthly environmental averages for temperature, wind speed, rainfall, humidity, cloud cover and total rainfall were received for the period between March 2010AD and October 2014 AD (inclusive), representing ‘high level’, regional weather trends.

# 3 Data Analysis

Analysis investigated which single or combination of environmental variables are utilised by both species of dune system lizards when above ground and in the open, hence available for observation.

‘High level’ regional environmental variables recorded across the Cardigan Bay area, throughout the duration of the field research and not just those recorded at the time of survey, were analysed from information obtained from the Meteorological Office. The data provides ‘over-arching’ trends across years, during the field research. Mean monthly averages for temperature, wind speed, rainfall, humidity, cloud cover and total rainfall are depicted in line graphs to provide visual reference.

Environmental variables recorded during Lacertidae encounters were initially analysed, then depicted within boxplots and line graphs. Boxplots summerise data and represent the sample by a box whose top and bottom values represent the upper and lower quartiles (i.e. the box covers the interquartile range) (Dythan, 2003; Field ,2009). The Boxplots and line graphs were created in the package Microsoft Excel v.14.0.7166.5000 (32-bit).

Secondly, environmental variables related to sand and viviparous lizard activity were analysed using both a Principal Component Analysis (PCA), and an information-theoretic approach implementing the Bayesian Information Criterion (BIC) (Schwarz, 1978). Both PCA and BIC analysis was undertaken using IBM’s SPSS (Statistical Package for the Social Sciences) version 22.

PCA was utilised to identify the environmental conditions at which the two species of lizards were observed, as it is the oldest and probably the most popular multivariate statistical technique, used by almost all scientific disciplines (Jolliffe, 1986; Abdi & Williams, 2010). The central idea of PCA is to reduce the dimensionality of the data set, in which there are a large number of interrelated variables, while retaining as much of the variation present in the data set (Jolliffe, 1986) for further analysis. Its goal is to extract the important information from the data table and to express this information as a set of new orthogonal variables called principal components/factors (Jolliffe, 1986; Jackson, 1991; Saporta & Niang, 2009; Abdi & Williams, 2010).

There are three steps involved in conducting factor (PCA) analysis, with the first step being an assessment of the suitability of the data for factor analysis. There are two main issues to consider in determining whether a data set is suitable for factor analysis: sample size and the strength of the relationships amongst the variables or items (Pallant, 2010). Regarding sample size, there is little agreement as to the size of the actual sample, with values of between 150 and 300 (Tabachnick & Fidell, 2007) quoted as a minimum. It is however generally accepted that the larger the samples size the better (Pallant, 2010). The second issue involves interrogation of the correlation matrix and reviewing the Bartlett's test of sphericity ($p < 0.05$ significance required for factor analysis to be considered appropriate, Bartlett, 1954) and the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy (> 0.6, minimum value for a good factor analysis, Kaiser, 1970 & 1974) to identify the strength of the intercorrelations among the items (Pallant, 2010).

Step two involves determining the smallest number of factors to be applied that best represent the interrelationships between the sets of environmental variables (Pallant, 2010). Determining the number of factors to be applied involves a trade-off between the need to find a simple solution with as few factors as possible and the need to explain as much of the variance in the original data set as possible (Field, 2009). There are several techniques used to assist in decision making regarding the number of factors to retain: Kaiser's criterion, scree test and parallel analysis (Field, 2009; Pallant, 2010) and all of these should be utilised in conjunction with each other before the final decision is made (Jolliffe, 1972, 1986; Stevens, 2002; Field, 2009).

Step three involves interpreting and determining the factors which have been identified as significant (Tabachnick & Fidell, 2007; Field, 2009; Pallant, 2010).

Yoccoz (1991), Cherry (1998), Johnson (1999), and Anderson & Thompson (2000), Anderson & Burnham (2002), Murtaugh (2014) and Schneider (2013, 2015) have outlined the overuse, misuse, and limitations of null hypothesis testing in wildlife research for some time. With support for null hypothesis testing and associated $P$-values having declined among statisticians over the decades (Anderson & Thompson, 2000) considerable weight has been given to information-theoretic approaches as a superior method of analysis over hypothesis testing (Burnham & Anderson, 2002; Whittingham *et al.*, 2006). Mazerolle (2006) proposed that the use of information-theory in model selection should be adopted by herpetologists. Though still underutilised compared to its more well-known counterpart (AIC), Bayesian or Schwarz Information Criterion

(BIC) also uses a ‘goodness-of-fit’ measure that is corrected for model complexity (Field, 2009; Vogt & Johnson, 2011). However, the penalty term of BIC is more stringent than the penalty term for AIC, consequently, BIC tends to favour smaller models than AIC (Cavanaugh, 2012). A ‘second-order Bayesian Information Criterion’ (BICc) is used to pick the simplest model that explains the data adequately (Cavanagh, 2012) when the ratio of data points/number of parameters is $k<40$. BIC is defined by the following mathematical equation, where $K$ = the number of free parameters to be estimated, $n$ = the number of data points and $L$ is the maximized value of the likelihood function of the model (Ernst *et al.,* 2012):

$$\mathbf{BIC = -2ln\ (\mathit{L}) + K\ log(\mathit{n})}$$

Individual BIC values are not interpretable as they contain arbitrary constants and are greatly affected by sample size. The values should be compared to values generated from further analysis, selecting the model with the smallest criterion value.

Further measures associated with BIC can be used and are probably better placed to compare models: ΔBIC and Akaike weights (Burnham & Anderson, 2004 and Fearnley, 2009). These were originally described by Mazerolle (2004) as easy to calculate, and interpret. The simplest, the delta BIC ($\Delta_i$), is a measure of each model relative to the smallest criterion value, calculated as:

$$\textbf{Delta BIC} = \Delta_i = \text{BIC}_i - \text{BIC}_{\min}.$$

Where BIC*i* is the BIC value for model *i*, and BICmin is the BIC value of the smallest criterion value (Burnham and Anderson 2004).

Akaike weights (*wi*) is a measure of the strength of evidence for each model, and represents the ratio of delta BIC (Δ*i*) values for each model relative to the whole set of candidate models. In effect, simply changing the scale of the Δ*i’s* to compare them on a scale of 1 (Mazerolle, 2004):

$$\textbf{Akaike weight} = w_i = \frac{\exp(-\Delta_i/2)}{\sum_{y=1}^{R} \exp(-\Delta_y/2)}$$

In keeping with parsimony (the simplest scientific explanation that fits the evidence), only models with three or less retained parameters were considered for analysis (Burnham & Anderson, 2002).

Data was randomly split into two sub-sets using Microsoft Excel. These two sub-sets were analysed utilising BIC: one as a ‘test’ sample, with the other used to validate the test. Comparison of the models generated from the ‘test’ and ‘validate’ sub-sets was undertaken to identify the most appropriate model, identifying those environmental variables most relevant to Lacertidae observation, relative to the whole set of candidate models.

# 4 Results

## 4.1 Weather Data Summaries

Table 2.1 presents summary values for the environmental variables recorded across the Cardigan Bay area (which includes Morfa Harlech, Aberdyfi and Ynyslas), between March 2010 AD and October 2014 AD (inclusive). Monthly averages for temperature, wind speed, rainfall, humidity, cloud cover and total rainfall, are visually presented in Appendix A, Figures. A2.1, to A2.6.

Analysis of mean monthly temperature values display a slight increase in temperature over the duration of the study, with the remainder of the variables remaining consistently even. Analysis identified seasonally better weather in 2013 AD compared to other years, with a trend for lower values over the autumn/winter period for temperature and total sunshine noted, with values peaking in spring/summer.

**Table 2.1:** Summary of weather conditions across the Cardigan Bay area received from Capel Curig, between March 2010 and October 2014 (Met Office, 2015).

| Environmental variable | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|
| Mean Temperature (ºC) | -0.1 | 16.5 | 8.9 | 4.2 |
| Average Lowest Temperature (ºC) | -3.6 | 12.1 | 5.7 | 5.5 |
| Average Highest Temperature (ºC) | 3.4 | 21.6 | 12.1 | 4.5 |
| Average Wind Speed (mph) | 6.6 | 24.9 | 14.5 | 3.8 |
| Average High Wind Speed (mph) | 25.3 | 62.1 | 43.9 | 8.0 |
| Monthly Total Rainfall (mm) | 33.8 | 616 | 203.6 | 120.3 |
| Humidity (%RH) | 74.9 | 90.1 | 83.6 | 3.7 |
| Average Cloud cover (%) | 48.8 | 92.5 | 76.1 | 10.1 |
| Monthly Total Sunshine (Hrs) | 23.7 | 297.3 | 132.6 | 65.7 |

## 4.2 Lizard Summaries

A total of 917.05 hours, over 202 days was invested surveying the three sites for both the reintroduced sand and viviparous lizards between 2010 AD and 2014 AD. 452.75 hours over 105 days at Harlech, 225 hours over 49 days at Aberdyfi and 239.3 hours over 48 days at Ynyslas. All habitats across the three sites received reasonably equal surveyor effort during survey. The earliest observations in any year were for a ‘yearling’ sand lizard and adult viviparous lizard on 02/03/2013 at Harlech. The latest observation in any year was for a hatchling sand lizard and neonate viviparous lizard on 27/10/2012, also at Harlech.

During this time, a total of 1,364 Lacertidae lizards were observed across the three sites, 237 (17%) of which were sand lizards, 1,059 (78%) were viviparous lizards and the remaining 68 (5%) were Lacertidae lizards which were not identified to species (unknown). Of the 237 sand lizards identified, 99 (42%) were males, 70 (29%) females and 68 (29%) unknown (due to the life stage of the individuals). The life stage of individuals observed were as follows: 135 (57%) were adults, 20 (8%) were sub-adults, 17 (7%) were juveniles, 16 (7%) were yearlings and there were 49 (21%) hatchlings.

Of the 1,059 viviparous lizards identified, 405 (38%) were males, 184 (17%) females and 470 (45%) were unknown (due to either the life stage of the individuals or the individuals not being able to be observed fully). The life stage of individuals observed were as follows: 546 (52%) were adults, 100 (9%) were sub-adults, 99 (9%) were juveniles, 67 (6%) were yearlings and there were 241 (23%) neonates. A total of 68 lizards were also partially observed where the species could not be confidently recorded. A breakdown of Lacertidae observations across the three individual sites is provided in Table 2.2.

A simple system of colour coding (olive green for sand lizards, red for viviparous lizards and blue for unidentified) has been applied to all information presented in Tables and Figures. This colour coding provides a quick visual reference to the species to which the information pertains.

A varied sand lizard population structure was observed across the three research sites. For populations that have been established for some time, such as Morfa Harlech and Aberdyfi, the structure presents more successional stage individuals than the structure of a research site Ynyslas which had just completed its reintroduction of sand lizards which has more founding stages.

**Table 2.2:** Sex and life stage results of sand and viviparous lizard observations across the three research sites (Harlech, Aberdyfi and Ynyslas) between 2010 and 2014.

| Sand lizard | Research site | | | | | |
|---|---|---|---|---|---|---|
| Sex /life stage | Harlech | | Aberdyfi | | Ynyslas | |
| | Number observed | Percentage | Number observed | Percentage | Number observed | Percentage |
| Male | 61 | 37% | 22 | 63% | 16 | 45% |
| Female | 56 | 34% | 6 | 17% | 8 | 22% |
| Unknown | 49 | 29% | 7 | 20% | 12 | 33% |
| Adult | 98 | 59% | 22 | 63% | 15 | 42% |
| Sub-adult | 15 | 9% | 2 | 6% | 3 | 8% |
| Juveniles | 7 | 4% | 4 | 11% | 6 | 17% |
| Yearling | 7 | 4% | 2 | 6% | 7 | 19% |
| Hatchling | 39 | 24% | 5 | 14% | 5 | 14% |

| Viviparous lizard | Research site | | | | | |
|---|---|---|---|---|---|---|
| | Harlech | | Aberdyfi | | Ynyslas | |
| Male | 256 | 38% | 81 | 42% | 68 | 36% |
| Female | 135 | 20% | 19 | 10% | 30 | 16% |
| Unknown | 285 | 42% | 93 | 48% | 92 | 48% |
| Adult | 339 | 50% | 105 | 54% | 102 | 54% |
| Sub-adult | 63 | 9% | 17 | 9% | 20 | 10% |
| Juveniles | 55 | 8% | 23 | 12% | 21 | 11% |
| Yearling | 38 | 6% | 12 | 6% | 17 | 9% |
| Neonate | 178 | 26% | 36 | 19% | 27 | 14% |

In contrast to that of the sand lizard, Table 2.2 indicates that a similar population structure exists for the viviparous lizard between the three research sites. The viviparous lizards have a typical population structure of 50-54% adults, 9-10% sub adults, 8-12% juveniles, 6-9% yearlings and 14-26% neonates. A similar sex structure is also observed between the three sites with males representing 36-42% of the population and females 10-20%.

Figure 2.5 provides a visual representation of the total number of observations per month and an indication of months where it is most favourable to observe sand and viviparous lizards on a species level. Figure 2.6 indicate that although sand lizards can be observed readily between the months of March and October, some months are more favourable than others, optimising surveyor effort. Adult sand lizards were more readily observed during the month of April, whereas hatchling sand lizards were more readily observed during the months of August, September and October, peaking in October. The total number of observations, including all life stages, indicates that April and August are the most favourable months in North West Wales.

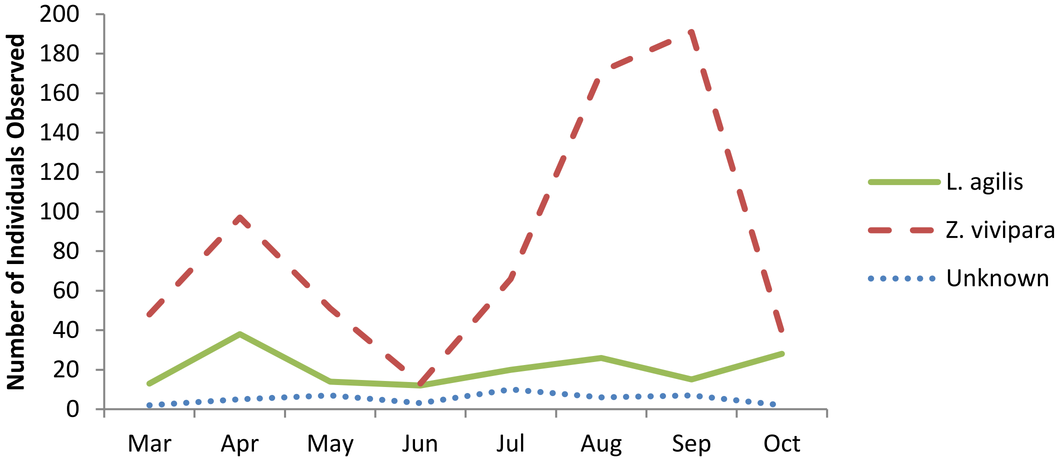


**Figure 2.5:** Monthly sand and viviparous lizard observations combined across the three research sites (Harlech, Aberdyfi and Ynyslas) between March 2010 and October 2014.

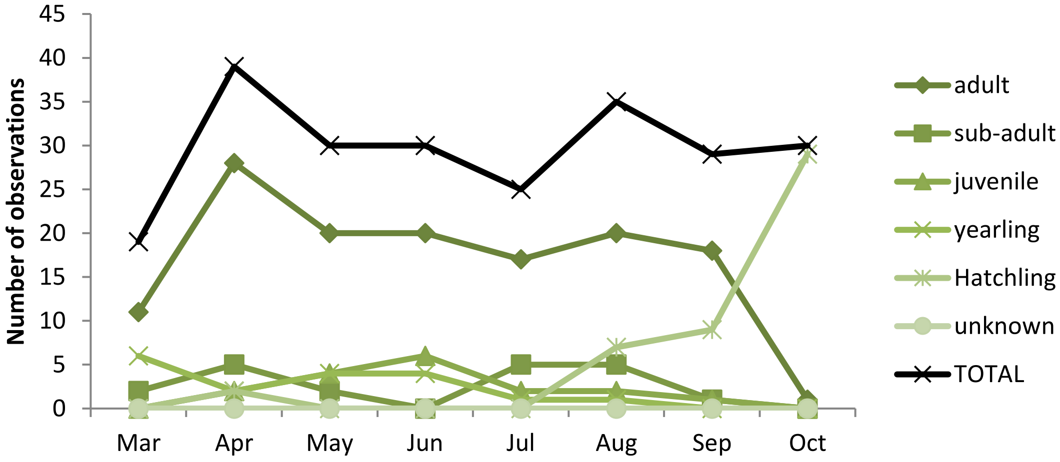


**Figure 2.6:** Number of sand lizard observations per month across all three research sites (Harlech, Aberdyfi and Ynyslas) between March 2010 and October 2014.

Figure 2.7, indicates that viviparous lizards are also readily observed between the months of March and October. Like sand lizards, some months are more favourable than others. Adult and sub-adult viviparous lizards were more readily observed during the months of April, July, August and September, whereas neonate viviparous lizards were more readily observed during the months of August, September and October; peaking in September. The total number of observations, including all life stages, indicates that April, August and September are the combined, most favourable months for observation.

Observation rates for each species and life stage, for the three individual research sites, were calculated. Figures 2.8–2.10 provide observation rates as a North-West Wales perspective (corrected for sampling effort). Rates of lizard observations indicate that between 2.25 – 6.11 Lacertidae lizards/hour could be typically observed. April, August and September represent the most favourable months for observing lizards with rates of 6.1, 5.4 and 5.5 Lacertidae lizards/hour observed. Across individual months, rates for lizard observations indicate that between 0.49 – 1.5 sand lizards/hour could be typically observed within March and October. Rates for viviparous lizard observations indicate a higher number of between 1.55 – 4.87 lizards/hour could be typically observed. Similar to those for sand lizards, periods at the beginning and end of the season, March and April – August and September represent the months with the highest observation rates (4.83, 3.6, 4.56 and 4.87 lizards/hour respectively).

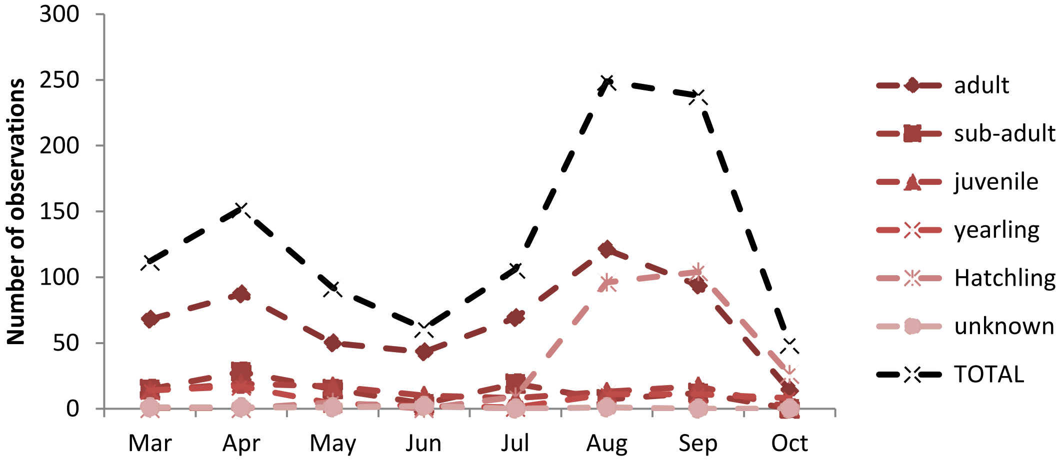


**Figure 2.7:** Number of viviparous lizard observations per month across all three research sites (Harlech, Aberdyfi and Ynyslas) between March 2010 and October 2014.

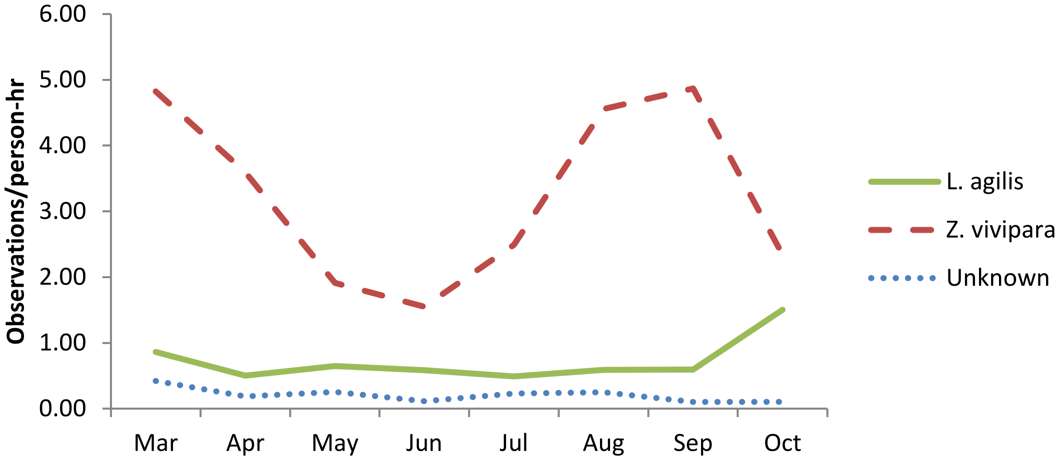


**Figure 2.8:** Lacertidae lizard observation rates across North West Wales calculated from observations made between March 2010 and October 2014.

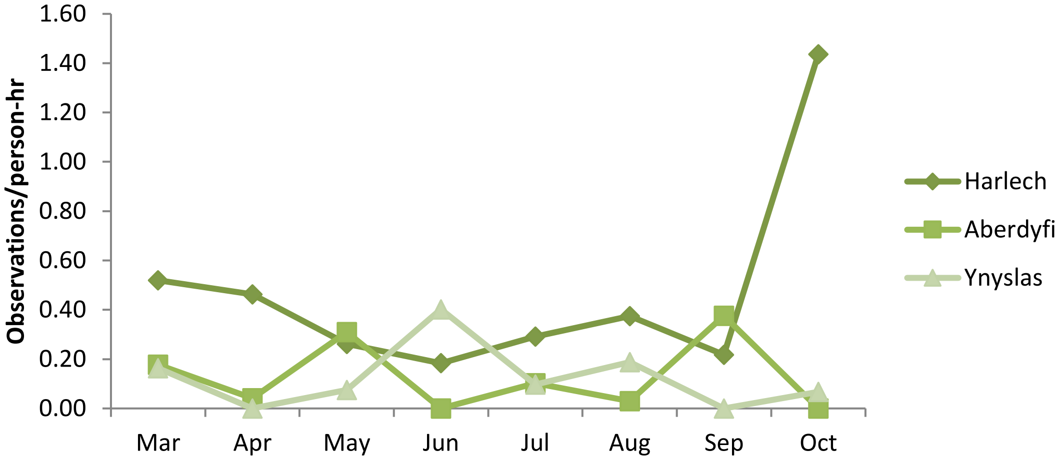


**Figure 2.9:** Sand lizard observation rates across North West Wales calculated from observations made between March 2010 and October 2014.

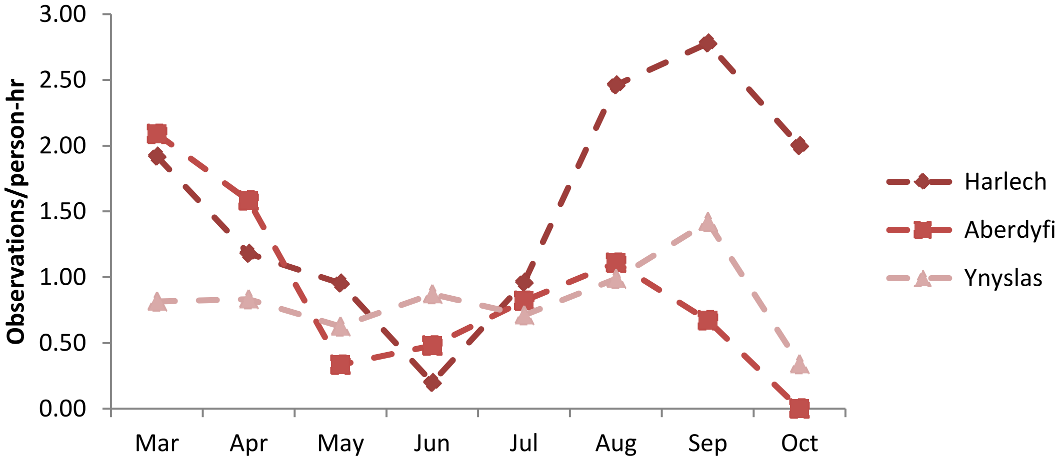


**Figure 2.10:** Viviparous lizard observation rates across North West Wales calculated from observations made between March 2010 and October 2014.

Mean weather conditions where both sand and viviparous lizards were observed appeared to be consistently similar between the two Lacertidae species. Mean sand lizard observations indicate that they are more tolerant of a generally warmer surface temperature $M = 25.28$, $df = 271$, $t(211) = 1.31$, $p \leq 0.05$ and slightly stronger wind speed compared to viviparous lizards $M = 7.9$, $df = 1282$, $t(1062) = 3.67$, $p \leq 0.01$. Table 2.3 show the results of environmental parameters recorded at the point and time in which all life stages of sand and viviparous lizards were observed between March 2010 AD and October 2014 AD across the three sites.

**Table 2.3:** Recorded values of environmental variables in which sand and viviparous lizards were observed across the three research sites between March 2010 – October 2014.

| Sand lizard | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|
| Air Temperature (ºC) | 6 | 27 | 18.3 | 3.9 |
| Surface Temperature (ºC) | 13.4 | 53.2 | 25.2 | 6.1 |
| Wind Speed (kph) | 0 | 48 | 5.5 | 8 |
| UV (mW/cm$^2$) | 0.1 | 2.1 | 1.2 | 0.4 |
| Humidity (%RH) | 31.5 | 75.5 | 52 | 8.6 |
| Cloud Cover (%) | 0 | 100 | 45.6 | 32 |
| Viviparous lizard | | | | |
| Air Temperature (ºC) | 3 | 32 | 18.3 | 3.9 |
| Surface Temperature (ºC) | 8.5 | 45.9 | 24.6 | 5.1 |
| Wind Speed (mph) | 0 | 54 | 8 | 8.7 |
| UV (mW/cm$^2$) | 0.1 | 2.2 | 1.2 | 0.4 |
| Humidity (%RH) | 25.6 | 93 | 52 | 9.1 |
| Cloud Cover (%) | 0 | 100 | 52 | 31.6 |

Figure 2.11 and Figures A2.7(a-f) in Appendix A depict the environmental variables air temperature, surface temperature, wind speed, UV, relative humidity and cloud cover, including time of observations recorded during Lacertidae observations. The boxplots give a five-number summary displaying differences between the species without making any assumptions of the underlying statistical distribution.

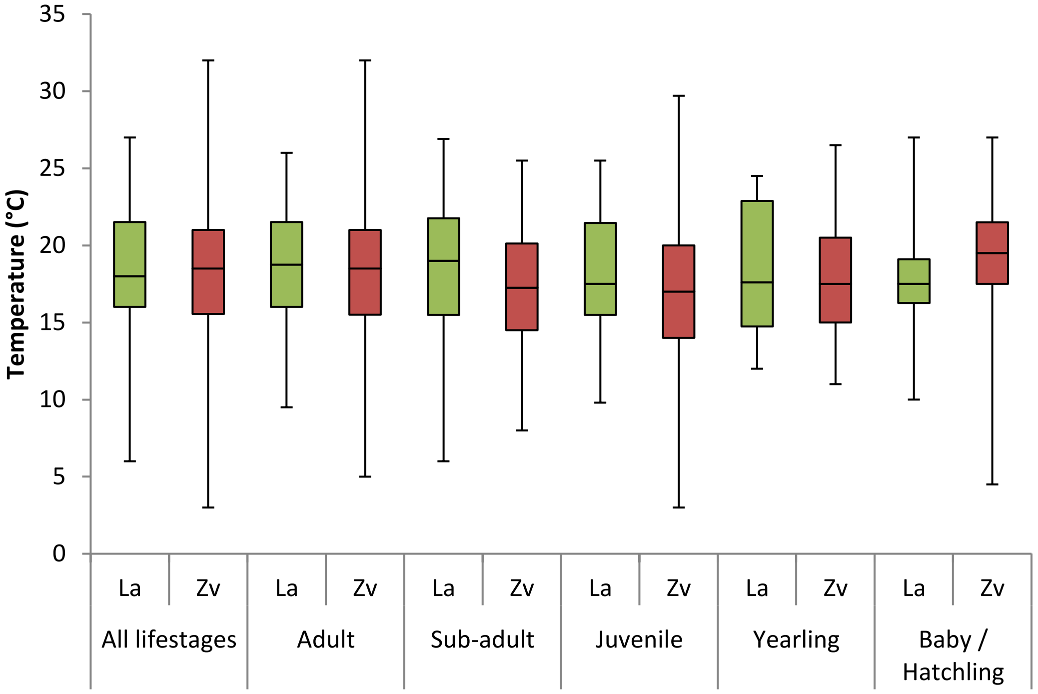


**Figure 2.11:** Basking air temperature associated with lizard observations across the three research sites between March 2010 and October 2014.

### 4.3 Lacertidae lizard observations in relation to environmental variables.

Prior to performing PCA and BIC analysis, the suitability of all data for factor analysis was assessed individually on a combined, site, life-stage and sex basis. Inspection of the individual correlation matrices revealed the presence of many coefficients of 0.3 and above (as per the guidance in Pallant, 2010), indicating several weak correlations, while no correlation above 0.49 was discovered. Kaiser-Meyer-Olkin values (a measure of sampling adequacy, providing an index of the proportion of variance among the variables) of between 0.474 and 0.579 were recorded for lizard observations across NWW: between 0.398 and 0.521 for sand lizards and between 0.417 and 0.526 for viviparous lizards, falling short of the recommended value of 0.6 (Kaiser, 1970, 1974) but indicating its suitability for assessment using factor analysis (shown in Table 2.4). All Bartlett's Tests of Sphericity (Bartlett, 1954) did however reach statistical significance ($p < 0.001$), supporting the factorability of the individual correlation matrices.

**Table 2.4:** PCA Correlation Matrix table for all life-stage records of combined, sand and viviparous lizards across NWW between 2010 – 2014 (*La* $\chi^2=176.25$, $df=15$, $p<0.001$. *Zv* $\chi^2=719.4$, $df=15$, $p<0.001$).

| Sand lizard (n = 237) | | Air temp | Surface temp | Humidity | UV | Wind speed | Cloud cover |
|---|---|---|---|---|---|---|---|
| **Correlation** | **Air temp** | 1.000 | -0.490 | -0.143 | 0.159 | -0.202 | 0.131 |
| | **Surface temp** | -0.490 | 1.000 | 0.160 | -0.465 | -0.079 | 0.052 |
| | **Humidity** | -0.143 | 0.160 | 1.000 | -0.111 | 0.063 | 0.145 |
| | **UV** | 0.159 | -0.465 | -0.111 | 1.000 | 0.026 | -0.406 |
| | **Wind speed** | -0.202 | -0.079 | 0.063 | 0.026 | 1.000 | 0.083 |
| | **Cloud cover** | 0.131 | 0.052 | 0.145 | -0.406 | 0.083 | 1.000 |
| Viviparous lizard (n = 1059) | | Air temp | Surface temp | Humidity | UV | Wind speed | Cloud cover |
| **Correlation** | **Air temp** | 1.000 | 0.460 | -0.083 | 0.040 | -0.079 | 0.252 |
| | **Surface temp** | 0.460 | 1.000 | -0.186 | 0.258 | -0.068 | 0.032 |
| | **Humidity** | -0.083 | -0.186 | 1.000 | -0.240 | 0.121 | 0.116 |
| | **UV** | 0.040 | 0.258 | -0.240 | 1.000 | 0.117 | -0.360 |
| | **Wind speed** | -0.079 | -0.068 | 0.121 | 0.117 | 1.000 | 0.143 |
| | **Cloud cover** | 0.252 | 0.032 | 0.116 | -0.360 | 0.143 | 1.000 |

Principal components analysis values typically revealed the presence of two or three environmental components with eigenvalues exceeding 1, which explained a total variance of between 49% - 78.6% for the individual life-stages and sexes. A summary of the individual percentages for each component: for all life stages and sexes (per species) are provided within Tables 2.5a and 2.5b, along with the total variance explained by the factors.

The summary tables were generated from an evaluation of the respective PCA results matrix tables, eigenvalues and percentage of variance explained from parallel analysis scree plots for the individual life stages and sexes (per species). Table 2.6 presents the individual component matrices (no Rotation of Factor) for environmental variables explained from parallel analysis as a North-West Wales perspective.

**Table 2.5a:** PCA results table summarising environmental conditions associated with detection of sand lizards across the three research sites between March 2010 and October 2014. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentage variance generated for each environmental variable.

| NWW | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable / Life stage** | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| **Air temperature** | -0.611 | 0.758 / 21.4% | 0.777 | 0.456 | -0.307 | -0.461 | -0.602 | 0.464 |
| **Surface temperature** | 0.808 / 31.5% | 0.834 / 32.5% | 0.230 | 0.784 / 36.9% | 0.657 | -0.279 | 0.708 / 31.4% | -0.100 |
| **Humidity** | 0.053 | 0.215 | 0.359 | 0.432 | 0.871 / 43.4% | 0.734 / 34.4% | 0.788 / 16.8% | 0.328 |
| **UV** | 0.167 | -0.215 | 0.351 | -0.364 | -0.766 | 0.805 / 23.7% | -0.466 | 0.807 / 34.7% |
| **Wind speed** | 0.884 / 18.3% | 0.660 / 18.4% | 0.795 / 24.3% | 0.504 | 0.887 / 32.0% | 0.470 | -0.123 | 0.926 / 16.9% |
| **Cloud cover** | 0.784 / 21.4% | 0.545 | 0.842 / 43.1% | 0.733 / 19.2% | 0.046 | 0.664 / 20.5% | 0.713 / 23.7% | 0.871 / 19.7% |
| **Total variance explained (%)** | 71.2 | 72.3 | 67.4 | 56.1 | 75.4 | 78.6 | 71.9 | 71.3 |

**Table 2.5b:** PCA results table summarising environmental conditions associated with detection of viviparous lizards across the three research sites between March 2010 and October 2014. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentage variance generated for each environmental variable.

| **NWW** | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable / Life stage** | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| **Air temperature** | 0.572 | 0.717 | 0.715 / 24.7% | 0.577 | 0.777 / 20.7% | 0.778 / 28.3% | 0.528 | 0.796 / 23.6% |
| **Surface temperature** | 0.776 / 28.0% | 0.835 / 27.9% | 0.115 | 0.784 / 32.1% | 0.654 | 0.715 | 0.750 / 28.1% | 0.677 |
| **Humidity** | -0.561 | -0.472 | 0.256 | -0.646 | -0.721 | -0.620 | -0.552 | -0.512 |
| **UV** | 0.602 | 0.461 | 0.792 / 34.6% | 0.469 | 0.807 / 33.4% | 0.723 / 23.4% | 0.646 | 0.786 / 26.4% |
| **Wind speed** | 0.932 / 18.1% | 0.907 / 18.4% | 0.774 / 17.8% | -0.557 | 0.783 / 18.7% | 0.900 / 19.5% | 0.936 / 18.8% | 0.940 / 18.9% |
| **Cloud cover** | 0.912 / 24.5% | 0.837 / 24.2% | 0.097 | 0.772 / 26.7% | -0.599 | 0.094 | 0.744 / 25.2% | -0.454 |
| **Total variance explained (%)** | 70.6 | 70.5 | 77.1 | 58.8 | 72.8 | 71.2 | 72.1 | 68.9 |

**Table 2.6:** Component Matrix (no Rotation of Factor) for Environmental variables explained from parallel analysis for all life-stage records of combined, sand and viviparous lizards across NWW between 2010 – 2014. (*La* Wilks' Lambda = 0.006, *F* (6, 188) = 5525.924, *p* <0.001. *Zv* Wilks' Lambda = 0.012, *F* (6, 986) = 14112.705, *p* <0.001). Note: major loadings for each item are highlighted in bold.

| Model | Component matrix | | | Component matrix | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| **Air temp** | -0.611 | 0.648 | -0.094 | 0.572 | 0.641 | -0.005 |
| **Surface temp** | **0.808** | -0.239 | -0.323 | **0.776** | 0.306 | 0.084 |
| **Humidity** | 0.404 | 0.053 | 0.343 | -0.561 | 0.230 | 0.188 |
| **UV** | -0.745 | -0.398 | 0.167 | 0.602 | -0.506 | 0.400 |
| **Wind speed** | 0.107 | -0.188 | **0.884** | -0.182 | 0.028 | **0.932** |
| **Cloud cover** | 0.371 | **0.784** | 0.254 | -0.204 | **0.912** | 0.136 |
| **% of variance** | 31.548 | 21.474 | 18.398 | 28.027 | 24.027 | 18.161 |

The summary results tables (Tables 2.5a and 2.5b) indicate that surface temperature, cloud cover and wind speed play a significant role in influencing sand lizard activity and availability for observation accounting for 70.6% to 71.2% of the variance ($\chi^2$=176.25, *df*=15, *p*<0.001). The variation explained within the correlation and component matrices indicate a positive influence of surface temperature, compared to the small negative influence of cloud cover and wind speed. The same environmental variables and effect were also identified for viviparous lizards, accounting for 70.6% of the variance ($\chi^2$=719.4, *df*=15, *p*<0.001): the percentage variance explained by each environmental variable for each life stage and sex varies within and between the two species.

The summary tables for the three individual research sites and total percentage variance explained by each environmental variable is provided in Appendix A, Tables A.3a and A.5b. In summary, Morfa Harlech, which represents the research site with the most Lacertidae observations, also identifies the environmental variables surface temperature, wind speed and cloud cover as playing a significant role in influencing both sand and viviparous lizards' activity and availability for observation. There is a higher degree in variation between the environmental variables identified, with surface temperature also identified as playing a role in observation. Ynyslas and Aberdyfi, with fewer Lacertidae observations, represent further variation with the exception of viviparous lizards at Aberdyfi, which predominantly identifies surface temperature, wind speed and cloud cover as those environmental variables playing a significant role in influencing both sand and viviparous lizards to be active and available for observation.

Further to the PCA analysis, which identified those environmental variables playing a significant role in determining whether both sand and viviparous lizards are active and available for observation, BIC analysis was undertaken in relation to the six environmental variables air temp, surface temp, wind speed, UV, relative humidity and cloud cover. Undertaken to identify and confirm the determining factors and further explain any variation accounted for in low observation numbers for any life stage or sex in PCA analysis, BICc was used to identify the simplest model that explains the data adequately when the ratio of data points/number of parameters is low (Cavanagh, 2012).

The criterion analysis for both the sand and viviparous lizards typically revealed between two or three environmental parameters. Tables 2.7a and 2.7b provide a summary of the simplest environmental models indicating when both sand and viviparous lizards are active and available for observation. The analysis was undertaken as a combined NWW perspective on the individual lizard life stages, along with a split between genders, as per the PCA analysis.

Individual criterion scores, used to inform Tables 2.7a and 2.7b, are provided in Tables 2.8a – 2.8c. In these tables the column titled “res dev/red df” (residual deviance of model/degrees’ freedom of the model) is equivalent to the variance inflation factor (ĉ) (Mazerolle. 2006; Fearnley, 2009). It approximates to a measure of over-dispersion with a value of 1.00 indicating a ‘good’ fit compared to larger ratio values which indicate model misspecification or an over-dispersed response variable, and ratios less than one, which may also indicate model misspecification or an under-dispersed response variable (Mazerolle, 2006).

**Table 2.7a:** BIC results table summarising environmental conditions, ('test' and 'validation' analysis results separated by a slash) associated with detection of sand lizards across three research sites between March 2010 and October 2014. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model.

| **NWW** | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable / Life stage** | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| **Air temperature** | 256.2 / 255.4 | 169.8 / 169.3 | 32.8 / 27.9 | 19.6 / 21.8 | 21.8 / 18.5 | 71.8 / 66.1 | 146.4 / 144.8 | 107.4 / 101.1 |
| **Surface temperature** | 525.5 / 521.4 | 318.2 / 289.8 | 36.5 / 24.3 | 21.8 / 21.8 | 21.8 / 21.8 | 94.7 / 80.2 | 232.5 / 204.8 | 154.9 / 145.2 |
| **Humidity** | 342.8 / 368.2 | 228.0 / 222.5 | 20.7 / 31.5 | 19.4 / 17.3 | 14.1 / 21.8 | 80.2 / 84.7 | 180.4 / 148.2 | 132.8 / 120.5 |
| **UV** | 672.5 / 652.8 | 357.2 / 357.2 | 36.5 / 31.5 | 26.6 / 21.8 | 21.8 / 21.8 | 99.4 / 89.1 | 248.1 / 251.8 | 161.2 / 166.1 |
| **Wind speed** | 126.1 / 131.5 | 99.7 / 74.8 | 21.5 / 16.9 | 19.4 / 11.2 | 11.2 / 7.5 | 27.4 / 31.5 | 81.4 / 70.6 | 74.0 / 55.7 |
| **Cloud cover** | 156.5 / 156.5 | 129.8 / 117.3 | 29.1 /24.3 | 23.1 / 21.8 | 21.8 / 21.8 | 62.6 / 60.9 | 102.6 / 118.1 | 81.2 / 86.9 |

**Table 2.7b:** BIC results table summarising environmental conditions, ('test' and 'validation' analysis results separated by a slash) associated with detection of viviparous lizards across, three research sites between March 2010 and October 2014. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model.

| **NWW** | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable / Life stage** | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| **Air temperature** | 585.5 / 577.2 | 437.7 / 445.8 | 139.6 / 155.6 | 159.3 / 155.1 | 112.6 / 112.7 | 227.8 / 208.9 | 406.5 / 407.0 | 199.6 / 193.8 |
| **Surface temperature** | 1746.2 / 1627.4 | 1114.4 / 1191.5 | 236.6 / 238.0 | 247.4 / 257.9 | 162.7 / 153.0 | 613.4 / 547.7 | 921.9 / 920.1 | 459.9 / 452.7 |
| **Humidity** | 833.2 / 815.0 | 646.1 / 651.4 | 221.8 / 211.2 | 168.0 / 203.1 | 118.3 / 113.5 | 374.2 / 381.7 | 579.5 / 586.5 | 316.9 / 328.1 |
| **UV** | 3584.0 / 3586.0 | 1840.9 / 1870.4 | 280.6 / 285.9 | 272.2 / 265.4 | 167.6 / 166.1 | 782.8 / 762.6 | 1396.8 / 1383.2 | 537.2 / 535.7 |
| **Wind speed** | 268.3 / 252.3 | 214.9 / 222.8 | 85.0 / 84.4 | 88.4 / 89.8 | 65.2 / 70.1 | 127.2 / 128.2 | 172.3 / 165.8 | 142.7 / 124.8 |
| **Cloud cover** | 246.3 / 229.4 | 201.1 / 208.7 | 122.5 / 132.4 | 115.3 / 108.7 | 81.7 / 87.7 | 160.7 / 155.0 | 204.9 / 189.9 | 142.6 / 147.9 |

**Table 2.8a:** Test (T) and validation (V) determining regression models for sand lizards in relation to environmental variables, recorded during observations across North West Wales. Where K = number of parameters (including intercept), BICc value = approximation of BICc values calculated using script provided in the data analysis section. ** res dev/res df is the residual deviance of the model divided by the residual degrees of freedom. It approximates to a measure of over-dispersion with a value of 1.00 indicated a ‘good’ fit compared to large ratio values which indicate model misspecification or an over-dispersed response variable and ratios less than one may also indicate model misspecification or an under-dispersed response variable (Mazerolle, 2004).

| **Predictor** | K | BICc | ΔBICc | Res dev/res df** | Likelihood | BIC weight | Evidence ratio | R^2 adj % |
|---|---|---|---|---|---|---|---|---|
| **All life stages** | | | | | | | | |
| **(T) Surface temperature & relative humidity** | 3 | 1438.925 | 1312.817 | 1.00 | 1.00 | 1.00 | 1.00 | 0.74 |
| **(V) UV & relative humidity** | 3 | 1397.552 | 1266.022 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 |
| **Adult** | | | | | | | | |
| **(T) Surface temperature & UV** | 3 | 727.251 | 627.529 | 1.00 | 1.00 | 1.00 | 1.00 | 0.76 |
| **(V) UV & Surface temperature** | 3 | 796.340 | 721.445 | 1.00 | 1.00 | 1.00 | 1.00 | 0.91 |
| **Sub-adult** | | | | | | | | |
| **(T) Cloud cover & UV** | 3 | 68.498 | 47.724 | 0.98 | 1.00 | 1.00 | 1.00 | 0.34 |
| **(V) Surface temperature & relative humidity** | 3 | 52.345 | 35.390 | 0.97 | 1.00 | 0.77 | 1.00 | 0.59 |
| **(V) Surface temperature & cloud cover** | 3 | 49.135 | 32.180 | 0.91 | 0.20 | 0.16 | 4.97 | 0.61 |
| **Juvenile** | | | | | | | | |
| **(T) Air temperature & wind speed** | 3 | 34.167 | 16.249 | 0.96 | 2.78 | 0.43 | 0.35 | 0.74 |
| **(T) UV & wind speed** | 3 | 32.120 | 14.202 | 1.00 | 1.00 | 0.15 | 1.00 | 0.96 |
| **(V) Air temperature & wind speed** | 3 | 28.085 | 16.789 | 1.00 | 1.00 | 0.18 | 1.00 | 0.90 |
| **Juvenile** | | | | | | | | |
| **(V) Surface temperature & wind speed** | 3 | 28.085 | 16.789 | 1.00 | 1.00 | 0.18 | 1.00 | 0.90 |
| **(V) Cloud cover & wind speed** | 3 | 28.085 | 16.789 | 1.00 | 1.00 | 0.18 | 1.00 | 0.90 |
| **(V) UV & wind speed** | 3 | 28.085 | 16.789 | 1.00 | 1.00 | 0.18 | 1.00 | 0.90 |

**Table 2.8a:** Cont.

| **Predictor** | K | BICc | ΔBICc | Res dev/res df** | Likelihood | BIC weight | Evidence ratio | R^2 adj % |
|---|---|---|---|---|---|---|---|---|
| **Yearling** | | | | | | | | |
| **(T) Air temperature & wind speed** | 3 | 28.085 | 16.769 | 1.00 | 1.00 | 0.16 | 1.00 | 0.90 |
| **(T) Surface temperature & wind speed** | 3 | 28.085 | 16.769 | 1.00 | 1.00 | 0.16 | 1.00 | 0.90 |
| **(T) Wind speed & Surface temperature** | 3 | 28.085 | 16.769 | 1.00 | 1.00 | 0.16 | 1.00 | 0.16 |
| **(T) Cloud cover & wind speed** | 3 | 28.085 | 16.769 | 1.00 | 1.00 | 0.16 | 1.00 | 0.90 |
| **(T) UV & wind speed** | 3 | 28.085 | 16.769 | 1.00 | 1.00 | 0.16 | 1.00 | 0.90 |
| **(V) Surface temperature & wind speed** | 3 | 29.870 | 22.305 | 1.00 | 1.00 | 0.20 | 1.00 | 0.79 |
| **(V) Relative humidity & wind speed** | 3 | 29.870 | 22.305 | 1.00 | 1.00 | 0.20 | 1.00 | 0.79 |
| **(V) Cloud cover & wind speed** | 3 | 29.870 | 22.305 | 1.00 | 1.00 | 0.20 | 1.00 | 0.79 |
| **(V) UV & wind speed** | 3 | 29.870 | 22.305 | 1.00 | 1.00 | 0.20 | 1.00 | 0.79 |
| **neonate** | | | | | | | | |
| **(T) Humidity & surface temperature** | 3 | 190.619 | 463.166 | 1.00 | 1.00 | 1.00 | 1.00 | 0.63 |
| **(V) Surface temperature & relative humidity** | 3 | 167.530 | 135.975 | 1.00 | 1.00 | 1.00 | 1.00 | 0.88 |
| **Male** | | | | | | | | |
| **(T) UV & surface temperature** | 3 | 1109.288 | 1027.844 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| **(V) Surface temperature & UV** | 3 | 515.700 | 445.044 | 1.00 | 1.00 | 1.00 | 1.00 | 0.77 |
| **Female** | | | | | | | | |
| **(T) UV & Cloud cover** | 3 | 300.155 | 226.140 | 1.00 | 0.94 | 0.46 | 1.05 | 0.94 |
| **(T) UV & relative humidity** | 3 | 300.270 | 265.255 | 1.00 | 1.00 | 0.48 | 1.00 | 0.91 |
| **(V) Surface temperature & air temperature** | 3 | 262.179 | 206.434 | 1.00 | 1.00 | 0.67 | 1.00 | 0.96 |
| **(V) Surface temperature & cloud cover** | 3 | 260.717 | 204.972 | 1.00 | 1.00 | 0.32 | 2.07 | 0.96 |

**Table 2.8b:** Test (T) and validation (V) determining regression models for viviparous lizards in relation to environmental variables, recorded during observations across North West Wales. Where K = number of parameters (including intercept), BICc value = approximation of BICc values calculated using script provided in the data analysis section. ** res dev/res df is the residual deviance of the model divided by the residual degrees of freedom.

| **Predictor** | K | BICc | ΔBICc | Res dev/res df** | Likelihood | BIC weight | Evidence ratio | R^2 adj % |
|---|---|---|---|---|---|---|---|---|
| **All life stages** | | | | | | | | |
| **(T) UV & Surface temperature** | 3 | 9898.464 | 9652.151 | 1.00 | 1.00 | 1.00 | 1.00 | 0.83 |
| **(V) UV & Surface temperature** | 3 | 9640.223 | 9387.860 | 1.00 | 1.00 | 1.00 | 1.00 | 0.88 |
| **Adult** | | | | | | | | |
| **(T) UV & Surface temperature** | 3 | 4777.704 | 4576.588 | 1.00 | 1.00 | 1.00 | 1.00 | 0.88 |
| **(V) UV & Surface temperature** | 3 | 4602.141 | 4393.346 | 1.00 | 1.00 | 1.00 | 1.00 | 0.95 |
| **Sub-adult** | | | | | | | | |
| **(T) UV & Wind speed** | 3 | 726.907 | 641.886 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| **(V) Relative humidity & UV** | 3 | 537.065 | 452.640 | 1.00 | 1.00 | 0.96 | 1.00 | 0.82 |
| **Juvenile** | | | | | | | | |
| **(T) Surface temperature & UV** | 3 | 600.340 | 511.914 | 1.00 | 1.00 | 1.00 | 1.00 | 0.77 |
| **(V) UV & Wind speed** | 3 | 629.904 | 540.018 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| **Yearling** | | | | | | | | |
| **(T) UV & Wind speed** | 3 | 312.877 | 247.620 | 1.00 | 8.93 | 0.90 | 0.112 | 0.91 |
| **(V) UV & Cloud cover** | 3 | 308.499 | 243.242 | 1.00 | 1.00 | 0.10 | 1.00 | 0.95 |
| **Yearling** | | | | | | | | |
| **(V) Surface temperature & cloud cover** | 3 | 310.724 | 240.535 | 1.00 | 1.00 | 1.00 | 1.00 | 0.89 |
| **Neonate** | | | | | | | | |
| **(T) Surface temperature & UV** | 3 | 1725.114 | 1597.875 | 1.00 | 1.00 | 1.00 | 1.00 | 0.79 |
| **(V) UV & Relative humidity** | 3 | 1698.333 | 1570.117 | 1.00 | 1.00 | 1.00 | 1.00 | 0.96 |

**Table 2.8b:** Cont.

| **Predictor** | K | BICc | ΔBICc | Res dev/res df** | Likelihood | BIC weight | Evidence ratio | R^2 adj % |
|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | |
| **(T) UV & Surface temperature** | 3 | 3282.043 | 3109.686 | 1.00 | 1.00 | 1.00 | 1.00 | 0.96 |
| **(V) UV & Surface temperature** | 3 | 3172.469 | 3006.584 | 1.00 | 1.00 | 1.00 | 1.00 | 0.97 |
| **Female** | | | | | | | | |
| **(T) Surface temperature & UV** | 3 | 1204.126 | 1061.354 | 1.00 | 1.00 | 1.00 | 1.00 | 0.80 |
| **(V) Surface temperature & UV** | 3 | 1168.083 | 1043.196 | 1.00 | 1.00 | 1.00 | 1.00 | 0.83 |

The summary results Tables 2.7a and 2.7b indicate that the environmental conditions surface temperature, relative humidity and UV significantly influence sand lizard activity and availability for observation. Similar environmental variables, surface temperature and UV, were also identified for viviparous lizards ((T) BIC=9898.464, $r^2$= 0.83, Relative likelihood=1, Akaike weight=1 and (V) BIC=9640.223, $r^2$= 0.88, Relative likelihood=1, Akaike weight=1).

The environmental conditions identified by the simplest models show greater variation for the sand lizards. For different life stages or sexes, where there were the fewest field observations, no clear models were identified although surface temperature, relative humidity and UV were identified within the models. For viviparous lizards, where there were a greater number of field observations, less variation in the environmental conditions identified by the simplest models was established.

The summary results tables for the three individual research sites are provided in Appendix A, Tables A6a. – A8b. In summary, the simplest models generated for Morfa Harlech, which represents the research site with the most Lacertidae observations, also identifies the environmental variables surface temperature and UV (*La* (T) BIC=958.288, $r^2$= 0.83, Relative likelihood=1, Akaike weight=1 and (V) BIC=938.728, $r^2$= 0.84, Relative likelihood=1, Akaike weight=1, *Zv* (T) BIC=5584.212, $r^2$= 0.95, Relative likelihood=1, Akaike weight=1 and (V) BIC=6007.361, $r^2$= 0.85, Relative likelihood=1, Akaike weight=1). A degree of variation between the environmental variables identified exists for the individual life stages or sexes, although surface temperature and UV are still identified. Similar environmental variables were identified by the models for viviparous lizards.

Ynyslas and Aberdyfi, with fewer Lacertidae observations, show further variation, although the environmental variables surface temperature and UV are always highlighted as those playing a significant role in sand and viviparous lizard activity and availability for observation.

## 5 Discussion

This chapter provides the first scientifically robust combination of environmental variables identified as being the most pertinent for when wild populations of dune system sand and viviparous lizards to be active and available for observation.

Widely available literature utilised by Foster & Gent (1996), JNCC (2007), and NCC (1983) encourages sand and viviparous lizard surveys to be conducted in 'favourable weather conditions' without scientifically elaborating on the definition of 'favourable'. The results of this Chapter detail 'favourable' environmental variables for wild populations of dune system sand and viviparous lizards. Fearnley's (2009) work identified a set of environmental variables for sand lizards focused on southern, heathland based and captive populations along with that of House *et al.* (1980). A summary of these previously published environmental variables in relation to lizard observations, along with further scientifically published guidelines is provided in Table 2.9, however, this is the first time that these parameters have been investigated for sand dune systems in the UK.

Fluctuations in the range and composition of favourable environmental variables were observed between the three research sites (see Table 2.3). These fluctuations indicate that the microclimates present at each site play an important role on the basking behaviours of the individual species, life-stages and sexes of the Lacertidae lizards. It would be impossible to establish these prior to the commencement of observational surveys at each site, therefore, a general set of environmental conditions (incorporating microclimate changes over a regional perspective) are therefore proposed.

The combination of environmental variables identified as being the most influential when undertaking surveys for wild populations of Lacertidae lizards across North West Wales were surface temperature, UV, cloud cover and wind speed. Cloud cover and wind speed were identified as having a negative influence on observation by the PCA analysis. Lizards were observed in the following environmental conditions:

Air temperature: 3 – 32 °C
Surface temperature: 8.5 – 53.2 °C
Wind speed: <58 mph (<93.3 kph)
UVA radiation: 0.129 – 2.29 mW/cm$^2$
Relative humidity: 25.6 – 93% rh
Cloud cover: 0 – 100%

The research does however suggest that viviparous lizard and the introduced populations of sand lizards are more readily observed during the following species specific environmental conditions:

*Lacerta agilis*

Air temperature: 16 – 21.5 °C
Surface temperature: 19.3 – 31.5 °C
Wind speed: <20 mph (<32.1 kph)
UVA radiation: 0.763 – 1.74 mW/cm$^2$
Relative Humidity: 45.5 – 62.3% rh
Cloud cover: 15 – 85%

*Zootoca vivipara*

Air temperature: 15.5 – 21 °C
Surface temperature: 21.4 – 27.4 °C
Wind speed: <12 mph (<19.3 kph)
UVA radiation: 0.848 – 1.54 mW/cm$^2$
Relative Humidity: 47 – 59% rh
Cloud cover: 30 – 75%

**Table 2.9:** Summary of published environmental variables in relation to *L. agilis and Z. vivipara*.

| **Reference** | **Environmental Variables detailed in Study** |
|---|---|
| Liberman & Pokrovskaja (1943), Russia | • Body temperature for active *L. agilis* of 33 °C |
| Spellerberg (1976), UK | • *L. agilis* mean basking temperature 26.3°C. Range 18.0 °C – 43.0 °C.<br>• *L. agilis* mean body temperature in normal activity 31.0 °C – 32 °C.<br>• Critical minimum temperature 5.9 °C or 3.0 °C when acclimated. |
| House *et al.* (1980), UK | • Mean body temperature of *L. agilis* 25.3 °C. Range of recorded values: 12.5°C – 34.0 °C.<br>• Mean air temperature of lizards 18.9 °C. Range: 7.0 °C – 28.5 °C.<br>• Mean ground surface temperatures in which lizards observed 21.9 °C. Range: 11.0 °C – 38.0 °C.<br>• March – May mean body temperatures of *L. agilis* 25.4 °C and June – September mean *L. agilis* body temperatures 29.0 °C. |
| Bauwens *et al.* (1995), SW Europe | • Critical maximum temperature of 43.9 °C.<br>• Median preferred body temperature of 34.7 °C.<br>• Lower preferred body temperature of 32.0 °C.<br>• Optimal temperature for maximum sprint speed 36.5 °C. |
| Fearnley (2009), UK | • *L. agilis* range of recorded values for captive individuals:<br>• Ground temperature: 3.1 °C – 30.1 °C (mean 19.0 °C)<br>• Relative humidity: 26% - 99% (mean 62.6%)<br>• Wind speed: 0 ms$^{-1}$ – 25.7 ms$^{-1}$ (mean 3.7 ms-1)<br>• Air temperature: 4.0 °C – 39.5 °C (mean 24.2 °C)<br>• UV: 0.0 wm$^{-2}$ – 1,230 wm$^{-2}$ (mean 564.4 wm$^{-2}$)<br>• Soil temperature: 3.0 °C – 36.5 °C (mean 20.5 °C) |
| Kraft (2012) | • *Morning (8:30 – 10:00)* Air temperature: 20 °C – 22 °C<br>• *Evening (17:00 – 19:00)* Air temperature: 23 °C – 30 °C |

Fearnley’s (2009) study on captive sand lizards also concluded UVB and temperature as the variables most strongly associated with sand lizard presence in captive populations, with sand lizards seen in warmer and brighter conditions, in comparison to those in which no lizards were observed (possibly due to the sand lizards having adapted to an environment which is safe from predation pressures). The surface temperature ranges observed are also in general accordance with those recorded during a study on captive Lacertidae lizards by House *et al.* (1980): see Table 2.9. House *et al*., (1980) collected their data between May and August in favourable weather conditions, limiting lizard counts to months and ‘favourable’ weather conditions. Fearnley (2009) however undertook her surveys between May 2005 and June 2006, incorporating unfavourable conditions and months.

Fearnley (2009) and House *et al*. (1980) further identified the significance of UVB in the detectability of Lacertidae lizards, with House *et al.* (1980) further stating that solar radiation is the most important source of heat for an ectothermic animal. The utilisation of UVB radiation by reptiles for vitamin D-3 production has been well documented (Herbert *et al*., 2006; De Lanuza & Font, 2007; Olsson *et al*., 2011; Molnar *et al*., 2012; Baines *et al*., 2016). UVA radiation (long wavelength ranging from 400 to 315 nm, Adlikns *et al*., 2003) is less well studied although Alberts (1989) has shown that certain behaviours are more likely to occur in the presence of UVA (Adlikns *et al.*, 2003) with UVA light appearing to have an influence on physiology. Gehrmann (1994) has also shown that in some species, UVA light initiates and maintains normal agonistic, reproductive, and signalling behaviour.

UVA is part of the visible spectrum for reptiles, they see colours and patterns differently to humans due to the possession of four types of cone cells in the eye (tetrachromacy). Honkavaara *et al.* (2002), Leal & Fleishman (2002), Loew *et al.* (2002), Sillman *et al*. (2001) and Thorpe & Richard (2001) have demonstrated that some reptiles rely upon UVA light to further identify individuals of their own species by their UVA-reflective markings; many plants and insects also have distinctive UVA reflectance and "patterns" which enable reptiles to recognise them. In sand lizards, De Lanuza & Font (2007) and Olsson *et al*. (2011) have demonstrated that males with higher UV reflectivity and brighter green flank colouration (Anderholm *et al*., 2004) have greater reproductive success (Russell, 2012).

In such an expansive habitat, where suitable basking opportunities are present throughout, the absence of individuals during surveys could provide false absence records when lizards are present. During field surveys sand lizards were only observed during 40.6% of surveys, viviparous lizards during 74.3% of surveys and unknown lizards on 27.2% of the surveys, illustrating how dune system Lacertidae lizard census surveys need to address the percentage chance of observation. These false absence records were mirrored during surveys of captive populations by Fearnley (2009) where on several occasions zero counts were returned even though they were known to be present.

To further maximise the opportunity of observation, not only should the surveys be undertaken under optimal environmental conditions, surveys should be undertaken during the optimal seasonal period. Across sites, rates for lizard observations indicate that between 0.49 – 1.5 sand lizards/hour could be typically observed within March and October providing the highest observation rates (0.86 + 1.50/hr likely observations, respectively). May, June and August also represented observation ratios above that observed within April. The high observation rate in May accounts for the higher observations of males at this time. This correlates with periods of prolonged basking undertaken by males for spermatogenesis (House & Spellerberg, 1983; Fearnley, 2009). Observation rates in June and August correlate with periods of mating and/or egg incubation by females (Dent, 1986; Edgar, 2005; Foster & Gent, 1996; JNCC, 2006; Robert *et al*., 2006) particularly if a second egg clutch is laid during the season: which is not uncommon in sand lizards (Hudson, 2013; Herbert *et al*., 2006; Fearnley, 2009). The high observation rates observed at the end of August - October related to adult and hatchling lizards condition basking prior to overwinter retreat (Amat *et al*., 2003).

Rates for viviparous lizard observations indicate a higher number of lizards. between 1.55 – 4.87/hour could be typically observed. Like those for sand lizards, periods at the beginning and end of the perceived season: March and April – August and September represent the months with the highest observation rates (4.83, 3.6, 4.56 and 4.87 lizards/hr respectively). Like that for sand lizards, these increased observation rates [per hour] correlate to periods of prolonged basking undertaken by males for spermatogenesis and with periods of mating and/or incubation by females. Unlike sand lizards, however, viviparous lizards were not readily encountered during October, having appeared to typically condition basked in August/September prior to overwinter retreat.

Previous surveys investigating environmental variables in relation to sand and/or viviparous lizard observations by House *et al.* (1980), Nuland & Strijbosch (1981) and

Fearnley (2009) were concentrated over a short field based research period of typically one year. Through limiting the surveys to ‘favourable’ periods and weather conditions it was anticipated that environmental variable ranges would be affected e.g. by a particularly wet, sunny or dry year etc. This would have been further exacerbated by the short duration of the surveys. In contrast, the current study was based on field research undertaken over a five-year period, along with monitoring of Meteorological Office data for the region, it can be demonstrated that long-term effects on observations as a result of over-arching environmental variable ranges have been considered.

These findings represent confirmation that the environmental conditions identified from previous studies are in-part applicable to environmental conditions utilised within a dune system environment in the UK. Furthermore, it identifies those environmental conditions which play a significant role as to when it is the most pertinent to undertake surveys for both sand and viviparous lizards. It refines these environmental conditions, to provide a specific set of conditions (specific to UK dune system habitats) which should be implemented when undertaking targeted presence/likely absence or monitoring surveys.

Through undertaking surveys during optimum survey conditions (identified earlier in this Chapter) and during optimum seasonal periods, the identification of true species presence, an indication of number and/or breeding success can be more accurately established. Conversely this will assist in identification of true species absence in areas surveyed in optimal conditions and times of the year without an observation.

# Chapter 3

## Utilisation of Dune System 'Suitable Habitat' by *Lacerta agilis* and *Zootoca vivipara* in North-West Wales, UK

# 1 Introduction

Sand dunes are shaped by a combination of physical, chemical, biotic and human factors. Within even a small dune system there can be marked gradients of instability, soil pH, moisture content and trampling. This wide range of conditions is reflected in the diversity of dune vegetation (Dargie, 1995). Three essential requirements must be fulfilled for dune formation on a beach: a prevailing onshore wind above the threshold wind velocity, a continuous supply of sand and an obstacle to reduce the velocity of wind to capture the sand load carried by the saltating cloud (Anwar Maun, 2009).

The crucial factor in the initiation of dune formation is the ability of certain plant species to grow in and stabilise wind-blown sand by growing up through it (Dargie, 1995). In Wales, this role is performed by marram grass (*Ammophila arenaria*), sand couch-grass (*Elymus farctus*) and sea lyme-grass (*Leymus arenarius*). Of these, marram is the main dune-building species as it can keep pace with up to 1 m of fresh sand deposition per annum, as well as producing far-spreading horizontal rhizomes binding the dune together (Gemmell, *et al.*, 1953; Ranwell, 1972; Dargie, 1995).

The subsequent course of succession depends on several factors. Initially, the vegetation is normally composed of only a few, highly specialised, species. As the rate of sand deposition declines, smaller grasses, annual and perennial herbs start to appear in greater number. These are joined by sand-binding mosses which are intolerant of sand deposition, and then by lichens. Simultaneously the vigour of the grasses such as marram decreases (Willis *et al.*, 1959; Hope-Simpson & Jefferies, 1966; Dargie, 1995).

The majority of dune habitat in Great Britain is now heavily protected through inclusion within SSSIs, SACs and/or NNRs, as a result of the habitats themselves being of value. Provided this level of protection can be maintained, a level of suitable habitat for this species is likely to persist (Russell, 2012). However, the level of anthropogenic pressure and influences on each site will only increase as the human population increases. As a result of increased anthropogenic pressures on sites a large proportion of the dune resource has already been lost to development (Rooney, 2001). Up to 2001 AD, the total area of dunes estimated to be lost in Great Britain, not including blown sand deposits, was about 56,000 hectares (Doody, 2005), compared to a total dune coverage of 11,897 ha in England, 8,101 ha in Wales and 1,576 ha in Northern Ireland, while the best estimate in Scotland is 48,000 ha (Rooney, 2001). Further loss is not desirable and the fragmenting effects of these losses are sometimes acute, although all natural habitats and populations of species are subject to natural change (Rooney, 2001a).

Sand and viviparous lizards are often associated with these areas containing a high number of vegetation interfaces. These interfaces provide structural diversity, through the combination of different plant species and a mixed sward height (House & Spellerberg, 1983; Moody, 2007; Blanke & Fearnley, 2015). The sand lizard in particular, requires a diverse mosaic of vegetation structures to support their daily and seasonal needs (Blanke & Fearnley, 2015), although it could be inferred that to a certain extent the same would apply to viviparous lizards.

Sand and viviparous lizards have also been found on mobile front dunes, fixed dunes and the transitional habitat between the two systems. However, Jackson (1979) reported that the lizards are most frequently found on mobile dunes where the topography is varied and there are large areas of bare sand (Blanke & Fearnley, 2015). In such habitats, the home range of sand and potentially viviparous lizards may be small and the population densities high. In contrast, sand lizards are very rarely present in larger open areas with small degrees of vegetation cover (<25%) and are rarely seen in areas with dense to complete vegetation cover (Gramentz, 1996 in Blanke & Fearnley, 2015).

The sand lizard is dependent on specific habitat communities, in this case dune systems, which receive high levels of anthropogenic influence. The conservation of sand lizards requires continuous input, particularly habitat management (Corbett & Moulton, 1999; Moulton & Corbett, 1999; Edgar, 2007; Russell, 2012). This is increasing important as this habitat has become more fragmented and isolated over time. It has been well documented by Sarre (1998), Diaz *et al*. (2000), Barrows & Allen (2007), Carvalho *et al.* (2008), Maura *et al.* (2011), Martinson *et al.* (2012), Russell (2012) and Cadenhead *et al*. (2015) that habitat fragmentation and decreased ‘patch’ size can influence the likelihood of occupancy and density of individual lizard species, including the European green lizard *Lacerta viridis,* Brazilian pygmy gecko *Coleodactylus amazonicus,* Trinidad gecko *Gonatodes humeralis,* Coachella Valley fringe-toed lizard *Uma inornata,* large psammodromus *Psammodromus algirus* and the tree dtella *Gehyra variegata*. However, lizards have also often been found to persist in small habitat patches for longer than other taxonomic groups (Burkey, 1995; Prugh *et al.*, 2008), provided the remaining habitat is of sufficient quality (Santos *et al.*, 2008; Russell, 2012; Betts *et al.*, 2014).

Problems associated with overuse, inappropriate management and dune system deterioration were described by Brooks & Agate (2008) as far back as the 1980s (Houston *et al.*, 2001). Across Europe, there has historically been a general lack of public understanding of dune system conservation. Dunes were seen as ‘wasteland’ and pine woods, which were planted across many dune sites, as ‘improvement’ (Houston *et al*., 2001). Over recent decades, attention has been drawn to the increasing problem of vegetation overgrowth and loss of bare sand on coastal dunes across Europe (Houston, 1997; Wanders, 1989). Dune reactivation work in the Netherlands and Denmark began in the late 1980s but the UK has been slow to adopt a similar approach (Smith & Lockwood, 2013). The development of strategic management prescriptions, improving conditions for key species, and carrying out management actions to protect dune habitats whilst raising awareness and support amongst visitors and locals is also required (Houston *et al*., 2001).

Where ongoing land use (maybe due to changes of practices, or fragmentation) is clearly damaging to a species, leading to decreases in the viability of a population in the area, a Member State is required to find ways to avoid this (European Commission, 2007; Blanke & Fearnley, 2015). The current conservation effort is governed by a Species Action Plan (HCT, 2009) under the auspices of the UK Biodiversity Framework (JNCC, 2012) and management activities include site protection and management, research and monitoring as well as captive breeding and reintroductions to restore its former range and viability (Corbett & Moulton, 1999; Moulton & Corbett, 1999; Russell, 2012; Blanke & Fearnley, 2015).

The utilisation of dune system habitats by native Lacertidae lizards is explored in this chapter, in relation to lizard observations. The research investigated which habitats are utilised and favoured by sand and viviparous lizards, contributing to our limited knowledge of the dune system habitat preferences of native Lacertidae lizard species. By furthering our understanding of which dune system habitat structures are utilised and favoured by both species, this study contributes to the development of more appropriate conservation and site management measures. The development of such, makes a direct contribution towards ARC’s reintroduction programme and NRW’s management of sites where reintroduced sand lizards and existing viviparous lizard populations are located. This chapter highlights the specific areas and habitats within the three reintroduction sites, subject to survey, reviews literature linked to the identification and utilisation of ‘suitable habitat’ of similar species, presents the methods and statistical/mapping tools used to explore this ‘suitable habitat’ and closes with a discussion of the findings.

# 2 Survey Methodology

To identify and model ‘suitable habitat’ for both sand and viviparous lizard species and how they utilise such habitat, a relatively rapid semi-natural vegetation (Phase 1) and detailed phytosociological classification assessment (NVC) was conducted on all three sites between late April 2013 AD and September 2015 AD. The assessment was undertaken to establish a detailed ‘habitat’ base-map to which observed sand and viviparous lizard sightings could be plotted and further analysis undertaken.

Figures 3.1, 3.2 and 3.3 present the specific research areas located within the three Welsh dune system sites, Morfa Harlech, Aberdyfi and Ynyslas, described in Chapter 2.

## 2.1 Phase 1 Study

All three study sites, identified in Chapter 2, were systematically walked and a Phase 1 survey assessment conducted as per the recommendations within the Joint Nature Conservation Committee Handbook for Phase 1 habitat survey (JNCC, 2003). The site-based mapping was undertaken to provide a relatively rapid record of the natural and semi-natural habitats found across the individual survey sites.

Each distinct unit or parcel of accessible land across and immediately adjacent to the research areas was visited and the vegetation types and features mapped on a set of Ordnance Survey MasterMap® at 1:1,250 scale. Standard record cards (as seen in Appendix B, Table B.3.1), which were based on the example provided within the Handbook for Phase 1 habitat survey (JNCC, 2003) were also utilised where required.

All habitat types and features encountered during the survey were highlighted on the maps using a combination of the alpha-numeric or letter codes, description and/or standard colour codes (see equipment, in Appendix B, Table B.3.2.) as detailed in the JNCC field manual section for Phase 1 habitat survey (JNCC, 2003).

Where access could not be gained to an area, satellite imagery was taken from Google Earth (utilised to identify the vegetation type associated with these features). It must be noted however, that some areas were impossible to accurately distinguish due to a number of factors (1) the age of the imagery (being surveyed by satellite between 12/09/2009 and 27/05/2010), (2) the quality of the imagery (pixilation due to low resolution satellite imagery), (3) the season of the year (imagery taken during the winter can be difficult to interpret due to the lack of vegetation cover) and/or

**Figure 3.1:** Morfa Harlech study area.

(4) time of day (shadows can greatly alter the look of vegetation and extent of vegetation area). In such cases a prediction of habitat type was made based on the surrounding habitat types and noted as such on the maps.

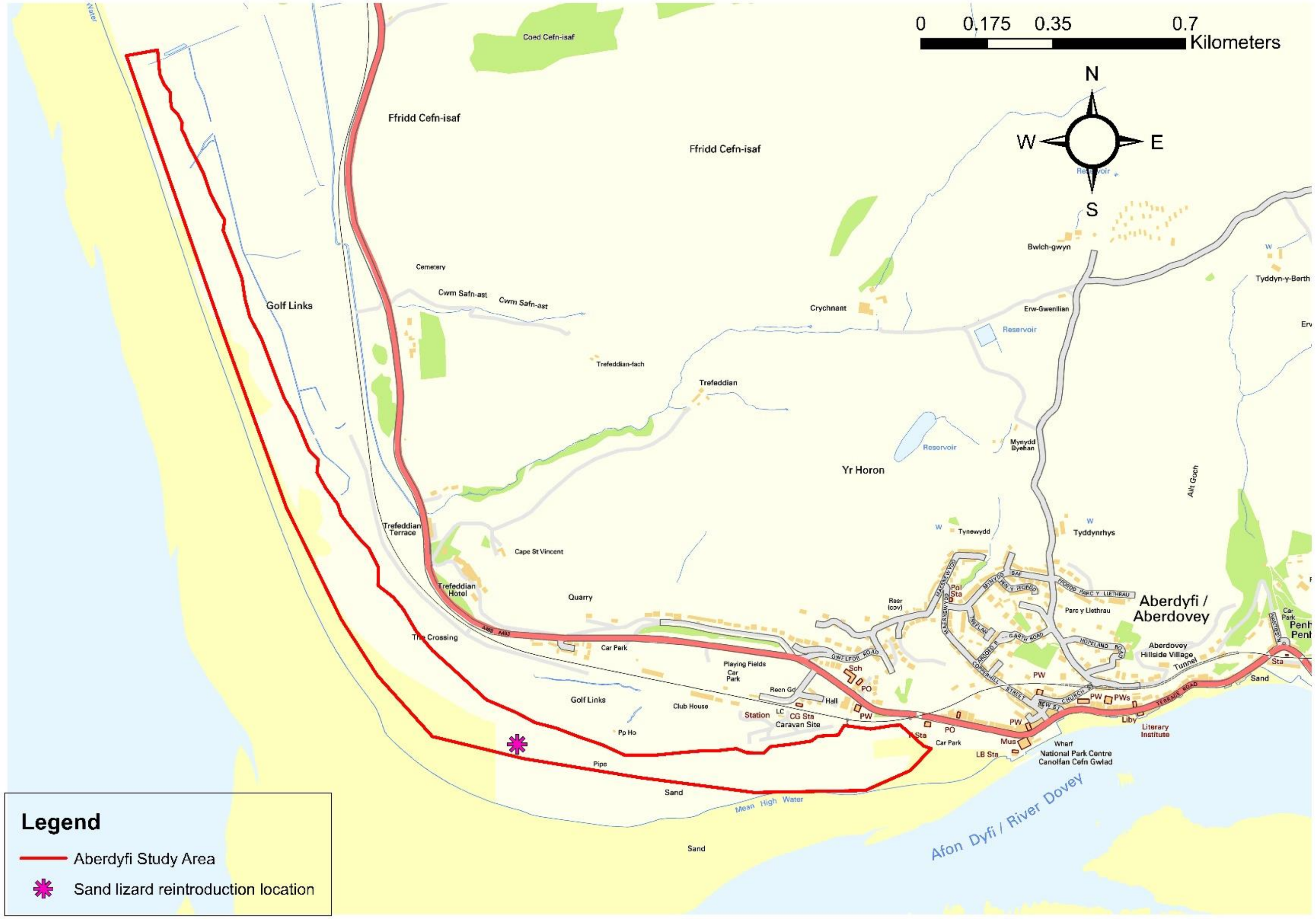


**Figure 3.2:** Aberdyfi study area.

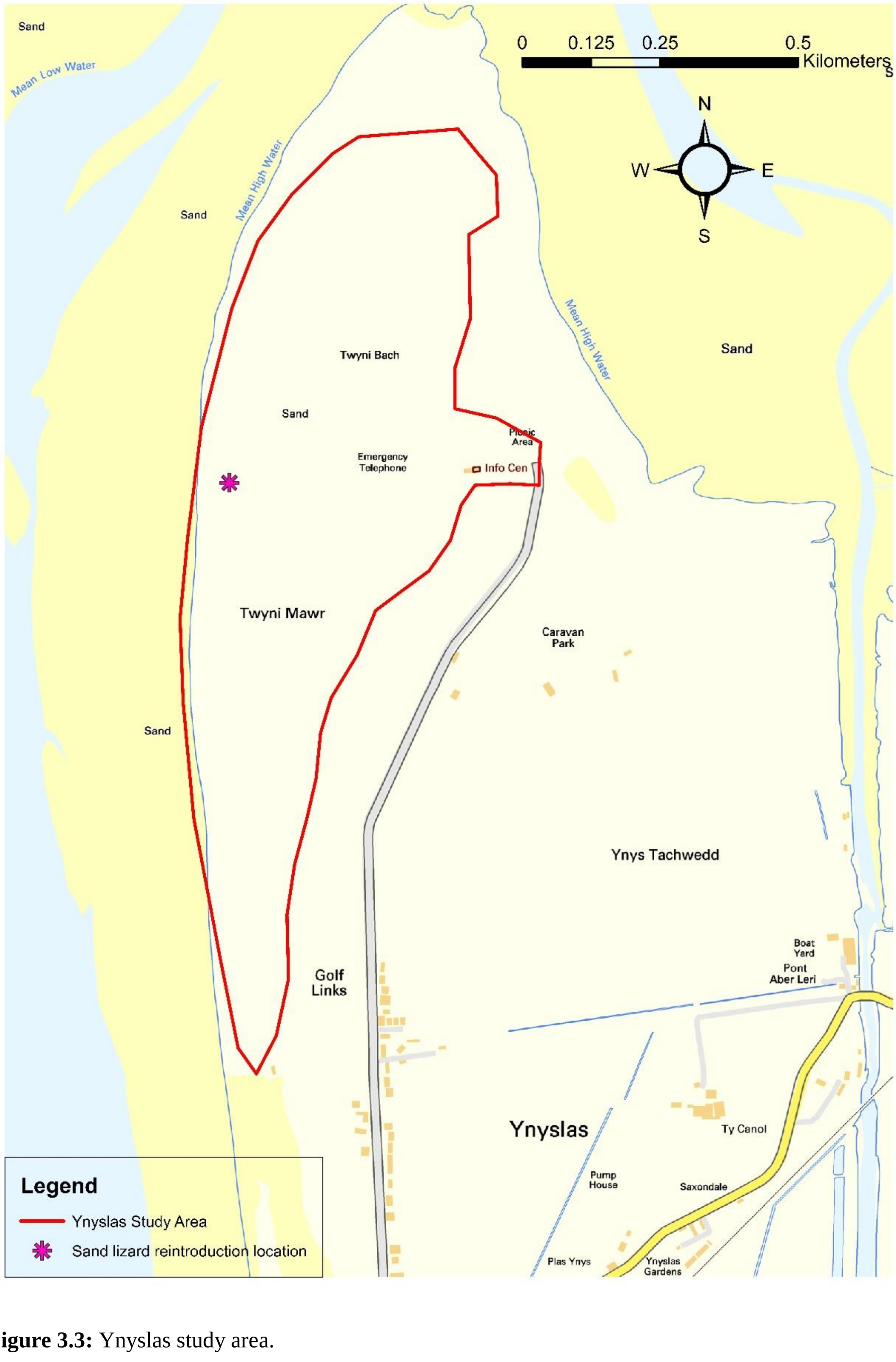


**Figure 3.3:** Ynyslas study area.

A number of descriptive 'target notes' were also included for areas where addition information of particular interest was deemed necessary to fully describe that particular habitat type, feature or observation.

## 2.2 Phase 2 study

The information and habitats identified throughout the Phase 1 surveys determined the extent of the study sites which warranted further investigation in the form of a ‘Phase 2’ survey. The Phase 2 surveys or National Vegetation Classification (NVC) were also conducted between late April 2013 and September 2015 adopting the principles detailed within the Sand dune vegetation survey of Great Britain: *A National inventory. Part 3: Wales* (Dargie, 1995), applying the method detailed in the 1995 sand dune vegetation survey of Great Britain. Whereas the NVC Manual for sand dunes (Rodwell, 2000) recommends that a minimum of five quadrats should be recorded from each stand type at each homogeneous stand, the 1999 sand dune vegetation survey of Great Britain ensured that one full quadrat was taken from each major stand type. Dargie’s (1995) survey methodology (used) comprised of three stages, as follows.

1) Field survey using results of Phase 1 survey and aerial photographs to map vegetation boundaries: With the use of the maps produced through the Phase 1 assessment of the sites and satellite imagery taken from ‘Google Earth’, the vegetation was divided into visually homogeneous stands.

2) Field analysis of vegetation according to NVC: On site, all visually homogeneous stands identified within the phase 1 study were identified and visited. All vegetative quadrat samples were recorded on record sheets (see Figure B.3.2), which were based on an example provided within from Rodwell (2006). Each sample was numbered and its location noted using site name and full eight figure grid reference. Where it was felt necessary, supplementary notes, and/or photos of the vegetation encountered were also taken/recorded to assist with interpretation of the data at a later date.

3) Analysis of NVC communities and cartography of results: This is explained further in the following sections.

At each stand type a typical sample area was selected and the vegetation contained within the quadrat recorded (JNCC, 1991a; Dargie, 1995). Suitably sized quadrats, either 2x2 m to sample areas of short herbaceous vegetation, or 4x4 m quadrats to sample areas of tall herbaceous and open vegetation (Rodwell, 2006), were used. For areas of vegetation smaller than the relevant sample size, Rowell’s (2006) methodology was adopted, whereby areas were recorded within their entirety, with mosaics treated as a single vegetation type.

**Table 3.1:** Summary of Domin scale/percentage cover utilised during the Phase 2 surveys across the three research sites.

| Domin | Cover |
|---|---|
| **10** | 91-100% |
| **9** | 76-90% |
| **8** | 51-75% |
| **7** | 34-50% |
| **6** | 26-33% |
| **5** | 11-25% |
| **4** | 4-10% |
| **3** | <4% (with many individuals) |
| **2** | <4% (several individuals) |
| **1** | <4% (few individuals) |

A quantitative measure of the abundance of every taxon was recorded using the Domin scale, shown in Table 3.1 and detailed in Dahl & Hadač (1941), with cover being assessed by eye as a vertical projection onto the ground of all the live, above-ground parts of the plant in the quadrat.

During survey, time was given to ensure the accuracy of scoring for all vascular plants and where possible bryophytes and macrolichens with all nomenclature for vascular plants followed that of Stace (1997, 2010). Some difficult bryophytes and crusts of lichen, even with the aid of reference material, had to be referred to just genus. It is also possible that some species may have been missed or misidentified and, with as much sampling taking place in summer, winter annuals and vernal perennials might have been missed on occasion.

### *Phase 1*

On completion of the site based survey assessment, an electronic version of the Ordnance Surveys (OS) MasterMap® was downloaded into ArcGIS ArcMap 10.3.1. (Esri, 2015). All habitat types and features encountered and mapped during the site based survey assessment were mapped in ArcGIS using the colour code detailed in the Handbook for Phase 1 habitat survey (JNCC, 2003) and supplied in AutoCAD format by the JNCC. The use of the colour codes on the final habitat map allows for a rapid visual assessment of the extent and distribution of different habitat types.

*Phase 2*

A blank copy of the site maps along with a copy of the final habitat maps produced from the Phase 1 survey, were used in the field to map out the extend of the individual homogenous stands. All habitat communities and features encountered and mapped during the Phase 2 survey assessment were also digitalised in ArcGIS ArcMap 10.3.1 using the list of National Vegetation Classification alphanumeric codes for community types commonly found on sand dunes, as shown in Rodwell (2000) and in Appendix B, Table B.3.4. Each group of communities is identified by a standard code. The code consists of three elements e.g. SD18b: NVC chapter code, i.e. SD for the sand dune and shingle chapter: Community number, i.e. 18, Sub-community letter, i.e. b.

*Lacertidae observations*

The data collected from lizard sightings (sand and viviparous lizards) were transferred from text sheets into an Excel spreadsheet, which was converted into Action Script Extension (.asc) files and imported directly into an ArcGIS database. Stored as discrete ‘points’ of data at precise moments in time, the data was transported as points into a GIS layer, and overlaid onto the Phase 1 maps.

## 2.3 Microhabitat

As detailed in Chapter 2, upon observation or capture of a lizard and following morphological, environmental data being recorded and DNA samples taken (in the case of sand lizards), microhabitat data was recorded at point of observation or capture.

Recording of microhabitat followed the methodology adopted for Phase 2/NVC surveys. Quadrats of 2x2 m were used for sampling area: suitable for areas of short herbaceous vegetation, as per Rodwell (2006). A quantitative measure of the abundance of every taxon was recorded using the Domin scale/percentage cover, shown in Table 3.1. Cover was assessed by eye as a vertical projection onto the ground of all the live, above-ground parts of the plant in the quadrat.

Further to those observations recorded during observation/capture of a lizard, the following environmental conditions relating to habitat utilisation were recorded: slope, direction of sun and wind direction.

# 3 Data Analysis

Environmental variables and habitat observations recorded during Lacertidae encounters were initially analysed and depicted within column/radar graphs. Both are a way of summarising data and represent a sample (Dythan, 2003; Field, 2009). The column/radar graphs were modelled in the statistic package ‘Excel’.

Sand lizard dispersal distances from reintroduction location(s), within each research site, were calculated. Measured from sand lizard observations, minimum and maximum dispersal distance were measured in ArcGIS ArcMap 10.3.1. From these measurements yearly ‘estimate’ dispersal distances from the reintroduction locations were estimated.

# 4 Modelling Habitat Utilisation

*Selection*

Lacertidae observations recorded during the survey of all three research sites were modelled using the Maximum Entropy Species Distribution Modelling package Maxent© (Version 3.3.3k). The software takes, as input, a set of layers or environmental variables, as well as a set of georeferenced observation locations, and produces a model of the range of the given species (Phillips, *et al*., 2010).

Initially, models for both sand and viviparous lizards were run utilising the ‘subsampling option’, whereby a proportion of the presence locations (25%) is set aside and used to test the model which was created based on the remaining 75% of the data. Initially, to produce several replicate runs of the model, the “random seed” option was applied to ensure that points apportioned as ‘test’ and ‘training’ were randomly selected for each model run (French *et al*., 2014).

To obtain the standard deviation of the individual model results, for the purposes of inspecting the reliability of individual model outputs, multiple model runs were performed utilising each set of environmental variables, so that the variation between their outputs could be assessed and the average of all runs obtained, as per the recommendations within French *et al.* (2014). While there is currently no published recommendation for the optimum number of model runs that should be selected, recommendations made by Phillips *et al*. (2006), Phillips & Dudik (2007) and the work undertaken by French *et al.* (2014), were followed, and 15 runs were completed.

Each model generated was formulated through analysing the ‘regularized training gain’, ‘test gain’, ‘percentage contribution’ and ‘importance’ outputs. This allowed for a backwards stepwise elimination of variables, whereas variables were removed and the reduced model re-run and re-checked for improvements until the optimal model was obtained (Elith *et al.*, 2011; French *et al.*, 2014).

Models generated in Maxent$^{©}$ were visualised in ArcGIS ArcMap 10.3.1. The visualised models were displayed utilising the ‘percent clip’ and ‘histogram equalize’ functions within the layer properties tab: stretch type. Percentage clip’ reduces the effects of outliers in the datasets, through cutting off a percentage of the minimum and maximum values defined. This visualisation represents the distribution of existing observations. ‘Histogram equalize’ redistributes the pixel values in an image. In contrast to ‘Percentage clip’, this visualisation presents a predicted distribution of based on the presence of statistically similar habitats present across the site.

*Interpretation*

Initially, inspection of the lambdas file (output by Maxent) for each individual model was undertaken by assessing the AUC values. The AUC value represents the ‘Area Under the Curve’ of a ‘Receiver Operating Characteristic’ graph, and is a threshold independent measure of the predictive power of a model. It displays how well the Maxent model predicts true presence background data (sometimes referred to as pseudo-absences) as per the methodology described in French *et al.* (2014). Merow *et al.* (2013) and French *et al.* (2014) discussed the use of the AUC value to evaluate model performance. While this has been questioned (Lobo *et al.*, 2008), there is a lack of suitable alternatives for non-threshold dependent model assessment, and AUC often provides the most conservative estimate of suitable habitat, appropriate for conservation planning. To ensure the most appropriate model and subsequent distribution maps were chosen and validate the model identified from the AUC outputs, secondary analysis using the ‘model selection’ function in ENMTools was undertaken. The ENMTools ‘model selection’ function allows criterion-based model selection using AIC and AICc (Warren & Seifert, 2011; Burnham & Anderson, 2002).

AIC and AICc are both measures of the relative quality of the models generated for a given set of data. Similar to BIC, described in Chapter 2, individual AIC and AICc values are not interpretable (as they contain arbitrary constants) and are greatly affected by sample size. The values provide a means for model selection and should be compared to other AICc values generated from further models, selecting the model with the smallest criterion value.

# 5 Results

## 5.1 Micro-habitat utilisation

As detailed in Chapter 2 a total of 1,364 dune system Lacertidae lizards were observed across the three sites: Morfa Harlech, Aberdyfi or Ynyslas. Of these observations, 237 (17%) were sand lizards and 1,059 (78%) were viviparous lizards. Of the 1,364 Lacertidae lizards observed, the micro-habitat at point of observation/capture was recorded for 1,091 lizards: 231 (21.2%) of which were sand lizards and 860 (78.8%) viviparous lizards. Micro-habitat structure utilised by both basking sand and viviparous lizards from a North-West Wales perspective is provided in Figure 3.4.

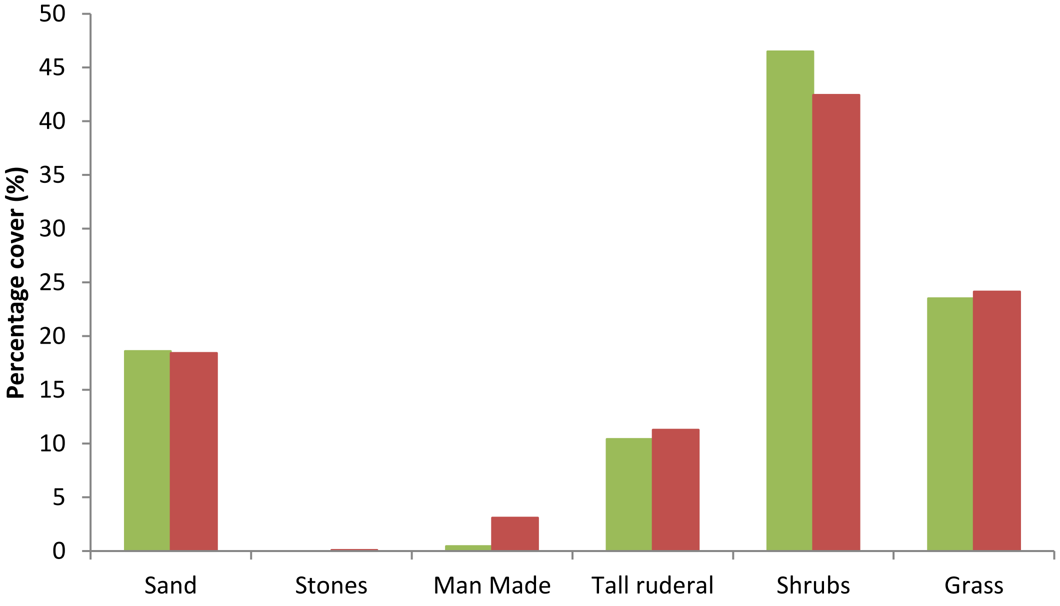


**Figure 3.4:** Percentage usage of habitat elements associated with sand and viviparous lizard observations across the three research sites between March 2010 and October 2014. n = 1091. Olive green represents sand lizards and red represents viviparous lizards.

The composition of habitat elements utilised by both species across the three research sites (NWW) was assessed and broadly described in relation to structure and height as basking sites are typically found in transition areas between high and low or sparse vegetation (House & Spellerberg, 1983; Blab *et al.*, 1991) or in the gaps of shrubs (Blanke & Fearnley, 2015).

Both species were found to utilise habitats of a similar composition with sand making up 18.4% to 18.6%, tall ruderal between 10.4% and 11.2% (vegetation of a typical height between 30cm – >100cm), grass between 23.5% to 24.2% (including marram) and shrubs between 42.4 and 46.5% (vegetation of a typical height between 5 cm – 30 cm). Stones and manmade objects (fence posts, plastic and unwanted lingerie) make up the remaining 0.46% – 3.1%. A list of species associated with each applicable habitat element is provided in Appendix B, Table B 3.5.

A further breakdown of the habitat composition utilised by both species for the three individual sites are provided in Appendix B, Fig. B.1; Morfa Harlech, Fig: B.2; Aberdyfi and Figure B.3; Ynyslas. Analysis for the three sites identified a similar compositional structure utilised to that across North West Wales, irrespective of sample size, indicating that these provide 'optimal dune system structure' at a high level of resolution.

Figure 3.5 provides a visual representation of the habitat types found across the Morfa Harlech research site, between late April 2013 AD and September 2015 AD. The figure is generated from areas identified during the targeted Phase 1 walkover surveys. A visual representation of the habitat types found across the Aberdyfi and Ynyslas research sites can be found in Appendix B, Figure B.4 and B.5.

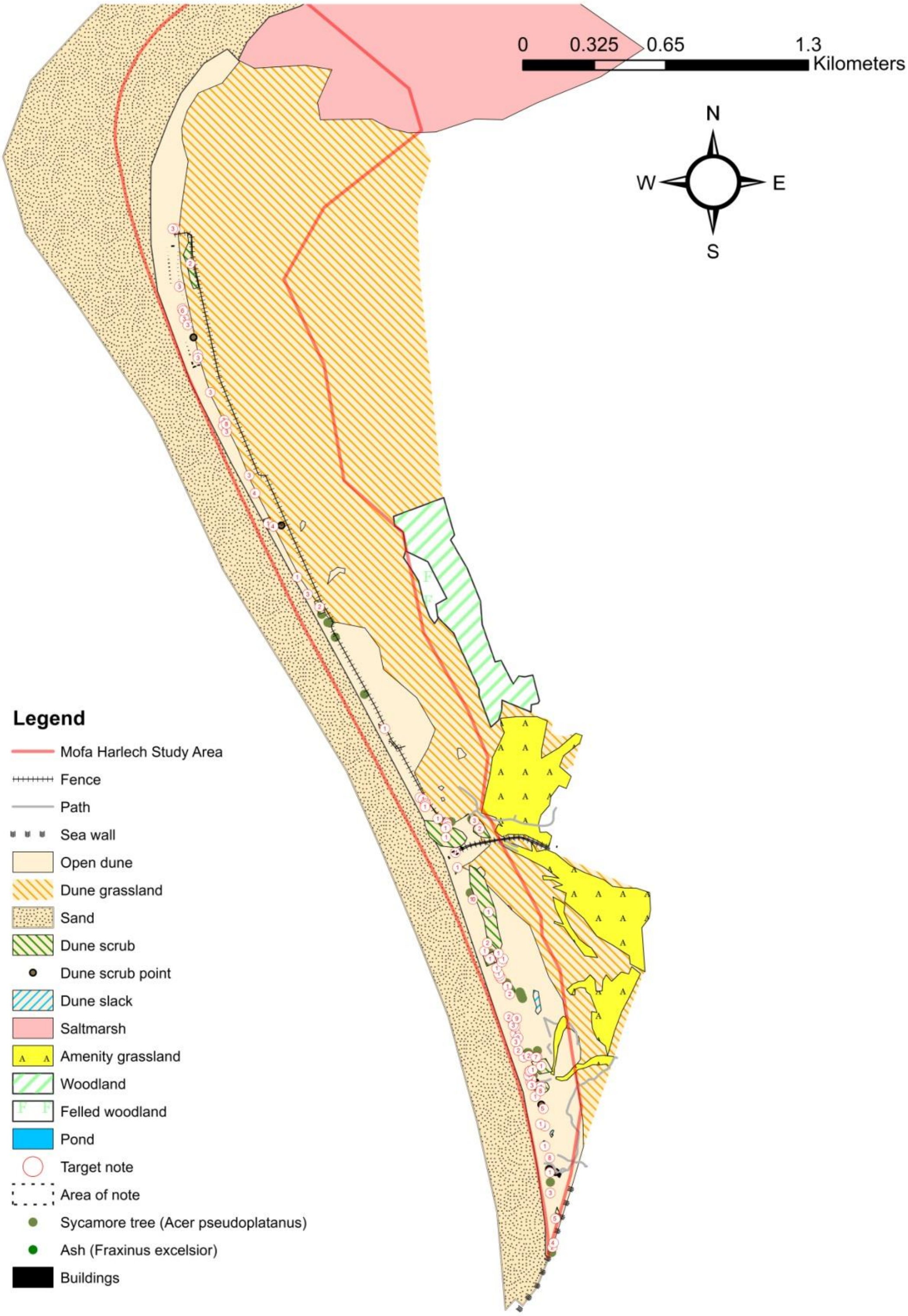


**Figure 3.5:** Morfa Harlech Phase 1 study map.

## 5.2 Habitat Summaries

The Phase 1 surveys identified the following habitat types/points of note as being present within or immediately adjacent to the survey sites study areas:

A1: Woodland
A4: Felled woodland
G1: Pond
H1.2: Intertidal shingle/cobbles
H2: Saltmarsh
H4: Shingle above high tide mark
H6.4: Dune slack
H6.5: Dune grassland
H6.7: Dune scrub and dune scrub point
H6.8: Open Dune
J1.2: Amenity grassland
J2.4: Fence
J3.4: Caravan site
J3.5: Sea wall
J3.6: Buildings/paths/roads
J5: Historical dune extent
J5: Sand

Habitats identified during the Phase 1 surveys, along with a yearly evaluation of Lacertidae lizard observations were utilised to identify those areas subject to the detailed NVC surveys. Figures 3.6-3.9 provides a visual representation of the detailed habitat types found across the Morfa Harlech research site between late April 2013 AD and September 2015 AD. The colour-coded habitats present within the study area should be read in conjunction with Table 3.2 (National Vegetation Classification legend).

A visual representation of the habitat types found across the Aberdyfi and Ynyslas research sites can be found in Appendix B, Figure B6 and B7(a - d).

**Figure 3.6:** Morfa Harlech National Vegetation Classification study map. To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

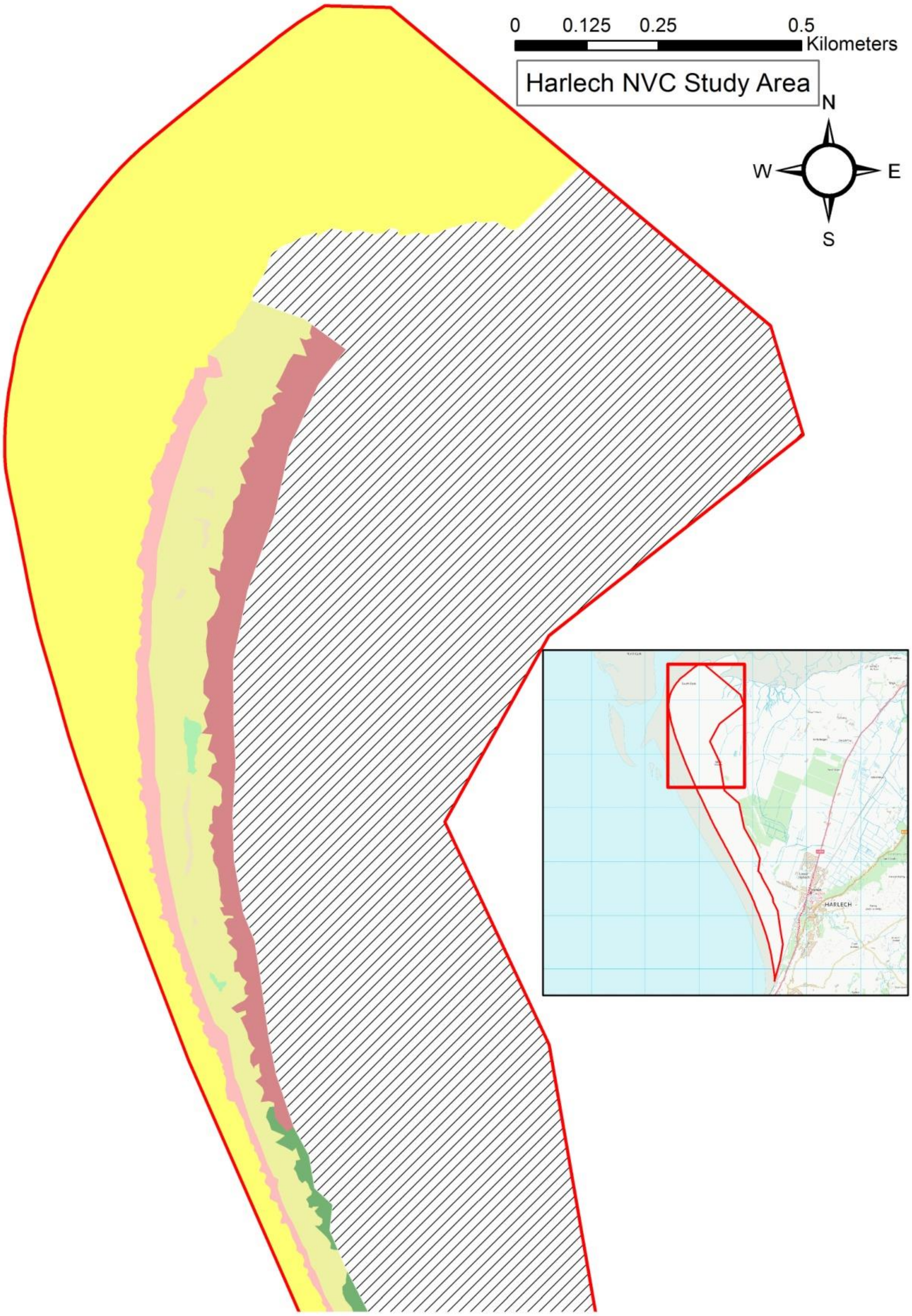


**Figure 3.7:** Detailed section of Morfa Harlech National Vegetation Classification study map (Northern section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

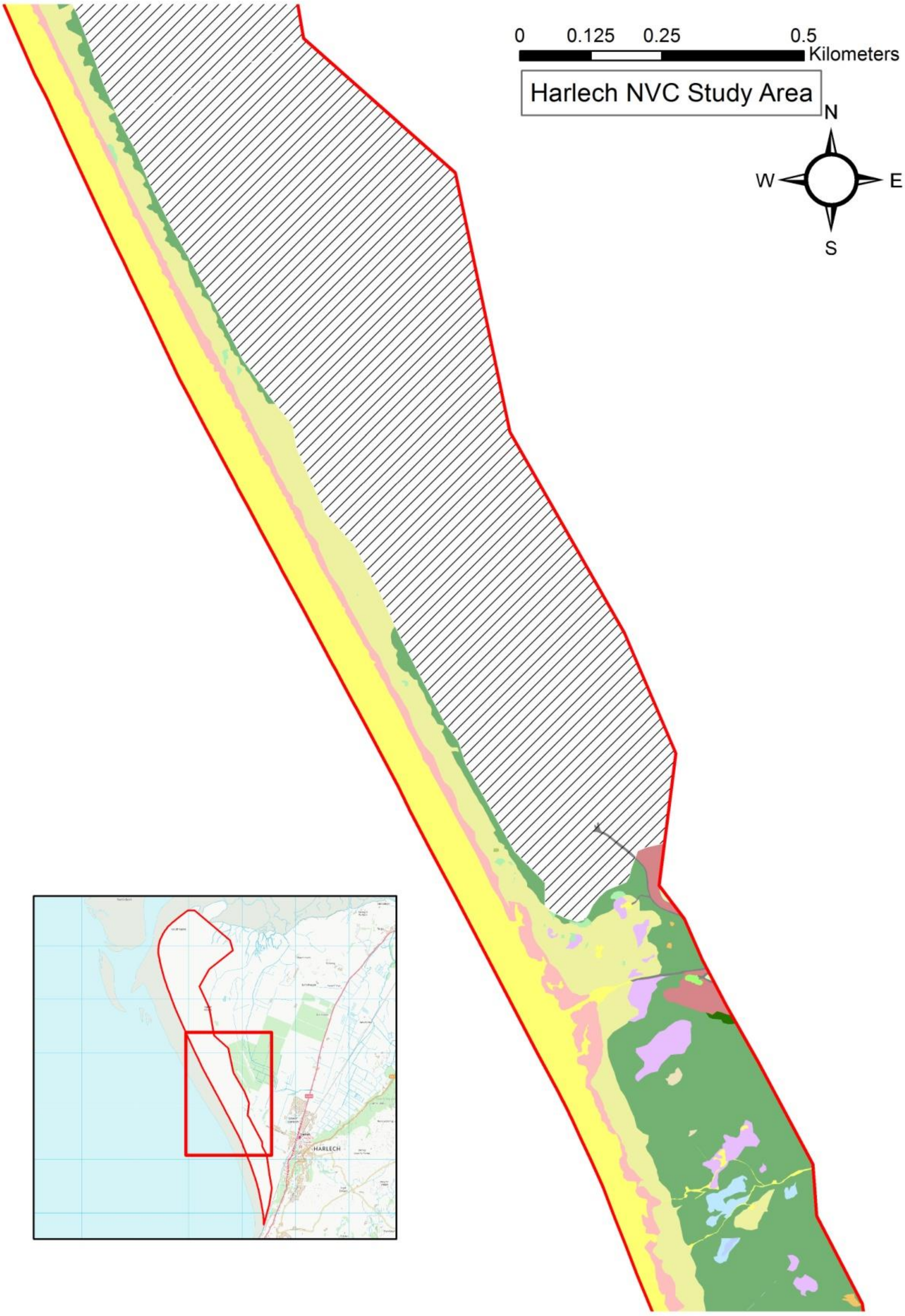


**Figure 3.8:** Detailed section of Morfa Harlech National Vegetation Classification study map (Central section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

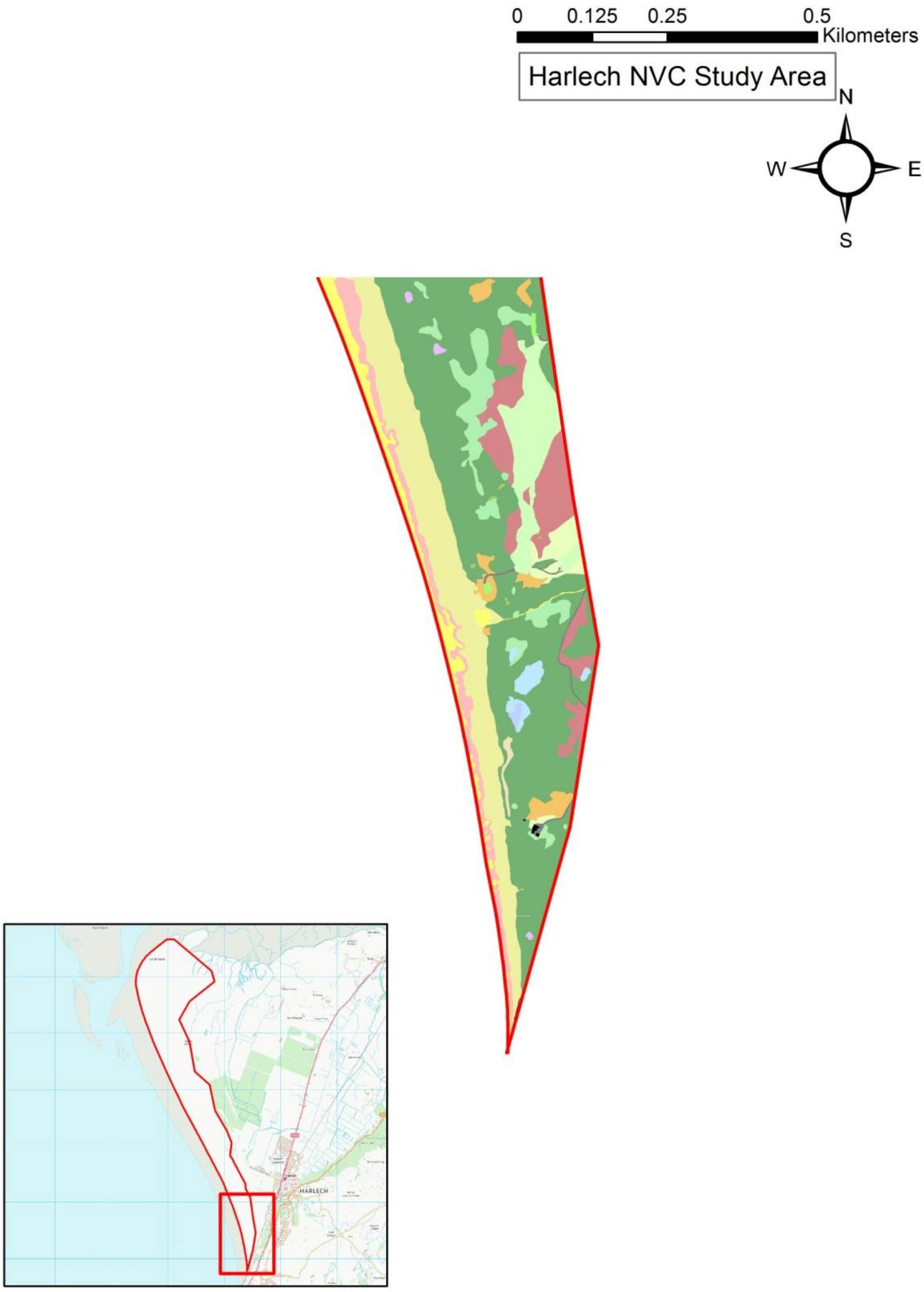


**Figure 3.9:** Detailed section of Morfa Harlech National Vegetation Classification study map (Southern section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

**Table 3.2:** National Vegetation Classification legend (– indicates no code available).

| Colour code | Alphanumeric Code | Description |
|---|---|---|
| **General** | | |
| — | - | Site study area |
| 🟨 | - | Bare sand |
| ⠿ | - | Boulders |
| ⬛ | - | Building |
| 🟩 | - | Golf tee |
| 🟩 | - | Green on golf course |
| ▒ | - | Shingle |
| ▓ | - | Road or track |
| —+— —+— | - | Fence |
| ▨ | - | No data |
| **Mobile dunes** | | |
| 🟥 | SD6a | Ammophila arenaria mobile dune, *Elymus farctus* sub-community. |
| | SD6d | *Ammophila arenaria* mobile dune, typical sub-community. |
| | SD6e | *Ammophila arenaria* mobile dune, *Festuca rubra* sub-community. |
| **Semi-fixed dunes** | | |
| 🟨 | SD7a | *Ammophila arenaria-Festuca rubra* semi-fixed dune, typical sub-community. |
| | SD7b | *Ammophila arenaria-Festuca rubra* semi-fixed dune, *Hypnum cupressiforme* sub-community. |
| | SD7c | *Ammophila arenaria-Festuca rubra* semi-fixed dune, *Ononis repens* sub-community. |
| | SD7d | *Ammophila arenaria-Festuca rubra* semi-fixed dune, *Tortula ruralis* ssp. *ruraliformis* sub-community. |
| **Dune grassland** | | |
| 🟥 | SD8a | *Festuca rubra-Galium verum* fixed dune grassland, typical sub-community. |
| | SD8b | *Festuca rubra-Galium verum* fixed dune grassland, *Luzula campestris* sub-community. |
| | SD8d | *Festuca rubra-Galium verum* fixed dune grassland, *Bellis perennis-Ranunculus acris* sub-community. |
| | SD8e | *Festuca rubra-Galium verum* fixed dune grassland, *Prunella vulgaris* sub-community. |
| 🟩 | SD9a | *Ammophila arenaria-Arrhenatherum elatius* dune grassland, typical sub-community. |
| | SD9b | *Ammophila arenaria-Arrhenatherum elatius* dune grassland, *Geranium sanguineum* sub-community. |

**Table 3.2:** Cont over page

**Table 3.2:** Cont.

| Colour code | Alphanumeric Code | Description |
|---|---|---|
| **Dune grassland** | | |
| 🟥 | SD10 | Carex arenaria dune community |
| 🟪 | SD11 | Carex arenaria-Cornicularia aculeate dune community |
| 🟨 | SD12a | *Carex arenaria-Festuca ovina-Agrostis capillaris* grassland, *Anthoxanthum oderatum* sub-community. |
| | SD12b | *Carex arenaria-Festuca ovina-Agrostis capillaris* dune grassland, *Holcus lanatus* sub-community. |
| **Wet heaths and mires** | | |
| 🟩 | M23 | *Juncus effuses/acutiflorus-Galium palustre* rush pasture, undifferentiated. |
| **Dune slacks** | | |
| 🟦 | SD16a | *Salix repens-Holcus lanatus* dune slack, *Ononis repens* sub-community. |
| | SD16b | *Salix repens-Holcus lanatus* dune slack, *Rubus caesius* sub-community. |
| 🟨 | SD17b | *Potentilla anserine-Carex nigra* dune slack, *Carex flacca* sub-community. |
| | SD17c | *Potentilla anserine-Carex nigra* dune slack, *Caltha palustris* sub-community. |
| | SD17d | *Potentilla anserine-Carex nigra* dune slack, *Hydrocotyle vulgaris-Ranunculus flammula* sub-community. |
| **Swamp and tall-herb fens** | | |
| 🟦 | S20 | *Scirpus lacustris* ssp. *tabernaemontani* swamp, *Agrostis stolonifera* sub-community. |
| **Scrub and woodland** | | |
| 🟧 | SD18a | *Hippophae rhamnoides* scrub, *Festuca rubra* sub-community. |
| 🟩 | W8 | *Fraxinus excelsior-Acer campestre-Mercurialis perennis* woodland, undifferentiated. |
| 🟧 | W22 | *Prunus spinosa-Rubus fruticosus* agg. Scrub, |
| 🟩 | W23b | *Ulex europaeus-Rubus fruticosus* agg. Scrub, *Rumex acetosella* sub-community. |
| 🟩 | W24 | *Rubus fruticosus agg.-Holcus lanatus* underscrub. |

With Blanke and Fearnley (2015) having identified the importance of ‘ideal sand lizard habitat being described primarily by its structural diversity’, the NVC habitats identified during survey were mapped by ‘community’. Furthermore, an absence of Lacertidae lizard observations within a proportion of the Morfa Harlech research site resulted in this being omitted from NVC survey and subsequent mapping (area indicated by hatched area on associated mapping).

Providing a ‘base map’ onto which Lacertidae lizard observations can be modelled, Table 3.3 presents a breakdown of the detailed habitat types identified across the Morfa Harlech, Aberdyfi and Ynyslas research areas during the NVC survey between late April 2013 and September 2015. A breakdown of the area ($m^2$) and percentage coverage for each habitat type represented is also provided.

**Table 3.3:** Habitat type areas ($m^2$) and percentage coverage present across the three research sites identified through the NVC survey (– indicates the absence of that habitat type).

| . | Research site | | | | | |
|---|---|---|---|---|---|---|
| Habitat type | Harlech | | Aberdyfi | | Ynyslas | |
| | $m^2$ | % | $m^2$ | % | $m^2$ | % |
| Bare sand | 676653 | 44.128 | 67001 | 16.753 | 66299 | 12.363 |
| Boulders | 123 | 0.008 | - | - | - | - |
| Building | 170 | 0.011 | 120 | 0.030 | 285 | 0.053 |
| Golf Tea | 882 | 0.058 | 1232 | 0.308 | 72 | 0.013 |
| Green on golf course | 18809 | 1.227 | 27921 | 6.981 | - | - |
| Shingle | - | - | 7135 | 1.784 | 16029 | 2.989 |
| Road or track | 2827 | 0.184 | 2144 | 0.536 | 2043 | 0.381 |
| Fence | - | - | - | - | - | - |
| No data | 1719591 | N/A | - | - | - | - |
| SD6 Mobile dunes | 91949 | 5.996 | 47483 | 11.873 | 31344 | 5.845 |
| SD7 Semi-fixed dunes | 310025 | 20.218 | 114242 | 28.566 | 114074 | 21.271 |
| SD8 Dune grassland | 100949 | 6.583 | - | - | 5021 | 0.936 |
| SD9 Dune grassland | 275557 | 17.970 | 98645 | 24.666 | 248593 | 46.355 |
| SD10 Dune grassland | - | - | 707 | 0.177 | 788 | 0.147 |
| SD11 Dune grassland | 15296 | 0.998 | 9405 | 2.352 | 24429 | 4.555 |
| SD12 Dune grassland | 5171 | 0.337 | 13421 | 3.356 | 12001 | 2.238 |

**Table 3.3:** Cont over page

**Table 3.3:** Cont.

| . | Research site | | | | | |
|---|---|---|---|---|---|---|
| Habitat type | Harlech | | Aberdyfi | | Ynyslas | |
| | m$^2$ | % | m$^2$ | % | m$^2$ | % |
| M23 Wet heaths and mires | - | - | - | - | 518 | 0.097 |
| SD16 Dune slacks | 7154 | 0.467 | - | - | 246 | 0.046 |
| SD17 Dune slacks | 3831 | 0.250 | - | - | - | - |
| S20 Swamp and tall-herb fens | 843 | 0.055 | - | - | - | - |
| SD18 Scrub and woodland | - | - | 5385 | 1.346 | - | - |
| W8 Scrub and woodland | 618 | 0.040 | - | - | - | - |
| W22 Scrub and woodland | 3397 | 0.222 | 2353 | 0.588 | 5312 | 0.991 |
| W23 Scrub and woodland | 302 | 0.020 | - | - | - | - |
| W24 Scrub and woodland | 18847 | 1.229 | 2735 | 0.684 | 9223 | 1.720 |

Observations of Lacertidae lizards between March 2010 and October 2014 are visually presented in Figures 3.10, 3.11 and 3.12. Mapping utilises baseline Phase 1 maps (due to ease of visual interpretation), however, NVC data informed site habitat utilisation modelling.

A visual representation of Lacertidae lizard observations between March 2010 and October 2014 found across the Aberdyfi and Ynyslas research sites can be found in Appendix B, Fig. B8 and B9(a – c).

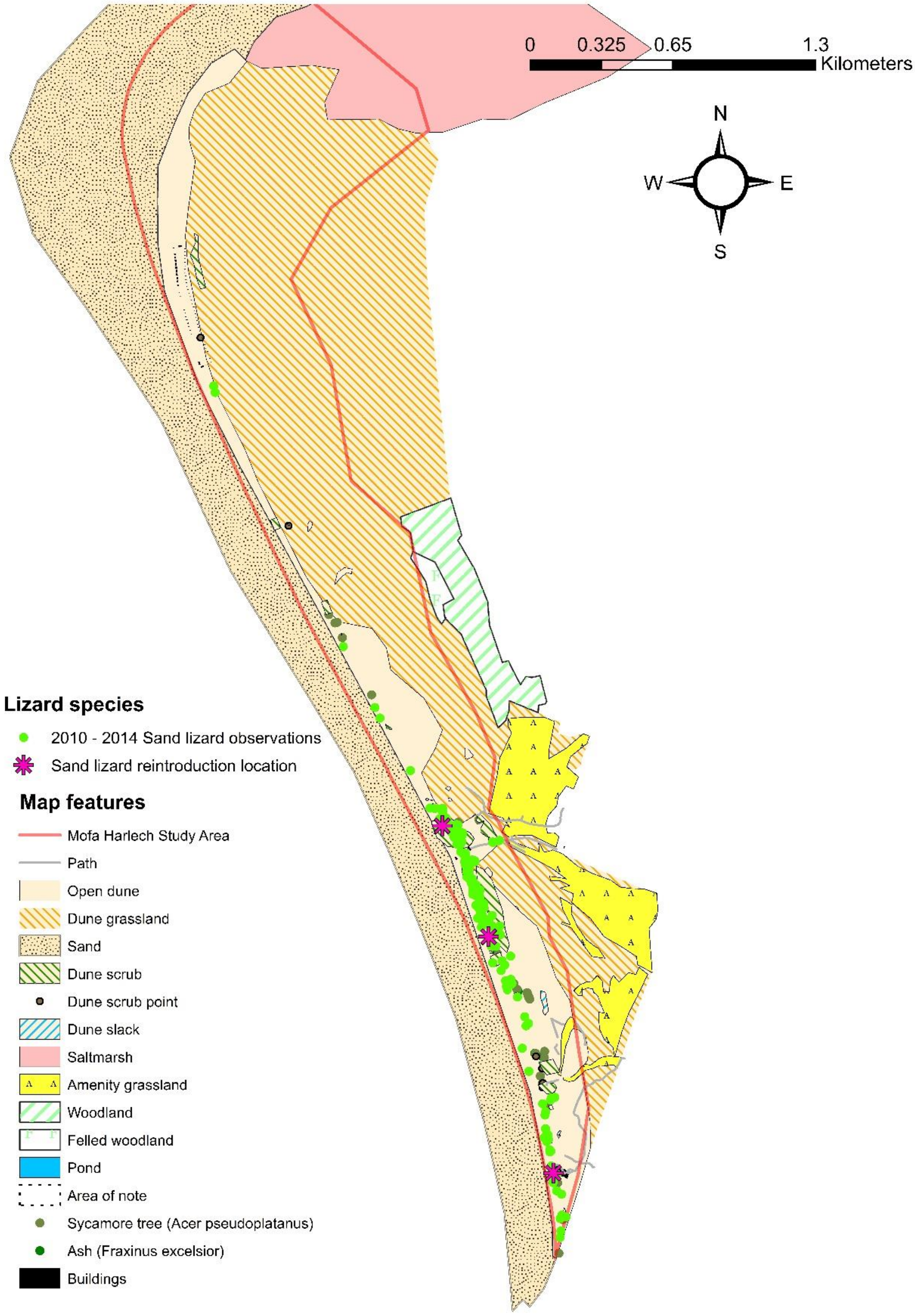


**Figure 3.10:** Visual representation of 2010 – 2012 sand lizard observations across Morfa Harlech study area.

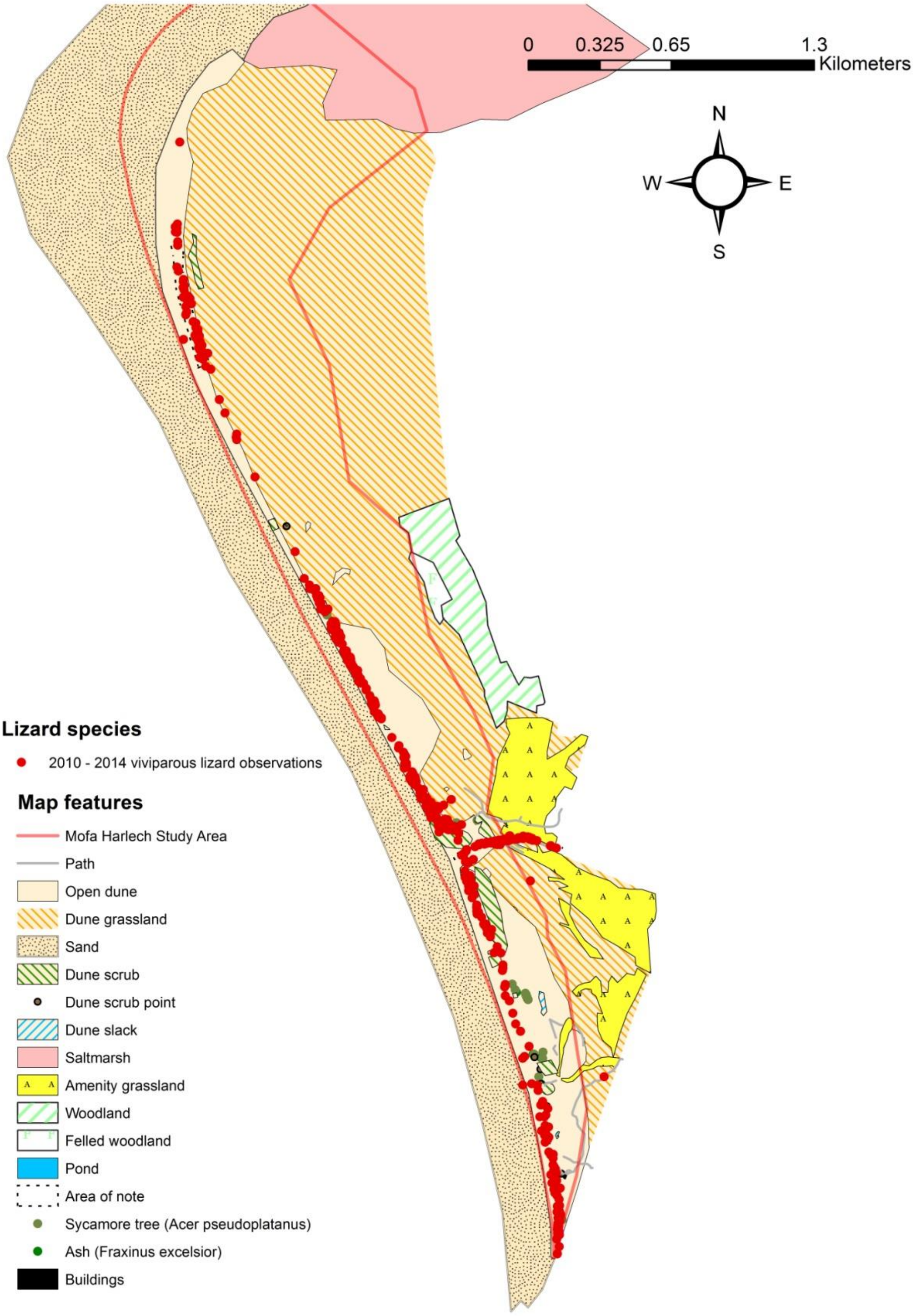


**Figure 3.11:** Visual representation of 2012 – 2014 viviparous lizard observations across Morfa Harlech study area.

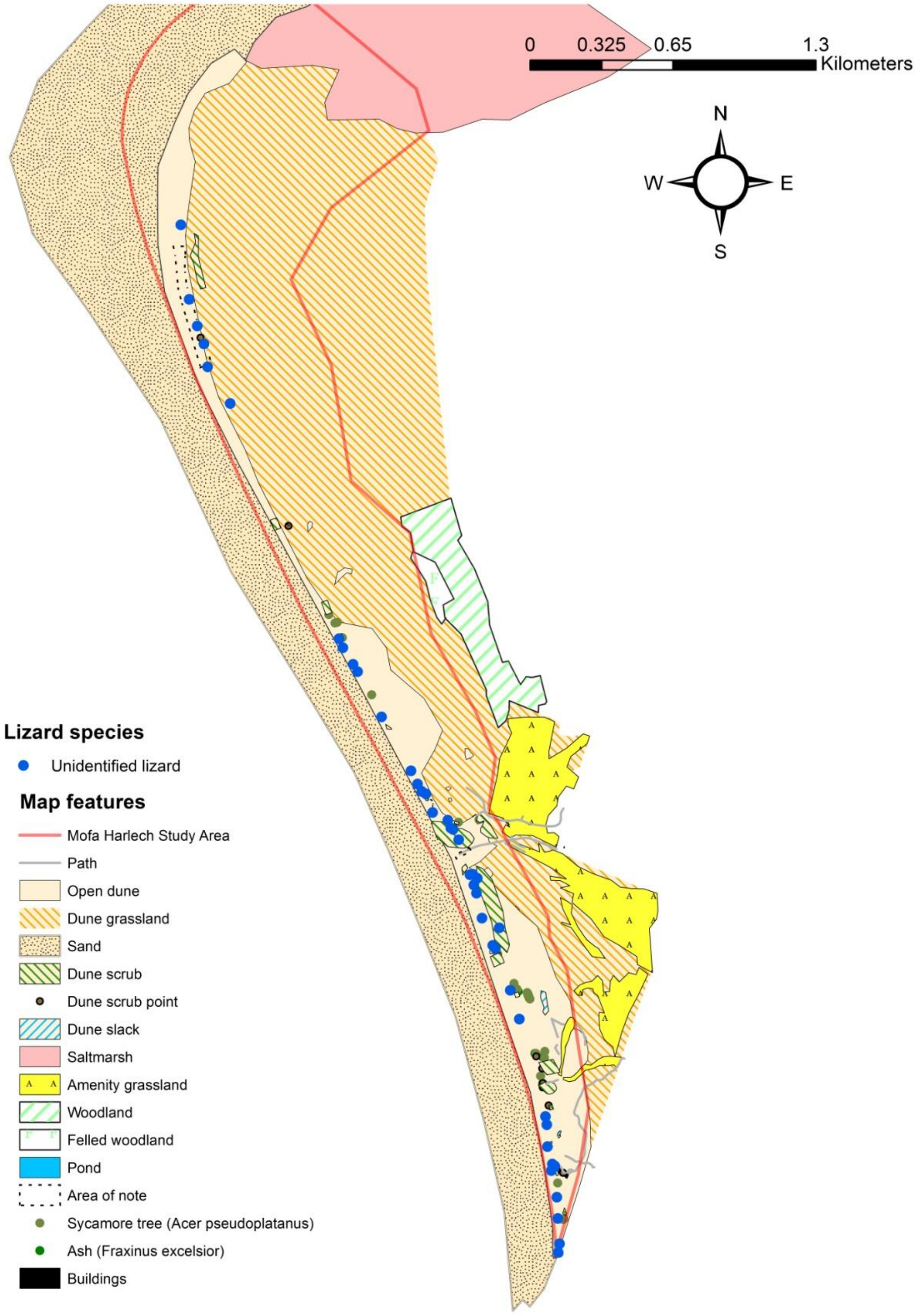


**Figure 3.12:** Visual representation of 2012 – 2014 unidentified lizard observations across Morfa Harlech study area.

### 5.3 Site utilisation modelling

Site utilisation models were developed for the three research sites for both sand and viviparous lizards. A single model based on current utilisation of the sites by each species is presented along with a model indicating areas of forecasted suitable habitat and therefore areas which are important for both species.

Figure 3.13 provides a visual representation of the current observed habitat utilisation and distribution of sand lizards across the Morfa Harlech site. Figure 3.14, also of Morfa Harlech, provides an indication of modelled suitable habitat and therefore areas which may be utilised by sand lizards. Representing the lowest AIC score: 5,745.87 (sample size 233, parameters 45.6, Log likelihood -2,827.33) five habitats or habitat features were identified as playing a significant part in the sand lizards modelled habitat selection. Table 3.4, provides a list of the identified habitats along with their measure of fit. Similar models for the Aberdovey and Ynyslas research sites are shown in Figures 3.17, 3.18, 3.21 and 3.22. These models too represent the lowest AIC scores, identifying four or five habitats or habitat features playing a significant part in the sand lizards modelled habitat selection (Aberdyfi AIC 1,019.63, sample size 50, parameters 15.6, Log likelihood -494.15. Ynyslas AIC 998.74, sample size 45, parameters 24.2, Log likelihood -475.17). These too have similarly high AUC values: Aberdyfi 0.980, Ynyslas 0.972. Habitats identified as playing a significant part in the sand lizard modelled habitat selection, include SD6 marram mobile dune (Aberdyfi PC 7.6%, PI 4.6%), SD7 marram fixed dune (Aberdyfi PC 10.3%, PI 1.3%; Ynyslas PC 31.6%, PI 10.4%) and terrain (Aberdyfi PC 17.9%, PI 2.9%; Ynyslas 23.1%, PI 9.6%: similar to those identified at Harlech. Further habitats or habitat features: SD8 red fescue grassland (Ynyslas PC 23%, PI 54.5%), W8 ash tree (Aberdyfi PC 64.2%, PI 91.2%) and W22 blackthorn and bramble (Ynyslas PC 22.4%, PI 25.4%).

Figures 3.15 and 3.16 provide similar maps for areas which are/may be utilised by viviparous lizards at Morfa Harlech. Representing the lowest AIC score: 16,090.20 (sample size 641, parameters 144, Log likelihood -7,901.1), all 24 habitat parameters were selected. Table 3.4 provides a list of those habitats with the highest permutation importance along with their measure of fit.

**Table 3.4:** Habitat type, percentage contribution and permutation importance identified for the Morfa Harlech site.

| Site | Species | Habitat | Percent Contribution (PC) (%) | Permutation Importance (PI) (%) | AUC | Standard deviation |
|---|---|---|---|---|---|---|
| Harlech | Sand lizard | SD7 marram fixed dune | 36.9 | 55 | 0.976 | 0.016 |
| | | Boulders | 35.8 | 11.7 | | |
| | | Terrain | 8.7 | 8.9 | | |
| | | SD6 marram mobile dunes | 7.5 | 9 | | |
| | | SD11 sand sedge and mosses | 11.2 | 15.3 | | |
| | Viviparous lizard | SD7 marram fixed dune | 31 | 47.3 | 0.961 | 0.006 |
| | | SD6 marram mobile dunes | 11 | 5.9 | | |
| | | SD17 *Carex nigra* dune slack | 10.5 | 2.3 | | |

Figures 3.19, 3.20, 3.23 and 3.24 provide ‘current observed habitat utilisation and distribution’ and ‘modelled suitable habitat’ maps for areas which are/may be utilised by viviparous lizards at Aberdyfi and Ynyslas. Unlike at Morfa Harlech, only five or six habitats or habitat features were identified as playing a significant part in the viviparous lizard modelled habitat selection at Aberdyfi and Ynyslas (Aberdyfi AIC 4,127.31, sample size 180, parameters 59, Log likelihood -2,004.66; Ynyslas AIC 5,596.09, sample size 229, parameters 64, Log likelihood -2,734.05). Habitats identified as playing a significant part in the sand lizards modelled habitat selection across these two sites include SD7 marram fixed dune (Aberdyfi PC 10.8%, PI 18.2%; Ynyslas PC 36.9%, PI 34.8%) SD12 (Aberdyfi PC 24.2%, PI 42.8%; Ynyslas PC 22.9%, PI 9.8%), road/track (Aberdyfi PC 11.5%, PI 14.8%; Ynyslas PC 12.7, PI 9.1%) some of which are similar to those identified at Harlech. Further habitats or habitat features: SD8 red fescue grassland (Ynyslas PC 24.1%, PI 33.5%), M23 common rush and Yorkshire fog (Ynyslas PC 10.2%, PI 5%) and SD18 seaberry and grassland (Aberdyfi PC 16.4%, PI 19.1%).

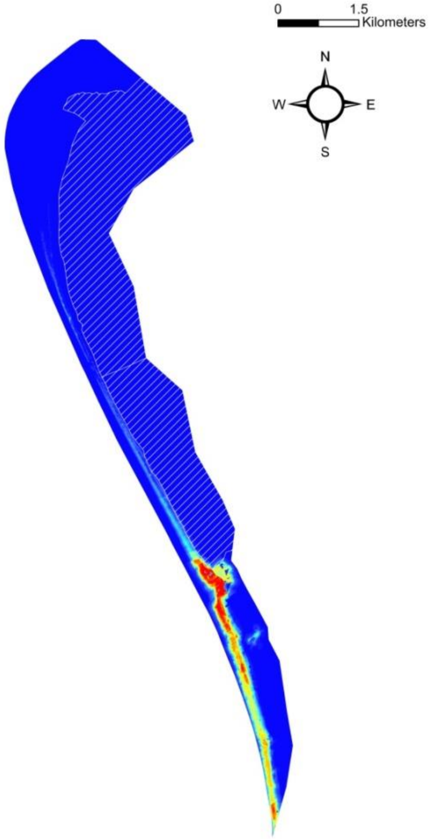


**Figure 3.13:** Current *L. agilis* habitat utilisation map of Morfa Harlech produced in Maxent. Warm colours indicate current observation localities and therefore areas which are more important for *L. agilis*. Cooler colours indicate a lower observed density and therefore areas which were observed to be less important for *L. agilis*.

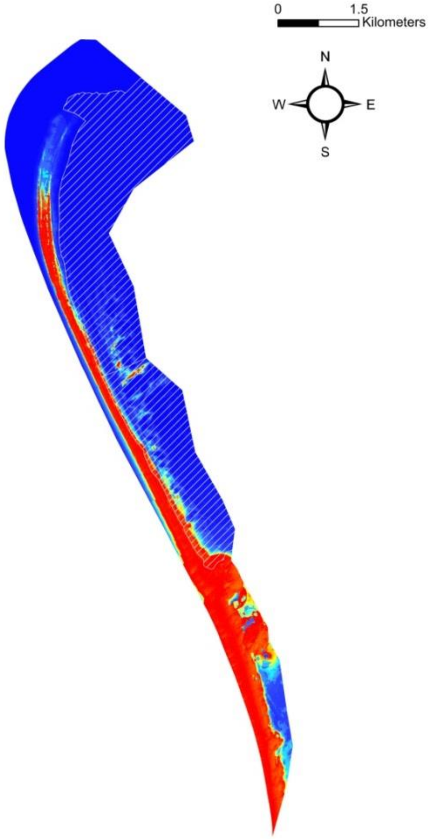


**Figure 3.14:** Habitat suitability map of Morfa Harlech produced in Maxent. Warm colours indicate areas of forecasted suitable suitable habitat and therefore areas which are important for *L. agilis*. Cooler colours indicate areas of less suitable habitat for *L. agilis*.

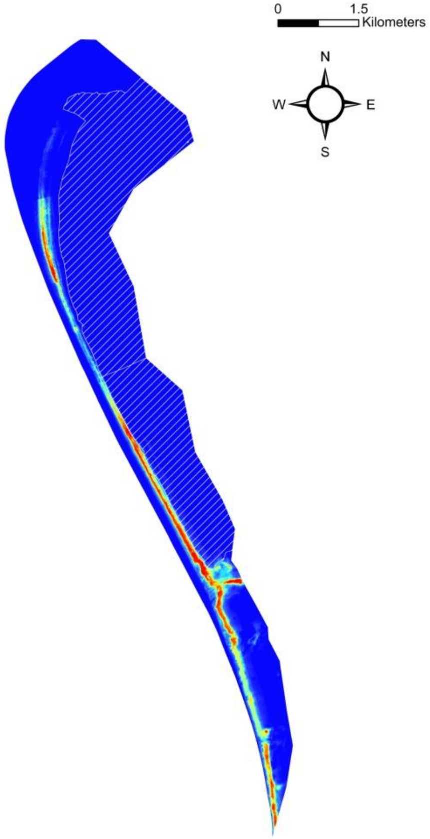


**Figure 3.15:** Current *Z. vivipara* habitat utilisation map of Morfa Harlech produced in Maxent. Warm colours indicate current observation localities and therefore areas which are more important for *Z. vivipara*. Cooler colours indicate a lower observed density and therefore areas which were observed to be less important for *Z. vivipara*.

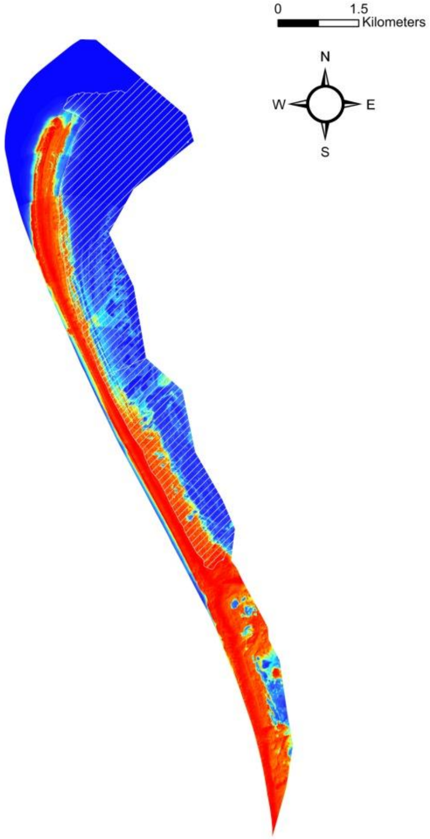


**Figure 3.16:** Habitat suitability map of Morfa Harlech produced in Maxent. Warm colours indicate areas of forecasted suitable suitable habitat and therefore areas which are important for *Z. vivipara*. Cooler colours indicate areas of less suitable habitat for *Z. vivipara*.

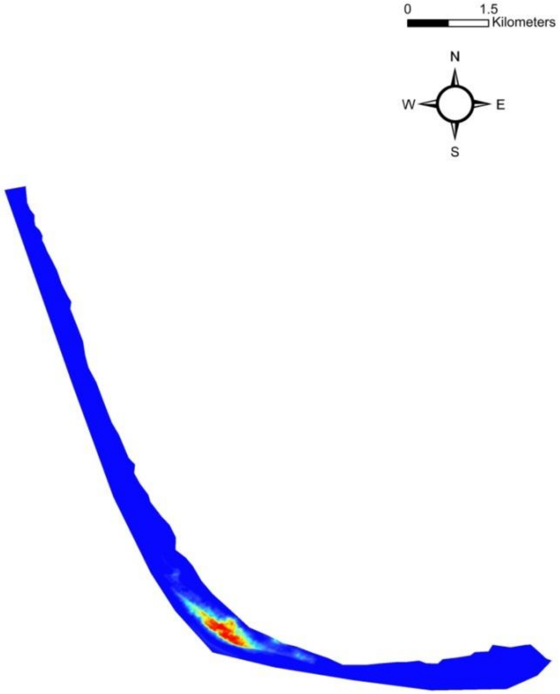


**Figure 3.17:** Current *L. agilis* habitat utilisation map of Aberdyfi produced in Maxent. Warm colours indicate current observation localities and therefore areas which are more important for *L. agilis*. Cooler colours indicate a lower observed density and therefore areas which were observed to be less important for *L. agilis*.

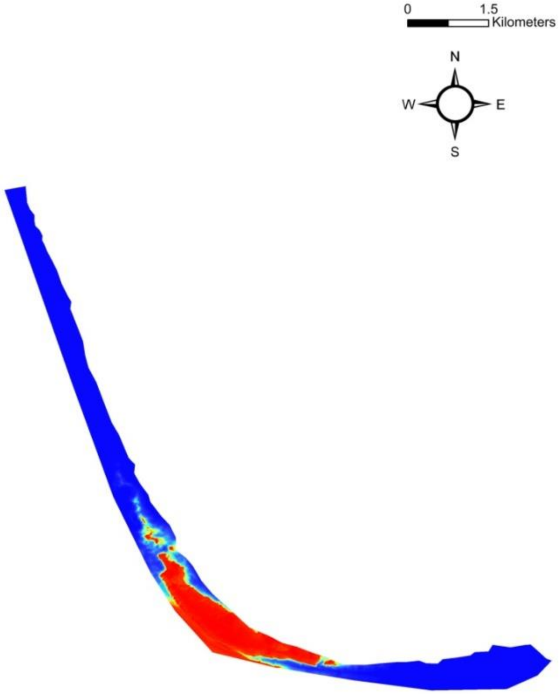


**Figure 3.18:** Habitat suitability map of Aberdyfi produced in Maxent. Warm colours indicate areas of forecasted suitable suitable habitat and therefore areas which are important for *L. agilis*. Cooler colours indicate areas of less suitable habitat for *L. agilis*.

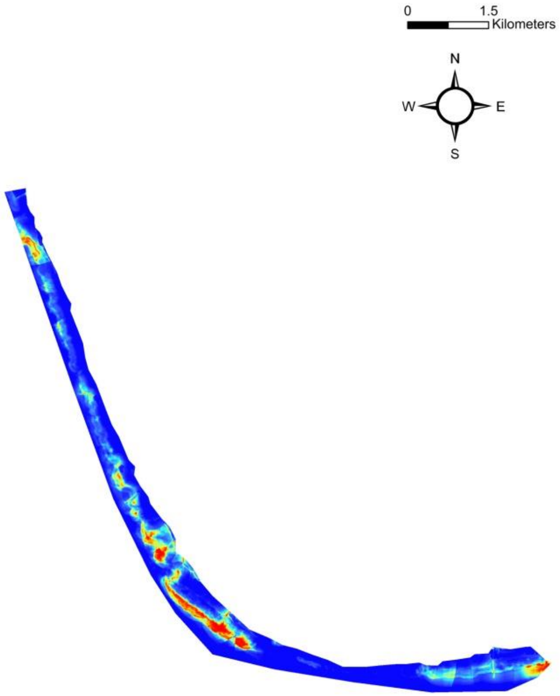


**Figure 3.19:** Current *Z. vivipara* habitat utilisation map of Aberdyfi produced in Maxent. Warm colours indicate current observation localities and therefore areas which are more important for *Z. vivipara*. Cooler colours indicate a lower observed density and therefore areas which were observed to be less important for *Z. vivipara*.

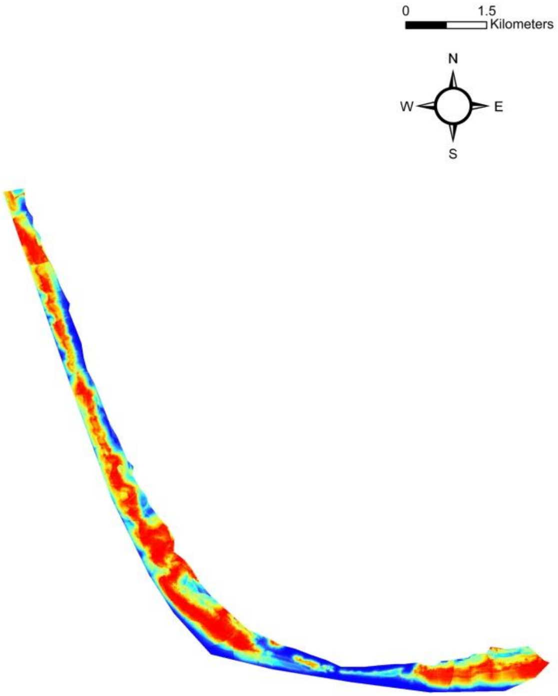


**Figure 3.20:** Habitat suitability map of Aberdyfi produced in Maxent. Warm colours indicate areas of forecasted suitable suitable habitat and therefore areas which are important for *Z. vivipara*. Cooler colours indicate areas of less suitable habitat for *Z. vivipara*.

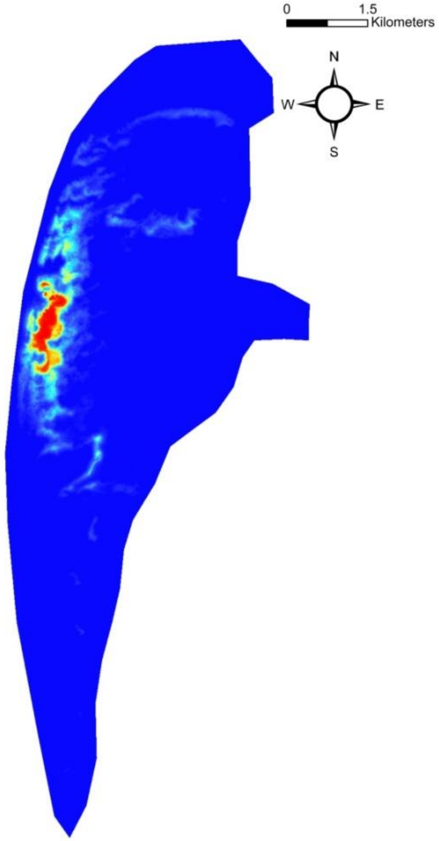


**Figure 3.21:** Current *L. agilis* habitat utilisation map of Ynyslas produced in Maxent. Warm colours indicate current observation localities and therefore areas which are more important for *L. agilis*. Cooler colours indicate a lower observed density and therefore areas which were observed to be less important for *L. agilis*.

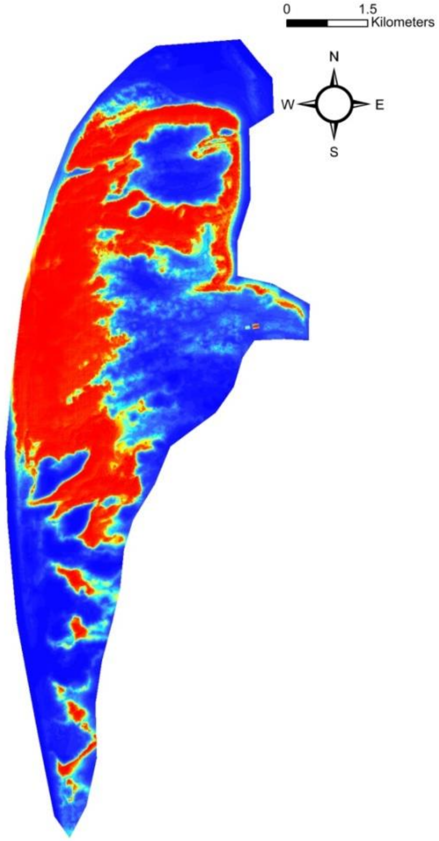
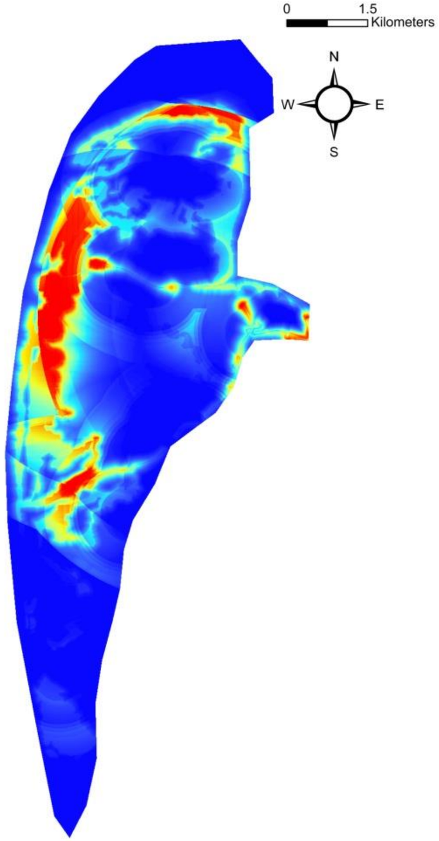


**Figure 3.22:** Habitat suitability map of Ynyslas produced in Maxent. Warm colours indicate areas of forecasted suitable suitable habitat and therefore areas which are important for *L. agilis*. Cooler colours indicate areas of less suitable habitat for *L. agilis*.



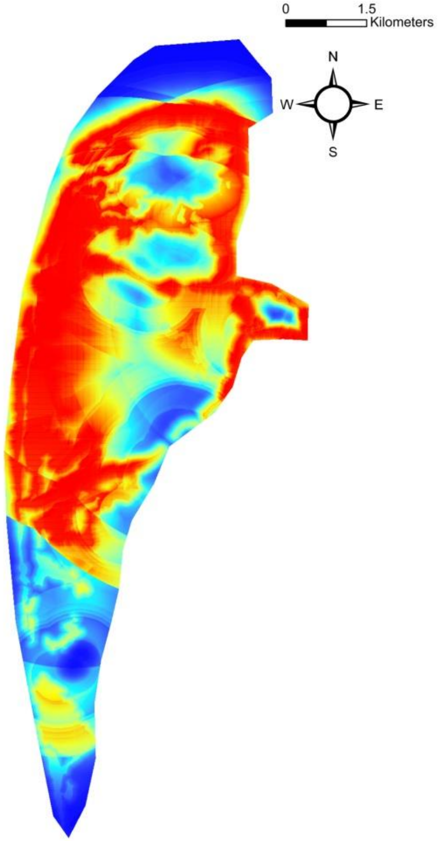


**Figure 3.23:** Current *Z. vivipara* habitat utilisation map of Ynyslas produced in Maxent. Warm colours indicate current observation localities and therefore areas which are more important for *Z. vivipara*. Cooler colours indicate a lower observed density and therefore areas which were observed to be less important for *Z. vivipara*.

**Figure 3.24:** Habitat suitability map of Ynyslas produced in Maxent. Warm colours indicate areas of forecasted suitable suitable habitat and therefore areas which are important for *Z. vivipara*. Cooler colours indicate areas of less suitable habitat for *Z. vivipara*.

With lizards identifying basking sites for their micro-habitat features and surroundings, against a backdrop that offers shelter (either vegetation or man-made), Figures 3.25, 3.26 and 3.27 provide a breakdown of additional environmental induced habitat factors influencing their thermal properties,

These figures indicate that both sand and viviparous lizards utilise varying aspects of the habitat and micro-habitat throughout the day, month and year. Adapting their habitat preference to optimise basking conditions, amongst other things, analysis of observations indicate that sand and viviparous lizards utilise the varied aspect of a site in slightly different ways. Sand lizards favour basking in an easterly to south-westerly direction; associated with position of the sun and timing of observations. Viviparous lizards were observed to favour basking in a more westerly to south-westerly direction, although still utilising aspects between the east and south-west.

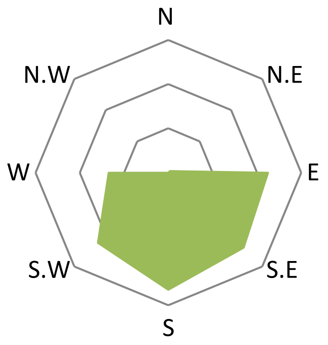


**Figure 3.25:** Basking direction associated with sand lizard observations across the three research sites between March 2010 and October 2014. n = 220.

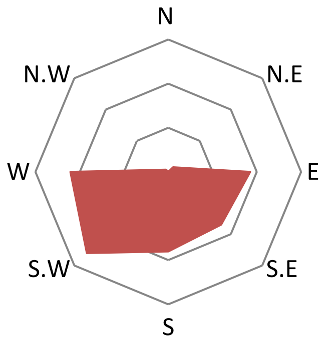


**Figure 3.26:** Basking direction associated with viviparous lizard observations across the three research sites between March 2010 and October 2014. n = 1038.

In addition to basking direction, the mean monthly basking degree of angle utilised was shown to fluctuate between months (see Figure. 3.5). Sand lizards utilise slopes with a higher degree angle towards the beginning and end of each survey year (44 – 48 deg), compared to a degree angle of 24 deg during the warmer summer months of June and July; when the sun is present at a higher degree angle in the sky. Viviparous lizards were shown to utilise slopes with a higher degree angle towards the end of each survey year (48 deg). In contrast to sand lizards, viviparous lizards utilise a shallower degree of angle towards the beginning of the year, with it increasing towards October.

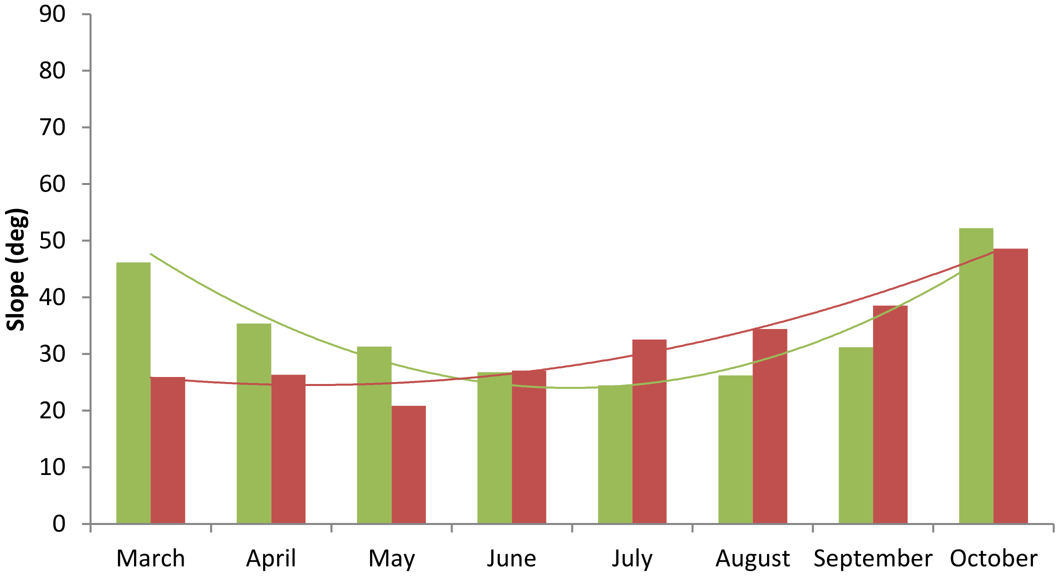


**Figure 3.27:** Monthly mean basking degree of angle identified within microhabitats associated with all sand and viviparous lizard observations across the three research sites between March 2010 and October 2014. (Polynomial trend line *La* $R^2$*=0.9058.* *Zv* $R^2$*=0.9425*). n = 1242.

## 5.4 Dispersal distance

Table 3.5 presents summary values for sand lizard dispersal distances from reintroduction locations across the three research sites. The table provides minimum, maximum and mean distances for all life stages and sexes, based on observations, not accounting for time since introduction.

With the three research sites having received sand lizard introductions over variable time periods, Table 3.6 provides estimated values for dispersal rates per year based on observations of natural animal movement and time since initial and final release.

Although different dispersal distances have been identified between life stages and sexes across the three research sites, the mean dispersal distances per year, expressed in meters, indicate a potential yearly dispersal distance average of 20 m – 28 m. The large mean dispersal distance indicated from Morfa Harlech may over-estimate actual distances as a result of all dispersal distances having been generated from the three release locations (indicated on Figures 3.1, 3.2 and 3.3). Although the large mean distances cannot be disproved/confirmed the mean yearly dispersal distance average of 20 m – 28 m represents an approximate trend across the three research sites.

**Table 3.5:** Summary of calculated dispersal distances across the Morfa Harlech, Aberdyfi and Ynyslas research sites, between March 2010 and October 2014 (– indicates absence of data).

| Site | Life stage/sex | Minimum (m) | Maximum (m) | Mean (m) | Standard deviation |
|---|---|---|---|---|---|
| **Harlech** | **All** | 2.09 – 14.94 | 2256.34 – 3872.17 | 475.66 – 1240.45 | 381.40 – 547.41 |
| | **Adult** | 2.09 – 14.94 | 2256.34 – 3872.17 | 533.67 – 1199.55 | 465.40 – 619.05 |
| | **Sub-adult** | 11.75 – 206.96 | 968.13 – 1746.24 | 375.24 – 1321.20 | 196.27 – 528.54 |
| | **Juvenile** | 8.24 – 44.73 | 1324.07 – 1864.67 | 436.40 – 1094.34 | 369.06 – 574.28 |
| | **Yearling** | 37.88 – 106.57 | 1193.77 – 1740.44 | 467.25 – 1243.93 | 387.29 – 643.10 |
| | **Hatchling** | 40.46 – 1340.79 | 322.34 – 1636.36 | 182.00 – 1497.68 | 94.66 – 590.70 |
| | **Male** | 2.38 – 14.94 | 2227.39 – 3871.34 | 607.39 – 1187.16 | 435.23 – 703.24 |
| | **Female** | 2.09 – 103.31 | 2256.34 – 3916.80 | 460.20 – 1265.73 | 428.63 – 593.52 |
| | **Unknown** | 18.56 – 44.17 | 1322.77 – 1869.97 | 401.93 – 1248.41 | 284.41 – 475.24 |
| **Aberdyfi** | **All** | 56.55 | 474.81 | 195.873 | 75.30 |
| | **Adult** | 89.11 | 474.81 | 199.56 | 81.36 |
| | **Sub-adult** | 318.03 | 356.51 | 337.277 | 27.20 |
| | **Juvenile** | 56.55 | 248.75 | 165.80 | 67.12 |
| | **Yearling** | 213.58 | 216.65 | 215.11 | 2.17 |
| | **Hatchling** | 136.12 | 218.09 | 188.75 | 37.94 |
| | **Male** | 89.00 | 425.03 | 204.16 | 83.93 |
| | **Female** | 115.33 | 248.54 | 193.45 | 50.33 |
| | **Unknown** | 143.46 | 193.52 | 166.35 | 21.42 |
| **Ynyslas** | **All** | 4.96 | 481.32 | 98.24 | 122.46 |
| | **Adult** | 5.11 | 481.32 | 137.01 | 164.55 |
| | **Sub-adult** | 12.09 | 87.82 | 52.04 | 38.03 |
| | **Juvenile** | 24.20 | 135.95 | 88.48 | 47.77 |
| | **Yearling** | 17.16 | 225.71 | 71.46 | 70.89 |
| | **Hatchling** | 5.29 | 130.61 | 41.39 | 51.97 |
| | **Male** | 4.96 | 481.48 | 96.75 | 121.44 |
| | **Female** | 10.55 | 477.12 | 155.85 | 157.84 |
| | **Unknown** | - | - | - | - |

**Table 3.6:** Sand Lizard estimated mean yearly dispersal rates across the three research sites, between initial releases and October 2012 (Harlech) and October 2014 (Aberdyfi and Ynyslas).Range of means represents mean values calculated from the combination of release locations for each site.

| Research site | Mean minimum per year (m) | Mean maximum per year (m) | Mean per year (m) |
|---|---|---|---|
| **Harlech** | 0.12 – 4.98 | 132.72 – 1290.72 | 27.98 – 413.48 |
| **Aberdyfi** | 7.06 – 8.07 | 59.35 – 67.83 | 24.48 – 27.98 |
| **Ynyslas** | 1.02 – 2.55 | 96.26 – 240.66 | 19.64 – 49.12 |

# 6 Discussion

In this Chapter, different methodologies have been applied to identify how sand and viviparous lizards utilise available, ‘suitable’ dune system habitat. Previous researchers focused on individuals within their natural range or within ex-situ, captive breeding enclosures (Jackson, 1979; Cooke, 1991; Blanke, 1995; Fearnley, 2009; Kraft, 2012) and not on sites which have received introduced populations of sand lizards, like this study.

This research models ‘suitable habitat’ for both species across the three dune system sites. This modelling is undertaken for the first time on UK dune systems. Furthermore, sand lizard dispersal distances have been calculated for each of the three populations to inform future management practices.

Management activities including research, site protection and management are required to restore sand lizards to their former range (Corbett & Moulton, 1998; Moulton & Corbett, 1999; Russel, 2012) with the extent, distribution and condition of habitats and species requiring monitored to check whether management objectives are being achieved (Rooney, 2001).

## 6.1 Micro-habitat utilisation

This research identifies that both sand and viviparous lizards were found to utilise habitats of a similar structural composition on UK dune systems. This has also been proved on studies undertaken or described by House & Spellerberg (1983), Blab *et al.* (1991), Cooke (1991) and Blanke & Fearnley (2015) within alternative habitats. Across the three research sites sand was shown to make up 18.4% to 18.6% of the micro-habitat, tall ruderal between 10.4% and 11.2% (vegetation of a typical height between

30 cm – >100 cm), grass between 23.5% to 24.2% (including marram) and shrubs between 42.4 and 46.5% (vegetation of a typical height between 5 cm – 30 cm). These values indicate that although reared in an ex-situ enclosure, the reintroduced sand lizards inherently utilise the same habitats components as those of a natural dune system population, although they are at the most north-westerly point of their distribution in Wales.

Similar research undertaken on native sand lizard populations on dune system habitat in the UK by Cooke (1991) in Merseyside (within their natural range) revealed a wider range of sand composition within the habitat structure (5 – 34% of open sand) was utilised. A higher percentage of medium-high vegetation cover was identified in this study compared to that of Cookes' (1991). This study however provides a more detailed prescription of the mosaic of habitats utilised compared to that of Cooke (1991), with Cooke utilising less categories.

Further to the vegetative component of the habitat, this study further identified that the reintroduced sand lizards also favour specific habitat features such as basking direction and the degree of angle of their basking position. Favouring an easterly to south-westerly basking direction and the mean monthly basking degree of angle of between 24 deg to 48 deg, the results of this study further confirm a sand lizards site component utilisation described by House & Spellerberg (1983), Cooke (1991), Blab *et al*. (1991) and Blanke (2010), identified in natural populations across their range in the UK and Germany. This study goes further to detail those habitat structures and features favoured by viviparous lizards. Similar to those favoured and utilised by the sand lizards, viviparous lizards were noted as utilising a different basking direction and degree of basking angle strategy to sand lizards. Favouring a more westerly to south-westerly basking direction as a North-West Wales perspective, viviparous lizards do however vary basking direction based on the orientation of the site and this should be noted when planning and undertaking monitoring surveys or planning site management and any remodelling/re-profiling of a site.

## 6.2 Site utilisation modelling

Species-specific modelling presented in this Chapter is the first provided for these species on a site-based scale. The models provide a level of site based utilisation detail, not previously explored for UK reptiles.

Habitat and site utilisation based on current observation localities models for the three research sites indicate that for highly mobile dune systems such as Morfa Harlech and Ynyslas, sand lizard dispersal across a site (from introduction locations) is not of concern. In the highly mobile or accreting dune systems the sand lizards have the availability of favoured habitats such as SD7, SD6, SD11, and SD17 and to a lesser extent SD8 and W22, whereas in static or eroding dune systems, such as that experienced at Aberdyfi, large, connected areas of these habitats are absent. In this situation, at Aberdyfi, sand lizard dispersal (from introduction locations) has been limited to a core area within the vicinity of the introduction locality resulting from dune system erosion during extreme winter storm events and lack of connectivity between favourable areas of habitat. Maps forecasting suitable habitat and therefore areas which are important for sand lizards for the three research sites provide a similar picture to that of the current observation localities models. On sites where the dunes are highly mobile, such as Morfa Harlech and Ynyslas, large areas of connected, suitable habitat are available for sand lizards to colonise, therefore sites such as these prove more favourable as reintroduction sites. Static or eroding dune systems, such as that experienced at Aberdyfi, lack this favourable connected habitat, therefore sites such as these prove unfavourable as reintroduction sites and should be avoided in the future.

Based on current observation locality models and habitat suitability maps, forecasting suitable habitat and therefore areas which are important for viviparous lizards for the three research sites is not a priority. Viviparous lizards would appear to be utilising the connectivity and habitats available to them to utilise a significant proportion of all of the individual sites.

The Sand Lizard Rapid Site Assessment (Green & Moulton, 2015) takes a qualitative approach to evaluating site conditions and the health of populations of sand lizards. Modelling species' habitat requirements are crucial to assess impacts of global climate change, for conservation efforts and to test mechanisms driving species presence (Peñalver-Alcázar *et al*., 2016). Habitat modelling adopted by this study demonstrates a quantifiable means for identifying those vegetative communities favoured by each Lacertidae species and visualises observed site utilisation, unlike the Sand Lizard Rapid Site Assessment (produced by ARC). Developemnt and alignment with other site assessment programmes should be undertaken, with the view to determining how this might be taken forward as a rolling programme to describe the sand lizard conservation status and enhance/drive targeted conservation management programmes.

## 6.3 Dispersal distance

Dispersal distance of reintroduced sand lizard populations have been investigated throughout its natural range by Yablokov *et al.* (1980), Gyovai (1984), Rahmel & Meyer (1988), Klewen (1988), Nöllert (1989), Blanke (1995), Gramentz (1996), Olsson *et al*. (1996b 1997), Märtens (1999), and Berglind (1999 & 2000). These capture and release studies indicate that towards the north, east, south and within the centre of their range that sand lizards can typically cover a distance of between 0 m – 400 m with mean distances of between 8.78 m – 140 m, when captured and released a set distance from the original position. Dispersal distances calculated for the three research sites as part of this study identified, dispersal rates [per year] based on observations of natural animal movement since initial and final release of between 0.12 m – 1,290.72 m. The large mean dispersal distance (from Morfa Harlech) may over-estimate actual distances as a result of all dispersal distances having been generated from the three release locations, although historical studies involving toe clipping of sand lizards in Germany recorded animals covering distances of up 1,200m (Strijbosch & van Gelder, 1997) to 4,000 m (Klewen, 1998).

The mean yearly dispersal distance average of 19.64 m – 28 m represents an approximate trend across the three research sites. This mean distance from the 'western-most population within its range' confirms more recent studies by Fearnley (2009) and Mayer & Elminger (2014) which have indicated that movements of between 5 m – 60 m (by a female) are a more realistic estimate of dispersal. This would also agree with studies undertaken by Yablokow *et al.* (1980), which indicate that 'over 70% of sand lizards move less than 30m from their hatchling site during their entire lifetime', which along with molecular phylogenetics studies by Godinho *et al*. (2005) and Russell (2012) implies limited dispersal ability. The dispersal ranges for individual life stages and sexes identified across the three research sites also corresponds with the ranges indicated within those studies investigated throughout its natural range by the likes of Yablokow *et al.* (1980), Gyovai (1984), Rahmel & Meyer (1988), Nöllert (1987), Blanke (1995), Gramentz (1996), Olsson *et al*. (1996b, 1997), Märtens (1999) and Berglind (2000).

Through a combination of favourable habitat types identified earlier in this Chapter and typical yearly dispersal distances identified for dune system reintroduction sites, conservationists and site managers can evaluate a site more accurately for its viability as a reintroduction site or areas requiring habitat modification (if required) to provide connectivity between areas of ‘suitable habitat’.

Further to site management, with typical yearly dispersal distances identified for dune system reintroduction sites, appropriate search areas can be identified for sand lizards to inform presence/likely absence and population/site monitoring surveys. A translocation can only be regarded as a success when a viable population persists for generations, therefore Blanke (2010) and Schneeweiß *et al.* (2014) suggest that any monitoring programme should be conducted at a reintroduction site for a minimum of five years (Blanke & Fearnley, 2015). Through identifying appropriate search areas at a site, combined with enhanced environmental variables associated with observation (detailed in Chapter 2), more accurate reporting on the conservation status of the species can be achieved and provided to the EU commission.

Dispersal distances for viviparous lizards were not investigated for the three research sites. The point(s) of introduction/dispersal onto individual sites could not be established, nor could the time taken between their establishment and dispersal across the whole sites established due to their wide-ranging distribution. Having established that viviparous lizards utilise a more varied habitat structure, and have greater ability to populate a site (shown in distribution maps), being able to negotiate more unfavourable area to colonise favourable areas, it is not anticipated that this would be a limiting factor for this species.

By furthering the understanding of which dune system habitat structures are utilised and favoured within this study, this will contribute to the development of more appropriate conservation and site management measures along with improving existing Habitat Suitability Index assessments being developed by ARC. The development of such will make a direct contribution towards ARC’s reintroduction programme and NRW’s management of sites where reintroduced sand lizards and existing viviparous lizard populations are located.

# Chapter 4

## Population Viability Analysis and Genetic Differentiation Between Reintroduced Sand Lizard (*L. agilis*) Populations and the Original Source Population

# 1 Introduction

Blanke and Fearnley (2015) have stated that across Europe "The remaining sand lizard populations are becoming increasingly scattered, isolated and often confined to small habitat patches. Big expanses or habitat that once supported large populations are becoming rarer and rarer". Russell (2012) identified that the size of a population of plants or animals can have significant implications for their long-term persistence and/or viability. Smaller populations are less viable and at a far greater risk of extinction than large ones as their long-term survival is dependent on the ability of relatively few individuals to survive and successfully reproduce (Frankham *et al.*, 2010). Smaller populations frequently inhabit smaller habitats, which can deteriorate in quality quickly (due to lack of habitat management). All of ARC's sand lizard reintroductions across the North-West Wales coast are within discrete, isolated locations, and Couvet (2002) and Russell (2012) highlighted the effects of isolation from other populations, preventing recruitment and the introduction of new genetic material from surrounding populations. This isolation leaves smaller populations significantly more vulnerable to stochastic events, from which larger populations are better able to recover (Lande, 1993; Russell, 2012). Stochastic events which affect population persistence fall into four categories (Shaffer, 1981; Boyce, 1992; Caughley, 1994; Henle *et al.*, 1999, Russell, 2012):

- **Genetic stochasticity** – changes in gene frequencies within a population brought about by processes such as genetic drift and inbreeding, which often result in reduced variability;
- **Demographic stochasticity** – changes in demographic parameters of a population such as random variation in survival and reproductive success between generations or random variation in sex ratios;
- **Environmental stochasticity** – changes in weather, climate, habitat and predation, parasitisation and disease, which affect survival and reproduction rates; and
- **Natural (or anthropogenic) catastrophes** – such as fires, floods, etc. These are essentially an extreme form of environmental stochasticity that occur infrequently and affect a very high proportion of a population. This category includes anthropogenic habitat loss. (Ewen *et al.*, 2012)

In wild populations, stochastic events are often interlinked. Genetic stochasticity within a population may increase a population's vulnerability to environmental stochasticity by reducing its ability to adapt to changing environmental conditions (Russell, 2012). Stochastic effects have also been demonstrated by Ralls *et al*. (2007) to occur by chance during the random sampling of alleles during reproduction; being an important element in inbreeding. It has been demonstrated that in-breeding and genetic diversity is closely related to population size and should increase with the number of breeding individuals in any population (Blanke & Fearnley, 2015). Smaller populations are specifically vulnerable to inbreeding and genetic drift, which is known to reduce genetic diversity and/or viability. This can lead to the fixing of disadvantageous deleterious mutations, inbreeding depression and generally reduced levels of adaptability and fitness (Veith *et al*., 1999; Russell, 2012; Blanke & Fearnley, 2015). Despite these factors, isolated and smaller populations have been recorded to survive, such as the pink and black-striped land iguana *Conolophus marthae* (Gentile & Snell, 2009; Gentile *et al*., 2016) which is known from 192 mature individuals over an area of 9 – 10.9 km$^2$ (Gentile *et al*., 2016).

Although a member of the most species-rich reptilian family in Europe (Cox & Temple, 2009), the sand lizard *Lacerta agilis* is at the north-western limit of its geographic range. At this northern extreme, small isolated populations of sand lizards have been recorded to survive on the German islands of Sylt and Föhr. Furthermore, the introduction of a small population to the Hebridean Island of Coll in 1970 (Arnold, 1995) is known to also persist (Edgar & Bird, 2005; Mouton, 2011, 2016), however, Blanke & Fearnley (2015) suggest that this time period might be too short to expect observable genetic effects. Furthermore, genetic analysis of these populations has not been undertaken.

Russell (2012) established that the Merseyside population, along with those populations present in Surrey, are genetically less diverse than the populations present in Dorset. Comparable to small, fragmented populations in Sweden, these are issues that have caused concern among conservation practitioners in relation to a wide range of endangered species, including sand lizards (e.g. Frankham, 1996; Olsson *et al*., 1996; Gullberg *et al*., 1998; Beebee & Rowe, 2001; Russell, 2012). Beebee & Rowe (2001) found a correlation between population sizes and genetic diversity in British sand lizards and emphasised the importance of conservation measures in their heathland and dune habitats (Blanke & Fearnley, 2015). Management practices have been developed and implemented for sand lizards in Britain through the ‘Conservation strategy for the sand lizard’ (Corbett, 1988), ‘Sand lizard conservation Handbook’ (Moulton & Corbett, 1999), ‘Action Plan for the conservation of the sand lizard in Northwest Europe’ (Edgar

& Bird, 2005), and the ‘Sand Lizard Rapid Site Assessment 2014’ (Green & Moulton, 2015). The species and their habitats currently receive maximum protection under both EU and UK law (Corbett & Tamarind, 1979; Moulton & Corbett, 1999; Agasyan *et al.*, 2010b), neither of which will necessarily alleviate the consequences of genetic impoverishment (Williams & Hoffman, 2009).

Anthropogenic factors detailed within previous Chapters have increasingly resulted in the modification of natural landscapes resulting in the loss of small, isolated and fragmented sand lizard populations in the UK and Europe. Although ARC’s sand lizard captive breeding and reintroduction programme has made reintroductions to several sites across the North-West Wales coast (UK), these reintroductions typically comprise between 120 - 220 individuals (over numerous years), bred from a small number (typically 2 to 20) of further isolated individuals (ARC, 2015c).

The genetic objective in any reintroduction programmes is to re-establish viable populations with the maximum possible genetic diversity, using individuals predicted to have maximum reproductive fitness under wild conditions (Frankham *et al.*, 2010). Reintroduction science has, however, significantly advanced over the last three decades. Starting with large vertebrates, often charismatic and with obvious sources of decline, the IUCN ‘Reintroduction Specialist Group’ database is now witness to a host of species including plant and animal, vertebrate and invertebrate, being returned to the ‘wild’ around the world (Ewen *et al.*, 2012).

Captive breeding programmes are often established to maintain the genetic diversity and fitness (Frankham, 2008) of a rare or endangered species, at least until reintroductions or a supplementation of wild populations have occurred (Utter & Epifanio, 2002; Willoughby *et al.*, 2015). These two genetic processes are particularly important regarding species reintroductions. All reintroduced populations experience some level of bottlenecking (Ewen *et al.*, 2012), i.e. periods of small population size, sometime several in a row. The first bottleneck often occurs before conservation measures are taken, when species are in their natural environment, endangered and hence, by definition, small. The second bottleneck typically occurs when a captive breeding population is then founded with a small number of wild-caught individuals which have already received a level of bottlenecking. Finally, the third bottleneck occurs when a limited number of animals are released back into their former range. Thus, all reintroduced populations have experienced periods when their population(s) size was small (Ewen *et al.*, 2012).

To be viable and hence successful in the long term, reintroduced populations need to be able to sustain themselves. This, in turn, requires that they are capable of adaptation to future environmental change (Ewen *et al.*, 2012). Successful reintroductions are exemplified by the black-footed ferret *Mustela nigripes,* California condor *Gymnogyps californianus*, Missouri river otter *Lontra Canadensis*, Przewalski's horse *Equus przewalskii*, and a range of herpetofauna (Miller *et al*., 1994; Russell *et al*., 1994; Toone & Wallace, 1994; van Dierendonck & Wallis de Vries, 1996; Fischer & Lindenmayer, 2000; Ewen *et al*., 2014; Mowry *et al*., 2015; Willoughby *et al*., 2015; Drag & Cizek, 2015). However, approximately one-third of reintroductions have been reported to have failed.

The 'Sand Lizard Captive Breeding and Reintroduction Programme' has been highly successful in re-establishing the species on sites within its former range (Corbett & Moulton, 1998; Moulton *et al*., 2011, Russell 2012). Communications with Nick Moulton (2012) identified that reintroduced lizards released at Ynyslas may have further included individuals from the Dorset race, hence the site was established without only using pure-bred Merseyside lizards; although the true genetic make-up of the original Welsh population cannot be established due to the absence of 'type' specimens. Nevertheless, an assessment of the genetic diversity of reintroduced populations has been advised by Russell (2012), due to a low genetic diversity within the Aberffraw population (details of its origins, such as establishment period and founder population size are unknown and may have a contributing factor).

This chapter explores population viability and makes genetic comparisons between three reintroduced *L. agilis* populations across Gwynedd and Ceredigion, ARC's captive breeding programme and original source population at Merseyside, England (now that the three reintroductions have been completed). The main objective of this Chapter is to explore individual populations viability and investigate the differentiation between the reintroduced populations through molecular phylogenetics. It is hypothesised that the small, isolated, reintroduced populations have reduced viability and there is a difference in genetic diversity between the reintroduced populations at Morfa Harlech, Aberdyfi and Ynyslas to those of the original source stock at Merseyside, England. It is predicted, that the genetic diversity of the reintroduced populations will be less than those of the source population at Merseyside, with observed bottlenecking of three introduced populations and the ex-situ captive breeding programme experienced.

# 2 Methodology

## 2.1 Population Viability Analysis

*Selection*

Parameters, established from site based recordings in Chapters 2 & 3, and a comprehensive literature review was utilised to evaluate population viability for sand lizard populations at each of the three research sites. VORTEX version 10.2.1.0 (Lacy & Pollak, 2015) was used to model sand lizard population viability for the three research sites.

Vortex is an individual-based, age-structured, population simulation model. The model includes carrying capacity, demographic stochasticity (in mortality and breeding structure), environmental stochasticity, catastrophes, density-independent reproductive rates, inbreeding depression, and allows a range of user-defined functions to replace fixed parameter terms (Miller & Lacy, 1999; Reed *et al*., 2003). Vortex has been extensively applied to endangered species conservation by the Conservation Breeding Specialist Group of the World Conservation Union (IUCN) and others (Seal *et al*., 1998; Reed *et al*., 2003; Carroll *et al*., 2014; Andersen *et al*., 2015; Kirchhoff *et al*. 2016).

Initially, models for each of the three sites were run utilising a set of 'default' programme options. Default values enabled a test scenario to be run to ensure Vortex was working with the magnitude and format of typical input values e.g., a percent, or an integer, or a number from 0 to 1 (Lacy *et al*., 2015). The number of repetitions was limited to 20 iterations over a period of 20 years to ensure that the simulated population was behaving in a manner that was expected. Vortex uses a random number generator to simulate random events in the life cycle, therefore no two iterations will be identical (Lacy *et al*., 2015).

Upon validation of Vortex and completion of test scenarios, analysis scenarios for the three sites were run utilising the simulation inputs detailed in Table 4.1. The number of repetitions was increased to 1,000 iterations over a period of 200 years to obtain a more rigorous description of the simulated population's viability and behaviour.

As indicated in Table 4.1, a number of simulation inputs were excluded from the analysis. Explanation for their exclusion is provided below:

- No **State variables** (global, population or individual) were developed for the model. All parameters were entered manually within individual simulation inputs;
- **Dispersal** has been excluded as each individual site is being considered as a single population (individual-based model);
- **Harvest** has been excluded as no known culling or removal of individuals for research, removal of young individuals for translocation programs, etc. has been undertaken and/or known to be proposed for the sites;
- **Supplementation** was excluded as Vortex assumes that individuals being added to a recipient population are unrelated to both each other and to any other individual in the recipient population. As supplementation within and between the populations present at the three research sites was unlikely through related individuals (See further in this Chapter), this was excluded from analysis; and
- **Genetics** was excluded as this evaluation was undertaken focusing on viability of populations in relation to suitable habitat. The impact of population genetics is considered further in this Chapter.

The three sites were modelled accounting for minimum and maximum ‘carrying capacity’ variables calculated from abundance estimates for sand lizards provided by studies undertaken in the United Kingdom, Netherlands, Hungary and Germany (House & Spellerberg, 1983; Hartung & Kock, 1988; Strijbosch & Creemers, 1988; Nöllert, 1987; Möller, 1996; Märtens & Stephan, 1997; Märtens, 1999). The three sites were also modelled with and without ‘inbreeding depression’ expressed. Although most diploid species that have been studied show depressed fitness when inbred (Lacy *et al.*, 2015), modelling with and without inbreeding depression allows impacts on the population viability to be documented.

*Interpretation*

The text output file (VOutput in Vortex) was inspected for each individual model, for the three research sites. Text and graphs displaying deterministic population growth rates (*det-r*) were projected from life table calculations. The following statistical parameters were documented across the analysis: Mean stochastic growth rate (*stoc-r*), Male and female generation time (*gen-m & gen-f),* ratio of adult males to adult females (*adu m/f*), probability of extinction (*PE*), median time to first extinction (median *TE*) and Mean time to first extinction (mean *TE*) supported by standard error and standard deviation values, calculated for the individual models.

**Table 4.1:** Vortex simulation parameters.

| Input | Simulation Input | | | | Source |
|---|---|---|---|---|---|
| | Site | Harlech | Aberdyfi | Ynyslas | |
| Scenario settings | Number of iterations | 1000 | 1000 | 1000 | Lacy & Pollak, 2015 |
| | Number of years (time steps) | 200 | 200 | 200 | |
| | Duration of each year in days | 365 | 365 | 365 | |
| | Run as population-based model | N | N | N | |
| | Extinction definition | Only 1 sex remains | Only 1 sex remains | Only 1 sex remains | |
| | Number of populations | 1 | 1 | 1 | |
| Species description | Inbreeding depression | Y / N | Y / N | Y / N | Lacy *et al.*, 2015 |
| | Lethal equivalents | 6.29 | 6.29 | 6.29 | O'Grady *et al.*, 2006 |
| | Percent due to recessive lethal alleles | 50 | 50 | 50 | Lacy *et al.*, 2015 |
| | EV correlation between reproduction and survival | 0.5 | 0.5 | 0.5 | Lacy and Pollak, 2015 |
| State variables | n/a | | | | |
| Dispersal | n/a | | | | |
| Reproductive system | Monogamous | N | N | N | Gullberg *et al.*, 1997. |
| | Polygynous | Y | Y | Y | |
| | Hermaphroditic | N | N | N | |
| | Long-term monogamy | N | N | N | |
| Reproductive rates | Long-term polygyny | N | N | N | Nicholson, 1980; Nöllert, 1987 |
| | Age of first offspring females | 3 | 3 | 3 | |
| | Maximum age of female reproduction | 19 | 19 | 19 | |
| | Age of first offspring males | 2 | 2 | 2 | Blanke & Fearnley, 2015 |
| | Maximum age of male reproduction | 21 | 21 | 21 | Blanke & Fearnley, 2015 |
| | Maximum lifespan | 21 | 21 | 21 | Blanke & Fearnley, 2015 |
| | Maximum number of broods per year | 2 | 2 | 2 | Phelps 2000; Blanke & Fearnley, 2015 |
| | Maximum number of progeny per brood | 17 | 17 | 17 | NCC, 1983; Strijbosch, 1987; Olsson & Shine, 1997 |
| | Sex ratio at birth – in % males | 50 | 50 | 50 | Blanke & Fearnley, 2015 |
| | % adult females breeding | 50 | 50 | 50 | Lacy & Pollak, 2015 |
| | SD in % breeding due to EV | 10 | 10 | 10 | Blanke & Fearnley, 2015 |
| | Distribution of broods per year | | | | |
| | 0 broods | 15 | 15 | 15 | Blanke & Fearnley, 2015 |

**Table 4.1:** Cont over page

**Table 4.1:** Cont.

| Input | Simulation Input | | | | Source |
|---|---|---|---|---|---|
| | Site | Harlech | Aberdyfi | Ynyslas | |
| | 1 broods | 60 | 60 | 60 | Blanke & Fearnley, 2015 |
| | 2 broods | 25 | 25 | 25 | Phelps, 2000 |
| | Use normal distribution | Y | Y | Y | Lacy & Pollak, 2015 |
| | Mean | 2.5 | 2.5 | 2.5 | |
| | Standard Dev | 1 | 1 | 1 | |
| Mortality rates | Mortality of females as % | | | | |
| | Mortality from age 0 to 1 | 50 | 50 | 50 | Strijbosch, 1988 |
| | SD in 0 to 1 mortality due to EV | 10 | 10 | 10 | |
| | Mortality from age 1 to 2 | 10 | 10 | 10 | |
| | SD in 1 to 2 mortality due to EV | 3 | 3 | 3 | |
| | Mortality from age 2 to 3 | 10 | 10 | 10 | |
| | SD in 2 to 3 mortality due to EV | 3 | 3 | 3 | |
| | Annual mortality after age 3 | 10 | 10 | 10 | |
| | SD in mortality after age 3 | 3 | 3 | 3 | |
| Mortality rates | Mortality of males as % | | | | |
| | Mortality from age 0 to 1 | 50 | 50 | 50 | Strijbosch, 1988 |
| | SD in 0 to 1 mortality due to EV | 10 | 10 | 10 | |
| | Mortality from age 1 to 2 | 20 | 20 | 20 | |
| | SD in 1 to 2 mortality due to EV | 3 | 3 | 3 | |
| | Annual mortality after age 2 | 15 | 15 | 15 | |
| | SD in mortality after age 2 | 3 | 3 | 3 | |
| Catastrophes | Frequency and extent of occurrence | | | | |
| | Number of types of catastrophes | 2 | 2 | 2 | Met Office, 2015 |
| | Local | Y / Y | Y / Y | Y / Y | |
| | Frequency % | 10 / 10 | 10 / 10 | 10 / 10 | |
| | Severity (proportion of normal values) | | | | |
| | Reproduction | 0.5 / 0.5 | 0.5 / 0.5 | 0.5 / 0.5 | Reed *et al.*, 2003 |
| | Survival | 0.5 / 0.5 | 0.5 / 0.5 | 0.5 / 0.5 | Reed *et al.*, 2003 |
| Mate monopolization | % Males in breeding pool | 75 | 75 | 75 | Lacy & Pollak, 2015 |
| Initial population size | Population size | 146 | 216 | 216 | ARC, 2015c |
| | Use stable age distribution | N | N | N | |
| | Use specified age distribution | Y | Y | Y | |
| | Enter proportional values for age distribution | N | N | N | |
| | Female / Male age distribution | | | | |
| | Age 1 | 60 / 66 | 100 / 116 | 70 / 73 | ARC, 2015c |
| | Age 2 | 10 / 10 | 0 / 0 | 0 / 0 | |
| Carrying capacity | Carrying Capacity (K) | 13 / 806 / 12897 / 21287 / 4800 / 4758 / 2838 / 4966 | 5 / 317 / 5070 / 8368 / 1887 / 1870 / 1116 | 12 / 12339 / 4592 / 2715 / 771 / 20365 / 4552 / 4751 | House & Spellerberg, 1983; Hartung & Kock, 1988; Strijbosch & Creemers, 1988; Nöllert, 1987; Möller, 1996; Märtens & Stephan, 1997; Märtens, 1999 |
| | SD in K due to EV | 0 | 0 | 0 | |

**Table 4.1:** Cont.

| Input | Simulation Input | | | | Source |
|---|---|---|---|---|---|
| | Site | Harlech | Aberdyfi | Ynyslas | |
| Carrying capacity | Future change in K | N | N | N | |
| | Over how many years? | 5 | 5 | 5 | |
| | % Annual increase or decrease | -10 | -10 | -10 | |
| | Implement K based on a limit on some population variable other than N | N | N | N | |
| | During K truncation, remove only individuals meeting criteria | N | N | N | |
| | Prioritize K truncation based on ISvar | N | N | N | |
| Harvest | Implement as Translocation | N | N | N | ARC, 2015c |
| Supplementation | Implement as Translocation | N | N | N | ARC, 2015c |
| Genetics | Genetic input | N | N | N | Lacy *et al.*, 2015 |
| | Genetic management | N | N | N | |
| | Genetic output | N | N | N | |

## 2.2 Sand Lizard DNA sampling

Lizards were caught either by hand or by noosing of the individuals (dependent upon exact location of individual in vegetation) following the methodology detailed within Gent & Gibson (1998) and Blomberg & Shine (2006): details can be found in Chapter 2. Sex was determined using guidance in Yablokow *et al*. (1980), Bischoff (1984), Gent & Gibson (1998) and Arnold & Ovenden (2004) also found in Chapter 2.

Images taken prior to DNA sampling were evaluated to ensure no individual was multiply sampled. Individuals were distinguished from a comparison of their natural markings. Nicholson (1980), Dent (1986), Märtens & Grosse (1996), Fearnley (2009) and Russell (2012) successfully utilised this technique to identify individual sand lizards in England, Wales and Germany. Automated photo identification software developed specifically for *L. agilis* identification by Conservation Research Ltd (utilised by Fearnley, 2009) was not utilised due to a match rate error of ca. 32% (Fearnley, 2009).

Buccal swabbing was utilised for sample collection. Swabbing has become a routine non-destructive technique for the DNA sampling of many species including reptiles (Beebee, 2008; Poschadel & Moller, 2004), producing no obvious signs of stress or tissue damage, and yielding good results during analysis (Beebee, 2008). Swabbing took only a minute, avoiding the need to handle the animal for a long period of time. Miller (2006) describes the procedure as simple, easy to implement in field situations and applicable to any medium-sized reptile (shown by Beebee's research using *L. agilis*).

All sand lizards sampled (excluding those held by the private captive breeders) were lightly but securely held with the upper body in the palm of the hand and the shoulders between the thumb and first two fingers. Each animal was allowed to bite and chew on the buccal swab (Isohelix™ SK-1 swab) for approximately one min (which they tended to do readily), after which the swabs were briefly air-dried (Beebee, 2008) and secured in the supplied sterile container with a sterile Dri-Capsule (Isohelix™ SGC-3). Under no circumstances were the mouths of lizards forced open to undertake the procedure. Upon completion of sampling, all lizards were released at point of capture. Appendix C, Table C.4.1 provides a list of sampled individuals.

Private captive breeders (excluding Chester Zoo) only permitted the collection of genetic material utilising an egg sampling approach (detailed below), with samples collected by the individual captive breeders. Upon hatching of the individual sand lizard clutches, each group of hatched eggs was collected using rubber gloves and placed into a sample jar/tub. All samples were frozen at the first available opportunity.

## 2.3 DNA extraction

DNA extraction utilised for buccal swabs is described in Appendix C, Table C.4.2, and is a modified version of the Isohelix™ DDK-50DNA Isolation Kit, Isohelix™; a division of Cell Projects Ltd (Kent, United Kingdom). Frozen amniotic fluid from four lizard eggs from each sample was extracted by digestion in a modified version of the methodology utilised by Fetzner (1999). DNA contained within amniotic fluid of the sand lizard eggs was extracted utilising the process described in Appendix C, Table C.4.3.

DNA extracted through both procedures was quantified using a NanoDrop nd1000 spectrophotometer (Thermo Fisher Scientific). Where required, each sample was diluted to 50ng/µl for genotyping.

## 2.4 PCR multiplex

Each sand lizard sample was amplified utilising a Polymerase Chain Reaction (PCR) method. Microsatellite loci were amplified using a series of primers (See Table 4.2) specifically developed for *L. agilis* by Gullberg *et al*. (1997) and Schwartz & Olsson

(2008). As per Russell's (2012) study, samples were amplified, with the exception of those primers, which failed to amplify, produced three alleles and/or produced null alleles in previous studies of Swedish (Gullberg *et al*. 1997) and British (Beebee & Rowe, 2001; Russell, 2010) sand lizards. In total, 15 microsatellite loci were utilised.

Three multiplex reactions comprising five microsatellite primer pairs were carried out (see Table 4.2). Each individual multiplex was amplified in a single 10µl multiplex reaction consisting of 2µl UPW, 4.5µl Qiagen multiplex PCR solution (dNTP, ddNTP, dUTP, biotin-11-dUTP, DIG-11-dUTP, fluorescent-dNTP/ddNTP), 0.5µl of 2.5pM of ABI Fam-, Pet- and Ned- and 5.0pM of Vic-labelled primer, 0.25µl of both reverse and forward primer mixes and 2µl of DNA sample.

Polymerase chain reactions were performed on a Bio Rad® DNA Engine Tetrad 2 thermocycler using the following cycling parameters developed by Williamson (2013): 95 °C for 15 min, followed by 13 cycles of 94 °C for 45 sec, 55 °C for 45 sec and 72 °C for 45 sec, followed by 25 cycles of 94 °C for 45 sec, 52 °C for 45 sec, and 72 °C for 45 sec. The profile was terminated with a 30 min extension at 60 °C.

PCR products were electrophoresed utilising an Applied Biosystems® 3130X1 Genetic Analyser, with sizing and genotyping of microsatellite data undertaken using GeneMapper® software (version 4.0). Samples which failed to amplify at any locus were repeated under original PCR conditions.

**Table 4.2:** Details of Polymerase Chain Reaction (PCR) primers and Multiplex reactions utilised. PCR primers were developed specifically for *L. agilis* by Gullberg *et al*. (1997) and Schwartz & Olsson (2008).

| | Primer sequence | Microsatellite repeat motif | Reference | Multiplex number | Allele size | Label |
|---|---|---|---|---|---|---|
| **La2** | GCTTAAATTGGAACCAGATTG AAGCAGCCAGAACACAGAG | $(GT)_{16}$ | Gullberg *et al.* (1997b) | 1 | 179 - 197 | Fam |
| **La3** | ACTAGGAGCGAGAAGAATCAG GACATATGGCAGAAGAGCAG | $(GA)_{28}$ | Gullberg *et al.* (1997b) | 1 | 160 - 194 | Vic |
| **La4** | CATGAGCAAAGCAATGAGC TGGAATGTGTCATTGAACTCTG | $(GT)_{19}$ | Gullberg *et al.* (1997b) | 1 | 138 - 160 | Ned |
| **La6** | GACTGGCGCATTCTATAAAAC GCCTTAAAGGGCCATCAG | $(GT)_{17}$ | Gullberg *et al.* (1997b) | 1 | 269 - 287 | Fam |
| **La9** | AGATGCTTTTATATATGCAACTTC GTGCCTTCATTTGTTTACTTC | $(GT)_{12}$ | Gullberg *et al.* (1997b) | 1 | 110 - 128 | Pet |
| **La01** | VIC-AACGGAGGTAGAATGTCATAGC CTTGAAGGGAAAGAGCTACTGC | $(GT)_{2}AT(GT)_{15}$ | Schwartz & Olsson (2008) | 2 | 87 - 123 | Fam |
| **La02** | FAM-TGCCTGCAAGACTATAATCCAAG GGAATGGCATGAGATATGGTG | $(GT)_{23}$ | Schwartz & Olsson (2008) | 2 | 216 – 244 | Fam |
| **La3E** | VIC-AAAGTTGGTCTGCACTGACG CAATTCAAAATGCACACAACG | $(GT)_{13}AT(GT)_{10}$ | Schwartz & Olsson (2008) | 2 | 220 - 258 | Vic |
| **La04** | VIC-CTAGGCATGGAGAATGGATGTG AGCCACTTCCCTAAGTGTGTCC | $(CA)_{20}$ | Schwartz & Olsson (2008) | 3 | 113 – 149 | Ned |
| **La10** | VIC-TAATAAAGCAGGCGCAAACC TGCAGCTAATCTTCATTTAGGATG | $(CA)_{5}(GA)_{4}GGGA(GACA)_{9}(CA)_{9}(GA)_{17}$ | Schwartz & Olsson (2008) | 3 | 170 - 223 | Vic |
| **La12** | CAGAGTTCATGGAAAGTGAAGGFAM-GGAGACTCTGCTGGTCATTC | $(CA)_{18}$ | Schwartz & Olsson (2008) | 3 | 195 - 225 | Pet |
| **La27** | VIC-AAATGCAAGCGAGCAACAAT ATCTGGCGGAGGGATGAG | $(GT)_{11}(AT)_{26}$ | Schwartz & Olsson (2008) | 3 | 112 – 160 | Fam |
| **La40** | GGGAACCGTTGTACTAAGTTTGGVIC-ATGCATTCAGATGTCTCCCAAG | $(CA)_{19}$ | Schwartz & Olsson (2008) | 3 | 187 - 209 | Ned |
| **La50** | FAM-AGGTAGCCCAGGTGTCATACAG TGGGTCTTACATGAGCTGAATC | $(GT)_{21}$ | Schwartz & Olsson (2008) | 2 | 99 - 123 | Pet |
| **La64** | PET-AGATGCTGAACTACCAGCTTGC GCTATCCTGGCTGACCATTAAG | $(CA)_{16}$ | Schwartz & Olsson (2008) | 2 | 184 - 195 | Pet |

## 2.5 Analysis of Microsatellite Data

*Screening for Scoring Errors and Loci under Selection*

Allele size(s) for each individual, at each locus were recorded and converted into file formats suitable for other programs using GENEPOP V4.6 (Rousset, 2016). The dataset was screened for scoring errors using the Brookfield 1 method (Brookfield, 1996) implemented in MICRO-CHECKER (Van Oosterhout *et al*., 2004) as it was possible that some alleles failed to amplify as a result of DNA degradation.

Compliance with Hardy-Weinberg Equilibrium (HWE) expectations was further tested using GENEPOP V4.6 (Rousset, 2016), as was linkage disequilibrium using a Markov chain method. Prior to undertaking full analysis of the dataset, each microsatellite locus was screened for its suitability for use in the study using a random sample of samples from the Source (Merseyside) population.

*Assessment of population structures*

Genetic diversity is measured using several metrics. The most common measure of genetic diversity used in conservation, heterozygosity, comes from population genetics theory (Frankham *et al*., 2010; Jamieson & Lacy, 2012).

Heterozygosity is the expected probability that an individual carries different alleles (heterozygote) at a single locus, or at an assay of different loci,

$$He = 1 - \frac{1}{m}\sum_{l=1}^{m}\sum_{i=1}^{k} p_i^2$$

where, $p_i$ is the frequency of the $i^{th}$ of $k$ alleles, and $m$ is the number of loci. The observed heterozygosity ($H_o$) in a population sample is frequently compared to that which would be expected ($H_e$) under conditions of random mating. Significant deviations will indicate that the population is experiencing an external driver or evolutionary force such as selection or inbreeding. Average heterozygosity is a measure of genetic diversity at the population scale and indicates the average proportion of individuals that are heterozygous for any given trait (locus) (Williamson, 2013).

Mean allelic richness is another commonly reported diversity index used in conservation genetics (Jamieson & Lacy, 2012). The method is useful as an alternative to heterozygosity as it is more sensitive to recent bottleneck events involving the loss of low frequency alleles.

GENEPOP V4.6 (Rousset, 2016) was utilised to check compliance with the HWE test for each locus, population, and genotypic linkage disequilibrium among loci pairs within each population (using a Markov chain method). GENEPOP V4.6 (Rousset, 2016) was further utilised to investigate genetic structure over all loci between sites and determine expected ($H_e$) and observed ($H_o$) heterozygosity. FSTAT v2.9.3 (Goudet, 1995) was used to calculate Allelic richness ($A_R$) for each sample site, and the inbreeding coefficient ($F_{is}$).

Population genetics studies frequently adjust critical *P*-values using the Bonferroni correction for multiple comparisons (Russell, 2012). This approach has been criticised as the power to correctly reject false null hypotheses is reduced. Therefore *P*-values were adjusted using a "False Discovery Rate" (FDR) procedure (Narum, 2006), a less conservative correction for multiple comparisons. This is calculated by dividing the desired critical level (e.g. 0.05) by the sum of one divided by the number of tests for each test (e.g. for five tests, $\alpha = 0.05/(1/1 + 1/2 + 1/3 + 1/4 + 1/5) = 0.022$) (Russell, 2012).

### *Detecting Bottlenecks*

Populations that have undergone a recent bottleneck event are known to suffer reductions in the number of alleles and a corresponding, but delayed reduction, in the observed heterozygosity. Genetic bottlenecks within the populations were tested for utilising the program BOTTLENECK v1.2.02 (Cornuet & Luikart, 1997) as BOTTLENECK is a program for detecting recent effective population size reductions from allele data frequencies (Cornuet & Luikart, 1997).

To determine if the populations exhibited a recent bottleneck event, two tests were applied: a "sign test" and a "Wilcoxon sign-rank test" (Luikart *et al.*, 1998), running 1000 replications. BOTTLENECK (Cornuet & Luikart, 1997) performs a third test, a "standardized differences" test (Cornuet & Luikart, 1997), but this could not be applied in this case as it requires a minimum of 20 loci and assumes normal distribution of heterozygosity across loci. The Wilcoxon sign test, however, does not assume this normal distribution and tests the hypothesis that the values of $H_e$ (expected heterozygosity) from the baseline and post selection cohorts (both separately and combined) are not different (Williamson, 2013). Furthermore, BOTTLENECK returns, for each population sample and for each locus, the distribution of the heterozygosity expected from the observed number of alleles (*k*), given the sample size (*n*) under the assumption of mutation-drift equilibrium. This distribution is obtained through simulating the coalescent process of *n* genes under three possible mutation models (Piry *et al.*, 1999): the “Infinite Allele Model” (I.A.M), the “Stepwise Mutation Model” (S.M.M), and the “Two Phase Model” (T.P.M). I.A.M is a mathematical model for calculating genetic mutations (Kimura & Crow, 1964), whereas S.M.M is a mathematical theory that allows for investigation of the equilibrium distribution of allelic frequencies in a finite population, where neutral alleles are produced in a step-wise fashion (Kimura & Ohta, 1978).T.M.P is described as an intermediate mutation model between I.A.M and S.M.M which is especially recommended for the study of microsatellite loci evolution (Di Rienzo *et al.*, 1994; Williamson, 2013). *P*-values for each observed heterozygote are calculated.

Results for all models are provided, however, emphasis is concentrated on the “Two Phase Model” since it is known to better model microsatellite evolution (Valdes *et al.*, 1993; Di Rienzo *et al.*, 1994; Williamson, 2013).

“Mode-Shift” analysis (Luikart *et al.*, 1998), implemented in BOTTLENECK, was utilised to further create allele distribution histograms. Low frequency alleles (indicated by L-shaped histograms in bottlenecked populations) are more likely to be lost during a bottleneck, hence a “mode shift” might be observed (Williamson, 2013).

# 3 Results

## 3.1 Population Viability analysis

Table 4.3 presents modelled summary values for sand lizard deterministic population growth rates, mean stochastic growth rate, male and female generation times, ratio of adult males to adult females, probability of extinction, median time to first extinction and Mean time to first extinction supported by standard error and standard deviation values, calculated for the individual models.

Table 4.3: Sand Lizard summary population viability values for the three research sites, between initial releases and October 2012 (Harlech) and October 2014 (Aberdyfi and Ynyslas). Yes (Y), No (N). Low (L) and High (H) values represent extremes calculated accounting for minimum and maximum carrying capacity variables.

| Site<br>Model | Inbreeding depression | Harlech | Aberdyfi | Ynyslas |
|---|---|---|---|---|
| ***det-r*** | Y | -0.0067 | 0.0061 | 0.0061 |
| | N | -0.0067 | 0.0061 | 0.0061 |
| ***stoc-r*** | Y | L: -0.0738<br>H: -0.0724 | L: -0.0678<br>H: -0.0592 | L: -0.0677<br>H: -0.0697 |
| | N | L: -0.0449<br>H: -0.0476 | L: -0.0447<br>H: -0.0362 | L: -0.0615<br>H: -0.0395 |
| ***gen-m*** | Y | 5.23 | 5.16 | 5.16 |
| | N | 5.23 | 5.16 | 5.16 |
| ***gen-f*** | Y | 6.86 | 6.77 | 6.77 |
| | N | 6.86 | 6.77 | 6.77 |
| ***adu m/f*** | Y | 0.894 | 0.913 | 0.571 |
| | N | 0.894 | 0.913 | 0.571 |
| ***PE*** | Y | L: 100%<br>H: 99.8% | L: 100%<br>H: 100% | L: 100%<br>H: 99.6% |
| | N | L: 100%<br>H: 95% | L: 100%<br>H: 100% | L:100%<br>H:91.1% |
| ***Median TE*** | Y | L: 12yrs<br>H: 43yrs | L: 4yrs<br>H: 54yrs | L: 10yrs<br>H: 40yrs |
| | N | L: 12yrs<br>H: 54yrs | L: 4yrs<br>H: 71yrs | L: 10yrs<br>H: 55yrs |
| ***Mean TE*** | Y | L: 13.5yrs<br>H: 48.9yrs | L: 5.6yrs<br>H: 66.2yrs | L: 11.4yrs<br>H: 48.3yrs |
| | N | L: 15.4yrs<br>H: 63.1yrs | L: 5.6yrs<br>H: 103.2yrs | L: 12.2yrs<br>H: 63.1yrs |

Median & Mean time to extinction (Median *TE*/Mean *TE*) with inbreeding depression expressed indicates that sand lizard populations across the three sites have been calculated to survive between 4 yrs/5.6 yrs (Aberdyfi 0.15 SE, 4.64 SD) and 54 yrs/66.2 yrs (Aberdyfi 1.52 SE, 48.10 SD). Population survival rates with inbreeding depression supressed provide similar trends across the three sites.

A slightly negative deterministic growth rate (*det-r*) of -0.0067 was observed at Harlech compared to slightly positive values of 0.0061 at Aberdyfi and Ynyslas with inbreeding depression expressed and supressed. This is not supported however by the stochastic results (*stoc-r*). Stochastic results indicate a negative growth rate of between 0.0362 – 0.0738 (with lower values representative of models with inbreeding depression supressed).

Male and female generation times (respectively) are similar across the three sites, although adult male/female ratios vary between 0.571 (Ynyslas) and 0.913 (Aberdyfi).

Further models were run utilising parameters from the individual dune systems (Strijbosch & Creemers, 1988). These models identified a probability of extinction of between 90.1% - 100% with a 100% *PE* identified for minimum and maximum values for the Aberdyfi site.

In the absence of phenotypical placicity Median & Mean time to extinction rates (Median *TE*/Mean *TE*) indicates that sand lizard populations across the three sites have been calculated to survive between 41yrs/49.3yrs (Ynyslas 1.07 SE, 33.83 SD) and 75 yrs/97.4 yrs (Aberdyfi 2.58 SE, 81.51 SD). Deterministic growth rates (*det-r*) and stochastic results (*stoc-r*) follow a similar patter to that described for the general results, indicating a negative growth rate with lower values representative of models with inbreeding depression supressed. Male and female generation times (respectively) are similar across the three sites, although adult male/female ratios vary between 0.571 (Ynyslas) and 0.913 (Aberdyfi).

### 3.2 Microsatellite

A total of 89 sand lizards were sampled for their genetics across the three research sites, the source population and captive breeders (31, 36 and 22 respectively). Of these, 98.87% of the samples successfully amplified at a sufficient number of loci to be included within the analysis. Blind re-genotyping identified only one mis-scored allele out of a total of 79, giving a genotyping error rate of 1.12%. An error rate of 2% or less is unlikely to significantly bias the results of the estimation of genetic diversity (Bonin *et al*., 2004) therefore the 1.12% error rate was considered acceptable.

During the initial screening *La*02, *La*3 and *La*64 failed to amplify for the majority of the sample sites and captive breeder populations (with the exception of the source stock population): these loci were consequently excluded from further use. This left a suite of 15 microsatellite loci for the source population and suite of 13 microsatellite loci for the research sites and captive-bred populations. These results reflected those of Gullberg *et al*. (1997) and Russell (2012) with *La*6 successfully amplified in this study whereas in a previous study of British *L. agilis* it produced no PCR products (Beebee & Rowe, 2001).

No significant deviations from expected Hardy-Weinberg proportions were detected at any locus in any sampling group. Furthermore, no deviations from Hardy-Weinberg expectations was observed when all the sampled groups were combined ($\chi 2$ (30) = 178, $p$ =0.116, > 0.005) after Bonferroni corrections. A familywise error rate (FWER) controlling procedures (Bonferroni correction) was adopted to reduce the number of Type I errors (Shaffer, 1995).

The total number of alleles sampled at each locus ranged from 1 (*La*64) to 5 (*La*2). In the introduced populations at Harlech, Aberdyfi and Ynyslas, the number of alleles per locus (allelic richness) ranged between 1 (*La*3, *La*6, *La*01, *La*02, *La*3e, *La*50, La64) and 4 (*La*2, *La*10, *La*12). In the source population, the numbers of alleles per locus ranged from 1 (*La*3, *La*64) to 5 (*La*2) and the captive-bred populations the numbers of alleles per locus ranged from 1 (*La*3, *La*01, *La*50, *La*64, *La*10, *La*27) to 4 (*La*2, *La*04) respectively (see Table 4.4).

A simple system of colour coding has been applied to all information presented in Tables and Figures in this Chapter. This colour coding provides a quick visual reference to the species groups which the information pertains too. Blue represents reintroduced, orange for source and yellow for ex-situ, captive bred sand lizard populations.

**Table 4.4:** No of alleles per locus observed for the reintroduced, source and ex-situ captive breeding programme (– indicates absence of allele at that population).

| Locus | Merseyside | Harlech | Aberdyfi | Ynyslas | Chester | Blackpool | Penrith |
|---|---|---|---|---|---|---|---|
| **La2** | 5 | 3 | 4 | 4 | 4 | 3 | 2 |
| **La3** | 1 | 1 | 1 | 1 | 1 | 2 | 2 |
| **La4** | 2 | 3 | 2 | 2 | 2 | 3 | 2 |
| **La6** | 3 | 1 | 2 | 3 | 4 | 3 | 2 |
| **La9** | 2 | 2 | 2 | 2 | 3 | 4 | 2 |
| **La01** | 3 | 1 | 1 | 2 | 1 | 1 | 1 |
| **La02** | 2 | 3 | 1 | - | - | - | - |
| **La3e** | 3 | 3 | 1 | - | - | - | - |
| **La50** | 2 | 1 | 1 | 2 | 1 | 1 | - |
| **La64** | 1 | 1 | - | - | 1 | - | - |
| **La04** | 3 | 3 | 3 | 3 | 4 | 3 | 2 |
| **La10** | 4 | 3 | 3 | 4 | 2 | 1 | 2 |
| **La12** | 3 | 2 | 5 | 4 | 3 | 3 | 3 |
| **La27** | 4 | 3 | 3 | 3 | 3 | 1 | 1 |
| **La40** | 4 | 3 | 5 | 2 | 2 | 2 | 2 |

The mean expected heterozygosity (all loci) for the introduced populations, source population and captive bread populations were 0.337 (Harlech), 0.304 (Aberdyfi), 0.348 (Ynyslas), 0.314 (Merseyside), 0.271 (Chester), 0.359 (Blackpool) and 0.342 (Penrith), and the mean observed heterozygosity values were 0.354, 0.367, 0.386, 0.317, 0.271, 0.359 and 0.342 respectively. Allelic richness (which, in this study, like that of Russell's (2012) is more appropriate than the mean number of alleles per locus, as it compensates for sample size), observed and expected heterozygosity and $F_{IS}$ was calculated for each sample site (see Table 4.5).

Genetic diversity was found to be higher in the source population (Merseyside $A_R$ = 4.351, $H_o$ = 0.317, $H_e$ = 0.314) in comparison to the three reintroduction sites Harlech ($A_R$ = 2.217, $H_o$ = 0.354, $H_e$ = 0.337), Aberdyfi ($A_R$ = 1.712, $H_o$ = 0.367, $H_e$ = 0.304) and Ynyslas ($A_R$ = 2.999, $H_e$ = 0.386, $H_e$ = 0.348). The levels of diversity for the reintroduction sites were all lower than those calculated in other British populations explored by Russell (2012). The captive-bred populations exhibited an even lower level of diversity than the source or reintroduction populations Chester ($A_R$ = 1.632, $H_o$ = 0.276, $H_e$ = 0.271), Blackpool ($A_R$ = 2.557, $H_o$ = 0.288, $H_e$ = 0.359) and Penrith ($A_R$ = 1.933, $H_o$ = 0.366, $H_e$ = 0.342).

Inbreeding was high across all populations, against those calculated for 23 other sand lizard populations by Russell (2012), with an average $F_{IS}$ of 0.375 at the source population (1.000 indicates a population which is fully inbred). 0.499 was observed across the three reintroduction sites and 0.782 with the captive breeders. The highest level of inbreeding was observed in the captive-bred populations ($F_{IS}$ = 0.450, 0.947 and 0.949). Of the reintroduced populations, Ynyslas indicates the highest level of inbreeding: $F_{IS}$ = 0.545 (see Table 4.5).

**Table 4.5:** Standard indices of genetic diversity for each sampling location. n = number of samples, N = average number of alleles per locus, $A_R$ = allelic richness, $H_o$ = observed heterozygosity, $H_e$ = expected heterozygosity, $F_{IS}$ = inbreeding coefficient.

| Sample site | *n* | *N* | $A_R$ | $H_o$ | $H_e$ | $F_{IS}$ |
|---|---|---|---|---|---|---|
| **Merseyside** | 36 | 2.800 | 4.351 | 0.317 | 0.314 | 0.375 |
| **Harlech** | 17 | 2.200 | 2.217 | 0.354 | 0.337 | 0.496 |
| **Aberdyfi** | 6 | 2.266 | 1.712 | 0.367 | 0.304 | 0.456 |
| **Ynyslas** | 8 | 2.133 | 2.999 | 0.386 | 0.348 | 0.545 |
| **Chester** | 15 | 2.066 | 1.632 | 0.276 | 0.271 | 0.450 |
| **Blackpool** | 4 | 1.800 | 2.557 | 0.288 | 0.359 | 0.947 |
| **Penrith** | 3 | 1.400 | 1.933 | 0.366 | 0.342 | 0.949 |

Neither source nor reintroduced sample groups returned significant heterozygote excess under the IAM, significant *P*-values (Wilcoxon-signed-rank test) under the T.M.P model, or by the sign rank test under S.M.M (Table 4.6). The only sample groups which have significant heterozygote excess under the IAM were those of the private captive breeders. Significant *P*-values were also returned by the Wilcoxon-signed-rank test for these samples under the T.M.P model and by the sign rank test samples under S.M.M (see Table 4.6).

**Table 4.6:** Bottleneck testing using excess heterozygosity. Infinite Allele Model (IAM), Two Phase Model (TPM), Stepwise Mutation Model (SMM). * Model most appropriate for use with microsatellites. Bold p-values are significant.

| Sample site | Test | I.A.M | T.P.M* | S.M.M |
|---|---|---|---|---|
| Merseyside | *Sign* | 0.523 | 0.205 | 0.061 |
| | *Wilcox one tail for HE* | 0.367 | 0.772 | 0.959 |
| Harlech | *Sign* | 0.069 | 0.100 | 0.157 |
| | *Wilcox one tail for HE* | 0.006 | 0.116 | 0.215 |
| Aberdyfi | *Sign* | 0.304 | 0.365 | 0.445 |
| | *Wilcox one tail for HE* | 0.285 | 0.500 | 0.589 |
| Ynyslas | *Sign* | 0.329 | 0.350 | 0.187 |
| | *Wilcox one tail for HE* | 0.206 | 0.415 | 0.740 |
| Chester | *Sign* | 0.304 | 0.351 | 0.596 |
| | *Wilcox one tail for HE* | 0.179 | 0.410 | 0.714 |
| Blackpool | *Sign* | **0.005** | **0.009** | **0.021** |
| | *Wilcox one tail for HE* | **0.001** | **0.001** | **0.001** |
| Penrith | *Sign* | **0.000** | **0.001** | **0.003** |
| | *Wilcox one tail for HE* | **0.000** | **0.000** | **0.000** |

No L-shaped distribution was provided for the sampling groups frequency distribution (Fig. 4.1a, b) or when cohort groups were combined (Figure 4.1c). Nevertheless, a progressive shift towards alleles of moderate to high frequency was observed across the groups, with low frequency alleles being typically less prevalent in each sampling group.

The program BOTTLENECK designates allele frequency categories: the proportion of the total number of alleles across all loci. Alleles that occur at frequencies < 0.1 are indicated in category 1 and similarly, the proportion of alleles across all loci that occur at frequencies ≥ 0.1 and < 0.2 are indicated in category 2 etc. (Williamson, 2013). In populations which have not experienced a recent bottleneck most alleles occur at low frequencies (category 1: <0.1). A shift towards higher frequency categories (categories 3 to 10), like those observed in the source, captive bred and reintroduced populations is observed when low frequency alleles are lost due to chance or selection. Such 'mode shifts' are observed in typically bottlenecked populations. Similar results were observed when the three captive bred populations at Chester, Blackpool and Penrith were considered independent or combined (see Figures 4.1c).

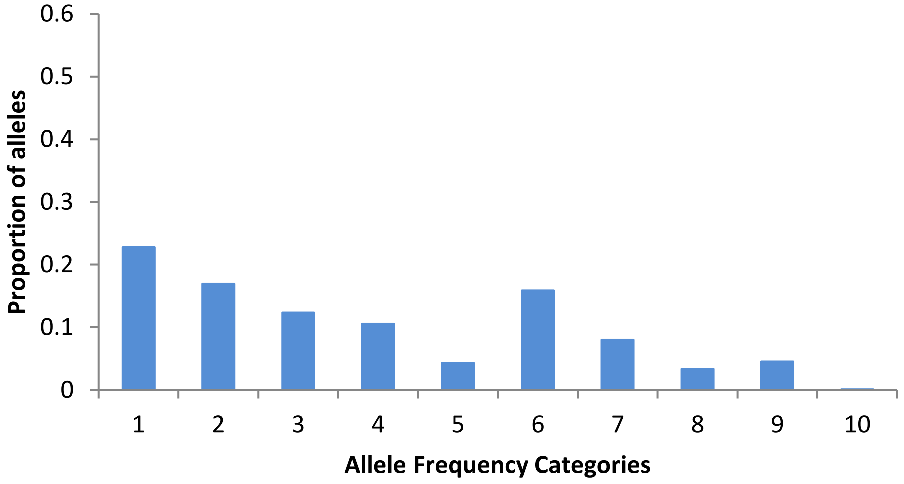


**Figure 4.1a:** Allele frequency graph indicating loss of low frequency alleles at the three reintroduction sites (Harlech, Aberdyfi and Ynyslas).

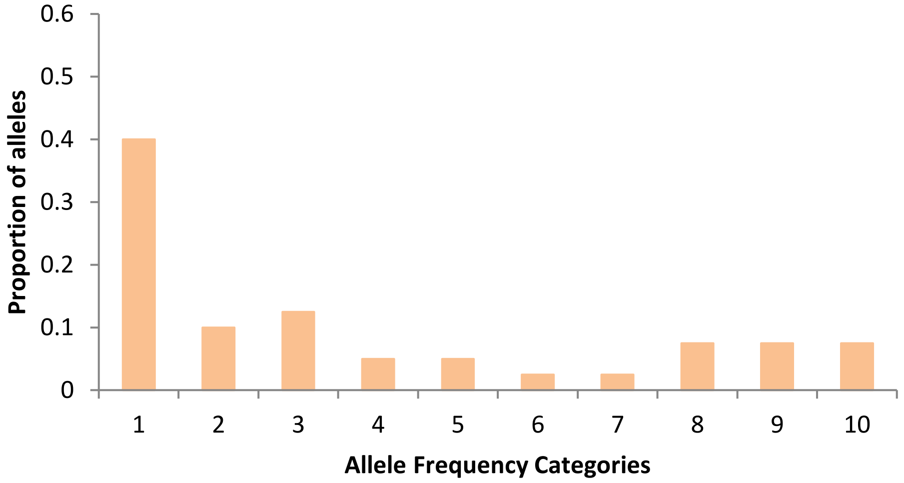


**Figure 4.1b:** Allele frequency graph indicating loss of low frequency alleles at the source population (Merseyside).

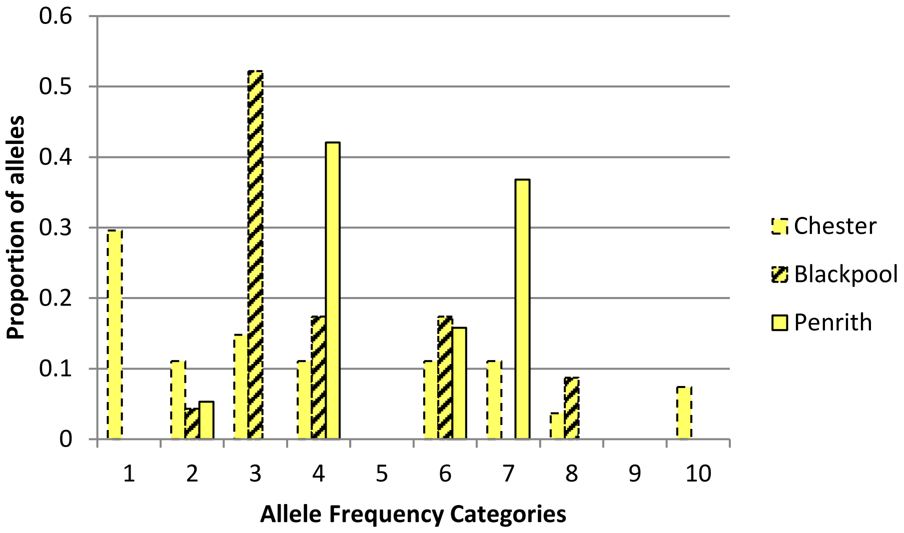


**Figure 4.1c:** Allele frequency graph indicating loss of low frequency alleles at the three captive bred populations (Chester, Blackpool and Penrith).

# 4 Discussion

Few studies have thoroughly investigated reintroduction populations specifically for their long-term viability and changes in key genetic parameters relative to their source population, including that undertaken for sand lizards in Wales by ARC. The scope of this Chapter was to investigate the viability of individual reintroduced populations and the genetic health of the individual sand lizard populations within the reintroduction program.

This study has revealed short to medium population viability between populations along with low levels of genetic diversity within the reintroduced sand lizard populations. These findings are in accordance with previous research undertaken in this field (Frankham *et al.*, 2010; Smallbone *et al.*, 2016 & Futuyma, 2013) that states that populations of conservation concern suffer low viability, accelerated inbreeding and loss of genetic diversity. Beck *et al.* (1994) considered only 11% of reintroduction programmes to be successful, based upon the criterion that reintroduced populations had reached at least 500 individuals, free from human support. A second study classified 26% of reintroductions, plus some translocations, as successful and 27% as failures (Fischer & Lindenmayer, 2000). Success rates were higher when larger numbers of animals were released and when the cause of the original decline was removed (Frankham *et al*., 2010). A brief review of the field of reintroduction biology by Ewen *et al.* (2012) indicated an early awareness of the need for translocation programmes to ensure adequate genetic diversity and viability in reintroduced populations.

There are many practical considerations when a reintroduction is contemplated. Genetics may play a relatively minor role in some decisions (Kleiman *et al*., 2009), but should not be ignored, as often occurs. There will be a higher probability of a successful reintroduction programme if all issues are initially considered. Conservation biologists and practitioners, including Government and Non-Government Organisations, need an understanding of how genetic diversity and a populations viability is maintained through natural processes, if conservation programmes or reintroduced populations of endangered species are to be managed effectively (Frankham *et al*., 2010). Unlike several other disciplines, genetics has the potential to provide this information without direct observation. This includes information about past changes in population size due to natural and anthropogenic impacts, suitability of likely source populations for captive breeding or translocations, inbreeding and relatedness of captively bred individuals, and identity of reintroduced individuals (Attard *et al*., 2016).

## 4.1 Population Viability Analysis

These smaller and isolated reintroduced sand lizard populations are vulnerable to stochastic effects such as changes in the population structure, mutations, catastrophes (high tides, strong winds and excessive build-up of winter sand) and external pressures due to changes of the immediate environment such as climate, disease, and habitat (Henle *et al*., 1999 in Blanke & Fearnley 2015).

Minimum and maximum ‘probability of extinction’ values for the three research sites identified a probability of extinction of between 90.1% - 100% with a 100% *PE* identified for both minimum and maximum values for the Aberdyfi site. Mean time to extinction estimates calculated for the Aberdyfi site indicates a population survival of between 5.6yrs (0.15 SE, 4.64 SD) and 66.2yrs (1.52 SE, 48.10 SD) with inbreeding expressed. With only 35 observations of individuals between March 2012 and October 2014, representative of a smaller number of individuals at the site, this aligns with lower extinction rates of five years (detailed above) modelled by Märtens & Stephan (1997).

Mean time to extinction estimates calculated for Harlech and Ynyslas indicate that the two populations are anticipated to survive between 11.4yrs (0.24 DE, 32.54 SD) and 48.9yrs (0.92 SE, 29.08 SD) with inbreeding expressed. In the absence of inbreeding depression, the upper values for survival at the two sites increases to 63.1yrs (1.40 SE, 43.14 SD).

The low upper survival values for the three sites is representative of negative mean stochastic growth rates forecasted for the sites: -0.0592 to -0.0738. This negative mean stochastic growth rate is evident with both inbreeding depression expressed and supressed. Blanke & Fearnley (2015) indicate that sex ratios do not generally deviate significantly from a 1 :1 distribution, however, these low upper survival values are further supported by an adult sex ratio of 0.571 : 1 - 0.913 : 1 (m : f) calculated for the sites. Sex ratios of 1 : 1 (Harlech), 3.6 : 1 (Aberdyfi) and 2 : 1 (Ynyslas) were observed during site surveys, however, Blanke & Fearnley, 2015 state that more males are observed earlier in the season and more females later in the season with Meister (2008) reporting a male : female ratio of 3.5 : 1.0 in May and 0.2 : 1.0 in October: which may explain discrepancies between the values.

Russell's (2012) research concluded that dune system sand lizard populations from Merseyside, England, which have had historically lower population sizes, were significantly less genetically diverse. A similar effect also noted in fragmented sand lizard populations from Sweden (Gullberg *et al*., 1998). Märtens & Stephan (1997) have modelled the persistence of different sized sand lizard populations. Their simulations indicate that from a population of 10 individuals (five males and five females) approximately 10% of the populations became extinct after five years, and approximately 50% of the populations were extinct after ten years. From their models, no populations survived beyond 35 years. Quite the opposite was found when a population scenario of 500 lizards (250 males and 250 females) was modelled. Only 5% of the populations were extinct after 45.4 years, 40% became extinct after 100 years, and some populations were persisting after 500 years (Blanke & Fearnley, 2015).

The sand lizard population at Aberdyfi on the brink of potential extinction (with a dramatic decline in numbers observed during field research), the Harlech and Ynyslas populations are approximately 28 – 43 years from a similar position. It is important to focus conservation effort on these smaller or isolated populations as although scattered, they are also important for the conservation of the species (Blanke & Fearnley, 2015).

## 4.2 Genetic diversity

The genetic diversity of the North-West Wales reintroduced populations was observed to be lower than the source population (Merseyside $A_R$ = 4.351, $H_o$ = 0.317, $H_e$ = 0.314). This loss of genetic diversity is indicated to have resulted from the extremely low levels of diversity in the origin of the reintroduced individuals within the captive bred populations of Chester, Blackpool and Penrith. The practice of breeding release individuals from the ex-situ populations held by the captive breeders over numerous years (i.e., until individuals die and are replaced) results in potentially advantageous alleles being lost.

With typically <200 individuals released at each reintroduction site, over numerous years, a further loss of advantageous alleles and genetic diversity would be anticipated post-release. This is supported by the genetic diversity of the reintroduced populations all being lower than those calculated in other natural British populations explored by Russell (2012). It is already known that smaller populations are vulnerable to inbreeding and genetic drift, which reduces genetic diversity. This reduction in genetic diversity is further known to lead to the fixing of disadvantageous deleterious mutations, inbreeding depression and generally reduced levels of adaptability and fitness (Veith *et al.*, 1999; Russell, 2012; Blanke & Fearnley, 2015).

With inbreeding high across all study populations, with an average $F_{IS}$ of 0.375 at the source population, compared to $F_{IS}$ 0.499 across the three reintroduction sites and $F_{IS}$ 0.782 with the captive breeders, this can potentially reduce population growth rates and increase extinction risk. This has been demonstrated in experimental fish, plant and insect populations (Leberg, 1993; Newman & Pilson, 1997; Bijlsma *et al.*, 2000; Ewen *et al.*, 2012). No L-shaped distribution was provided for the sampling groups frequency distribution (Figures 4.1a, b & c) even when cohort groups were combined (Figure 4.1b). A time-based relationship shift towards alleles of moderate to high frequency was observed across the groups, with low frequency alleles being typically less prevalent in each sampling group. An evaluation of allele frequency categories and the $F_{IS}$ values revealed a shift towards higher frequency categories in the source, captive bred and reintroduced populations. Such observations are typically observed when low frequency alleles are lost. Such 'mode shifts' are observed in bottlenecked populations.

In contrast to this general loss of genetic diversity, the genetic diversity of the Ynyslas reintroduction population ($A_R$ = 2.999, $H_o$ = 0.386, $H_e$ = 0.348) was observed to be higher than the other release sites anticipated to have not received individuals of the

Dorset race. With no background information pertaining to the original location, number, or sexes of the Dorset individuals released, however, an evaluation of the effect of this mixing of ‘races’ should be investigated further.

Based on the genetic findings, an investigation into the genetic status of further sand lizard populations should be undertaken. Similar to the genetic findings of this study, research undertaken by Beebee and Rowe, as early as 2001, and by Blanke & Fearnley (2015) identified substantial recent declines of sand lizard effective population sizes (bottlenecks); Merseyside and Surrey. This independent genetic assessment of the fate of British sand lizards undertaken by Beebee and Rowe (2001) concurs with conclusions derived from direct field studies throughout the 1990’s (Corbett, 1994; Moulton & Corbett, 1999). Elsewhere, surveys of genetic diversity in serially bottlenecked populations of dice snakes *Natrix tessellate* revealed significant loss of genetic variation and increased frequency of scale abnormalities, implying that the effects of multiple bottlenecks incurred as a consequence of conservation management cannot be ignored (Gautschi *et al*., 2002).

Russell (2012) undertook further studies on UK and Swedish sand lizard populations, investigating their genetic diversity and structuring. Russell (2012) found that the Dorset (UK) populations sampled were genetically diverse, even those with a small effective population size Levels of genetic diversity were similar to those from the continent (Bergherbos, with an $H_e$ of 0.74) and a large population in (Hungary with an $H_e$ of 0.67), Gullberg *et al*., 1998; Schwartz & Olsson, 2008; Russell, 2012). Russell (2012) also described the Dorset populations as having a considerably higher genetic diversity than a number of populations in Sweden, which averaged $H_e$ of 0.451 across the ten surveyed sites (Gullberg *et al*., 1998). However, the populations from Surrey and Merseyside (Merseyside being the source stock population for reintroductions to Harlech, Aberdyfi and Ynyslas), which have had historically lower population sizes, were significantly less diverse (Russell, 2012).

Beebee & Rowe (2001) and Russell (2012) identified a correlation between population sizes and genetic diversity in British sand lizards emphasising the importance of conservation measures in their heathland and dune habitats (Blanke & Fearnley, 2015). Furthermore, Russell’s (2012) research identified that there was a loss of genetic diversity in the reintroduced populations investigated compared to their source population(s). However, the loss seemed unlikely to have a significant effect on the long-term persistence of the populations as the absence of evidence of a bottleneck and the comparable levels of genetic diversity in these reintroduced populations (compared to the Dorset populations) suggests that a sufficient number of animals were translocated to avoid a founder effect and levels of genetic diversity were sufficient to limit the effects of genetic stochasticity.

A genetic evaluation of a recently discovered sand lizard population on the Isle of Anglesey (North Wales), undertaken by Russell (2012), concluded that it was ‘highly unlikely’ to be of natural origin. Divergence times between the Aberffraw and Ainsdale populations suggested that it was inconceivable that *L. agilis* colonised Anglesey ca. 9,000 years BP, or reached Anglesey by rafting. Low levels of differentiation supported this when compared with other pairwise comparisons between sample sites (Surrey, Dorset and Merseyside). STRUCTURE, BAPS and Ima2 analysis supported the results, indicating a recent, human-mediated origin for the Aberffraw population; most likely an introduction of animals originating from Merseyside.

Despite these factors, under certain circumstances, sand lizards seem to be able to survive for long periods of time in small and isolated populations, an example of which are those found on the German islands in the North Sea. Furthermore, in 1970, 51 Dorset-race sand lizards were introduced on to the small Hebridean Island of Coll. Arnold (1995) and Edgar & Bird (2005) confirm this population still exists (Blanke & Fearnley, 2015), although no genetic evaluation of this population has been undertaken, its continued presence could be an indication that this time period might be too short to expect the loss of such a population.

## 4.3 Reintroduction

One of the main genetic goals for the establishment phase of an introduction is for the released individuals to be as representative a sample of the source population as possible. In an ideal situation, the founders would be fully representative genetically of the source population (Ewen *et al*., 2012). This study identifies the lower genetic diversity of populations held within the ex-situ captive breeding programme and subsequently released onto reintroduced sites than the source population in Merseyside. This is with the exception of the Ynyslas population which was proven to be higher than the other release sites anticipated to have not received individuals of the Dorset race.

This study further demonstrates the findings of Saccheri *et al*. (1998), Frankham (2005) and Ewen *et al*. (2012) in that the loss of genetic diversity and increased levels of inbreeding in a reintroduced population can lead to problems associated with inbreeding depression, a reduced ability to adapt and, consequently, an increased extinction risk.

The evolutionary impact of a deliberate or unintentional introduction into wild populations of non-native and/or domesticated individuals, such as the mixing of races released at Ynyslas, is a growing concern for the management of endangered species (Allendorf, 2001; Facon, 2006; Attard *et al.*, 2016). It must be noted that the genetic make-up of native Welsh *L. agilis* (pre-extinction) cannot be established due to the absence of a 'type' species. With the reintroduced population at Ynyslas established without only pure breed Merseyside lizards (Moulton, Pers Coms., 2012), Ewen *et al.* (2012) propose a number of considerations when deciding whether multiple source populations should be merged for releases in a single reintroduction project:

1. If the potential source populations fragmented less than ~ 150 years ago, then there may not be significant genetic divergence, especially in allele diversity, so sourcing individuals from the largest or closest source population is probably a safe strategy;
2. If two or more populations have been separated historically (more than ~ 1000 years), they are likely to show significant genetic divergence and possibly locally adapted gene complexes if their local environments and habitats are significantly different (this cannot be confirmed for Welsh *L. agailis*). If so, then it would be best to source from the population in the habitat that is most similar to that at the reintroduction site. Local adaptations can originate rapidly, even in mobile species, and generate genetically differentiated ecotypes (Musiani *et al*., 2007; Mucci *et al*., 2010);
3. If the reintroduction is to occur to an area that is well outside the current range of the available source populations, or the habitat is highly altered relative to the environment in which the source population had evolved, then it would often be better to release a genetically mixed population (one that has the best chance to adapt to that altered habitat); and
4. Mixing of separate subspecies in a single reintroduction should normally be avoided because it could lead to outbreeding depression (Frankham *et al*., 2011). Unfortunately, subspecies designation is often not supported by good evidence, but instead is based on minor morphological variants that do not reflect isolated

> geographical populations with separate evolutionary histories. For example, Culver *et al.* (2000) found no molecular genetics support for the Florida panther (*Puma concolor coryi*) being designated a separate subspecies from the rest of the pumas in the USA. The remnant Florida population was so damaged genetically that it could not have been recovered as an isolated population. The mixed reintroduction resulted in a dramatic decline in observed frequency of deleterious genetic traits in animals as well as a rapid recovery of numbers (Hedrick & Fredrickson, 2010).

Beebee & Rowe (2001) suggested that for conservation purposes, populations in Dorset, Surrey and Merseyside should be considered as distinct clades, worthy of protection in their own right (in the absence of a 'type' specimen from the pre-extinction Welsh population(s)). Estimators of genetic differentiation revealed significant differences between the three areas that are consistent with separation of the regions at roughly the same time, presumably soon after postglacial colonization when forest development eliminated intervening open habitats (Vincent, 1990). Therefore, it is crucial to document and understand the consequences of supplementation on the genetic makeup of populations and life-history traits of individuals to mitigate threats to wild populations, maximizing their evolutionary potential over the long term (Attard *et al.*, 2016).

Considering the findings presented in this Chapter and that of previous studies and conservation theories, conservation practitioners involved in the sand lizard reintroduction programme need to further evaluate the cost benefit analysis approach of mixed populations for future reintroductions. Conservation strategies for the development of the reintroduction programme are discussed in Chapter 5, which include mixed race populations, re-enforcement (IUCN, 1998) or post hoc secondary releases (Armstrong & Ewen, 2001).

### 4.4 Species Sampling l

A strong case for the sampling of lizards to support this research was presented to the *Lacerta agilis* captive breeders (supported by ARC) on numerous occasions. Refusal of sampling all individuals held within the captive bred populations may have effected calculations on the genetic diversity of the populations. Except for Chester Zoo, who permitted the sampling of individuals using this methodology, the private captive breeders only permitted the collection of genetic material utilising an egg sampling approach, with samples collected by the individual captive breeders, even though evidence had been provided to the contrary (detailed below). To fully understand the genetic diversity of these ex-situ populations and any level of bottlenecking experienced, a survey of all individuals is required, which would contribute to the development of more appropriate conservation measures which are discussed further in Chapter 5.

Toe-clipping has been historically used to individually mark and take genetic samples from a wide variety of organisms including amphibians and reptiles (e.g. Clarke, 1972; Waichman, 1992; Donnelly *et al*., 1994; Beebee & Rowe, 2001; Davis & Ovaska, 2001; Funk *et al*., 2003; Phillott *et al*., 2007. The use of the procedure has declined in many taxonomic groups (Perry *et al*., 2011). In herpetology, however, the method remains very common for marking amphibian and reptiles (e.g. Guarino *et al*., 2015; Sousa *et al*., 2016). Over a decade ago, Parris & McCarthy (2001) and McCarthy & Parry (2004) used statistical models to re-evaluate previous data and concluded that removal of multiple toes results in measurable reduction in return rates in mark–recapture studies of frogs, 4–11% per toe removed (Perry *et al*., 2011). Although not necessarily utilising mark-recapture methods many of the earlier genetic surveys of British reptiles and amphibians, including those undertaken by the likes of Beebee & Rowe (2001) and Cunningham *et al.* (2005) utilised toe-clipping as a methodology.

Non-invasive and non-destructive tissue sampling methods for DNA analysis are preferred in the field of conservation genetics (Pidancier *et al*., 2003). With welfare being of increased concern (Perry *et al*., 2011), buccal swabbing has become a routine non-destructive technique for the DNA sampling of reptiles in the UK, including sand lizards (Poschadel & Moller, 2004; Beebee, 2008; Russell, 2012), producing no obvious signs of stress or tissue damage (Beebee 2008; Russell, 2012). It has also been established that swabbing also, ‘only’ takes a couple of minutes avoiding the need to handle the animal for a long period of time (Russell, 2012). Research on DNA extraction, through buccal swabbing, as a methodology also showed that no deaths as a result of the procedure were encountered during 15 days post-sampling monitoring. Poschadel & Moller (2004) monitored individuals after the buccal swabbing for several months. While in captivity, they were inspected daily for fungal or bacterial infections of the oral cavity. None of them showed any such infections or any apparent changes in behaviour, with growth and condition parameters not affected compared to those of un-sampled individuals.

Genetic issues in captive populations are of central importance to reintroduction biology (Robert, 2009; Ewen *et al*., 2012). Without careful pedigree or genetic marker monitoring, genetic composition or reintroduced populations may be distorted, as occurred in both the Mauna Kea silverswords *Argyroxiphium sandwicense ssp. sandwicense* and Galápagos tortoises *Chelonoidis nigra* (Milinkovitch *et al*., 2004; Frankham *et al*., 2010). Low numbers of samples provided by the private captive breeders (Blackpool 4, Penrith 3) compared to the number of individuals held, may have provided a skewed representation of the genetic diversity present within the captive bred populations. Although detailed within the Natural England species conservation licence, full access should be provided by private captive breeders to facilitate sampling of populations maintained, along with future individuals recruited. Sampling procedures are discussed further in Chapter 5.

# Chapter 5

## Conservation Considerations and Prescriptions for *Zootoca vivipara* and *Lacerta agilis* in Wales

# 1 Introduction

This thesis has increased the understanding of UK dune system Lacertidae lizards through standardising field survey techniques for the monitoring and evaluation of dune system sand and viviparous lizard populations (Chapter 2), identifying habitat management prescriptions (Chapter 3) and increasing the knowledge on reintroduced populations viability and their conservation genetics (Chapter 4). Thesis findings should inform the future conservation of sand *Lacerta agilis* and *Zootoca vivipara* in north-west Wales. This Chapter evaluates methodologies employed for mammal and reptile species and their habitats, which could be implemented directly or adapted to improve the management of existing and future reintroduced sand lizard populations. Based on the results of the previous Chapters. This Chapter further details limitations in existing conservation strategies related to reintroduced sand lizard populations and prescribing strategies to be utilised to increase the favourable conservation status of the sand lizard and halt declining viviparous lizard numbers.

## 1.1 Site Protection

Locations where sand lizards have been reintroduced across north-west Wales are subject to EU and/or UK protection, however, this is not due to species presence. Guidelines for the selection of biological SSSIs are provided by the Joint Nature Conservation Committee (JNCC, 1989), which include reptiles. These Guidelines are under revision (expected 2017) but currently include the following recommendations:

*Reptile sites, at least for the endangered species, will be sand-dunes and lowland heaths. Where there is contiguous, open, semi-natural habitat, this should be included even though reptiles may not have been recorded in all parts of the site. Suitable man-made structures (e.g. tumuli, embankments and stone walls) should also be included. There should be a presumption for selection of reptile sites on the following grounds.*

- *In Dorset all important and established populations of smooth snake* Coronella austriaca *and sand lizard* Lacerta agilis *should be selected; for sand lizards, sites might be considered "important" because of the overall strength of a dispersed population or because of the presence of an apparently discrete colony or colonies. In other counties all established populations should be selected.*

With only 56 individual sites in the UK (Edgar, 2015) (representing 1.35% of designated sites in the UK) notified for their reptile assemblages, none of the research sites is included in here. Reptiles, are infrequently listed as a ‘Reason for Notification’ in SSSIs or as an Annex II ‘species that is a primary reason for selection of the site/ species present as a qualifying feature, but not a primary reason for site selection’ in SACs. Lack of protection within the designation of the site frequently results in habitat management targeted towards ‘target’ species or habitats and not necessarily targeted to maintaining or increasing the habitat available to the reptile species or assemblage present on a site.

This finding reveals the importance of reviewing the ‘Reason for Notification’ in SSSIs. Species and their associated habitat management should be a compromise addressing all species and their habitat requirements. In the absence of the requirement within a sites citation, management has to be target to species which will maintain the ‘conservation status’ of the site.

Under the terms of the Bern Convention on the Conservation of European Wildlife and Natural Habitats, the United Kingdom (2012), as a contracting party, is required to take the necessary legislative and administrative measures to ensure the conservation of habitats important for sand lizards (JNCC, 1989; Edgar & Bird, 2005). Consultation with the Department for Environment, Food & Rural Affairs (DEFRA) and the Joint Nature Conservation Committee (JNCC) is required, to influence future decision making regarding the protection of these species and sites containing ‘said’ species once the UK has exited the European Union. This should be driven by Amphibian and Reptile Conservation (ARC) and the Amphibian & Reptile Groups of the UK (ARG) as a unified approach.

## 1.2 Habitat Assessment

Based on the thesis findings, it is recommended that concentrated site conservation prescriptions and management actions for both sand and viviparous lizard species are required across reintroduction sites. Current site based conservation, action and management plans etc. are provided in individual County Biodiversity Action Plans for *Zootoca vivipara* (JNCC, 2010b), the ‘Sand lizard conservation handbook’ (Moulton & Corbett, 1999), the ‘Convention on the Conservation of European Wildlife and Natural Habitats’ ‘Action Plan for the Conservation of the Sand Lizard *Lacerta agilis* in Northwest Europe’ by Edgar & Bird (2005) and the *Lacerta agilis* UKBAP (JNCC, 2010a & b).

The above typically lack detailed habitat descriptions to inform management prescriptions. To identify and model 'suitable habitat', to inform these concentrated site conservation prescriptions and management actions across reintroduction sites, detailed site assessments are a basic requirement. As detailed in Chapter 3, Phase 1 and National Vegetation Classification (NVC) surveys are the current method of assessment. Both these methods are labour intensive, involve specialists competent in habitat surveying and depending on the size of the individual sites, this habitat assessment approach is not feasible on a anual basis. With ever increasing computational capabilities, new approaches to mapping, modelling and monitoring sites and 'suitable habitat' should be adopted.

Considering the key surveillance 'outputs' and output 'activities' (Gent *et al*., 2015), studies into vegetation structure and ground surface elevations (Turner *et al*., 2003), habitat characteristics and modelling (Vierling *et al*., 2008; Deng *et al*., 2017), species distribution for conservation planning (Farrell *et al*., 2013), conservation management (Flaherty *et al*., 2014), biodiversity mapping (Zlinszky *et al*., 2016) and ecological connectivity (Casalegno *et al*., 2017), have adopted the following methodologies of habitat mapping:

- **Landsat:** Landsat represents a series of satellites providing a temporal record of moderate resolution multispectral data of the Earth's surface on a global basis. The Landsat record has remained remarkably unbroken, proving a unique resource to assist a broad range of specialists (agriculture, geology, forestry, regional planning, education, mapping, and global change research) in managing the world's food, water, forests, and other natural resources (DOI, 2017).
- **Light Imaging, Detection, and Ranging (LiDAR):** a surveying method that measures distance to a target by illuminating that target with a pulsed laser light, measuring the reflected pulses with a sensor (Kramer *et al*., 2016).
- **Remotely Piloted Aerial Systems (RPAS), Unmanned Aerial vehicles (UAVs) or drones:** Site scale monitoring utilising quadcopters and small fixed wing aircraft takes 100s to 10,000 of GPS-referenced photographs (Gent *et al*., 2015).

Landsat and LiDAR both predominantly utilize satellite technology. Landsat imagery is inexpensive and has been useful at a regional scale, but lacks the higher spatial resolution required for local project decisions or smaller scale conservation efforts (Næsset, 2002 & 2009; Hummel, 2011). LiDAR has historically receiving more attention because of its detailed structural information and established accuracy in research studies. The high cost of data collection and difficulties in processing LiDAR data have limited their application beyond the research community, however, with neither of these methods are easily accessible for most land managers or conservation practitioners (Kramer *et al*., 2016).

More recently, RPAS and UAVs have been increasingly utilized by land managers or conservation practitioners to collect site data for interpretation (Ezequiel *et al*., 2014; Ivoševic, 2015; Joppa, 2015; Rey, 2016; d'Oleire-Oltmanns *et al*., 2016; Gonzalez *et al*., 2016). Requiring no licenses (in most cases) for equipment operation or software for analysis, a visual interpretation of aerial photography by field teams can be more readily achievable (Gent *et al*., 2015). Comparable in accuracy with LiDAR and Digital Surface Mapping, data collected by RPAS and UAVs allows for quantification of vegetation height and cover (applicable to multiple taxa), allowing habitat modelling with pre-existing GIS layers which may be present for a site (Gent *et al*., 2015).

Habitat modelling utilizing RPAS and UAVs has already been utilized, by conservation organizations, on a small scale, to provide targeted management operations, a more effective way to monitor habitat change over time, rate of regrowth or succession, responses to management tasks and a yearly comparison of site utilization by species (if survey frequency is applicable) (Gent *et al*., 2015) as a more cost-effective option.

Considering the above, it is recommended that RPAS and UAVs be trialed and adopted by statutory authorities and conservation organizations as a more cost effective and achievable method to undertake habitat assessments of sites.

## 1.3 Species and Habitat Management

Based on the findings in Chapters 3 & 4, viability, habitat and site utilisation models indicate that for highly mobile dune systems sand lizard viability and dispersal across a site is of less concern. For static or eroding dune systems, however, large, connected areas of favoured habitats are absent. In this situation, sand lizard viability is significantly reduced and dispersal is limited.

With sand lizard reintroduction sites found across North-West Wales being isolated units, there is an increased degree of population isolation, with no potential of natural immigration or emigration from an existing population. It is important to focus conservation effort on these smaller or isolated populations. In line with conservation recommendations made by Edgar *et al.* (2010) and Hill *et al*. (2016) through a

'Suggested draft annex on sand dune works to the Reptile Habitat Management Handbook', the following habitat management prescriptions are provided in support:

- Undertaking future sand lizard releases on sites which have received habitat modifications through dune re-mobilisation;
- 'Site utilisation modelling' should be adopted to inform and identify habitat management requirements: large-scale interventions verses 'mosaic management';
- Model short-term and long-term risks and benefits to sand and viviparous lizards prior to habitat management;
- Habitat management works involving dune manipulation should be undertaken during periods of sand lizard activity: avoiding hibernation periods when sand lizards are unable to escape works activities;
- Shoreline Management Plans (SMPs) which provide the policy and strategy for dune system risk management, should be adopted when formulating habitat management strategies;
- The provision of connectivity between known areas of sand lizard presence and areas of suitable habitat identified in Chapter 3 through 'mosaic management' should be investigated. Provision of such connectivity will allow further expansion across a site and reduce any impact of anthropogenic or environmental events; and
- Post habitat management monitoring should be undertaken for the species and site utilisation modelled to inform future habitat management prescriptions.

The conservation of sand lizards essentially means maintaining, and where possible enhancing, individual populations. Adoption of the management prescriptions provided will assist in delivering targeted habitat management prescriptions and assist in the further progression towards achieving Favourable Conservation Status for these species on a site and country level.

### 1.4 Reintroduction Strategy

The 'sand lizard captive breeding and reintroduction programme' has been described as highly successful in physically re-establishing species presence on sites within its former range (Corbett & Moulton, 1999; Moulton *et al.*, 2011; Russell, 2012; Woodfine *et al.*, 2017) across the North West-Wales coastline.

Genetic analysis undertaken in this thesis enriched our knowledge on the genetic diversity, or lack thereof, for reintroduced sand lizard populations in North-West Wales. It showed a lower genetic diversity in reintroduced populations compared to those calculated for naturally occurring British populations, including the source population (as shown in Chapter 4). This is a matter of conservation concern for the long-term survival of populations and 'conservation status' of the species in North-West Wales.

Further to re-establishing a species presence at a site, the genetic objective in any reintroduction programme should be to re-establish populations with the maximum possible genetic diversity, using individuals predicted to have the maximum reproductive fitness under wild conditions (Frankham *et al*., 2010). Since all populations will experience environmental change in their future (Environmental Stochastic, see Chapter 4), reintroduction biologists must ensure the reintroduced populations retain high evolutionary potential (Groombridge *et al*., 2002; Jamieson & Lacy, 2012; Ewen *et al*., 2012). However, some programmes have been rather cursory in that animals have been reintroduced and left to fend for themselves (Frankham *et al*., 2010).

The sand lizard, to a certain extent, is able to avoid inbreeding through various mechanisms such as mate selection and sperm competition (Olsson *et al*., 1999; Olsson *et al*., 2003) which may compensate for low genetic diversity (Russell, 2012). Nevertheless, with low levels of genetic diversity observed at the three reintroduction sites [compared to the source population at Merseyside] and no new recruitment, re-enforcement of the existing reintroduced populations must be undertaken.

Re-enforcement (IUCN, 1998), also termed restocking (IUCN, 1987), supplementation (IUCN, 1998) or augmentation (Maguire & Servheen, 1992), involves the release of individuals into an existing population in order to increase population size and reduce the risks of genetic collapse (Ewen *et al*., 2012). Such *post hoc* secondary releases have been termed follow-up translocations (Armstrong & Ewen, 2001) and are used to enhance genetic diversity, avoid inbreeding depression (Jamieson *et al*., 2006) and should be seen as supplementation of a reintroduction. Follow-up translocations should strictly be considered part of the original, but not yet successful, reintroduction attempt (Seddon, 2010; Ewen *et al*., 2012).

The supplementation of reintroduced sand lizard populations at Morfa Harlech, Aberdyfi and Ynyslas is required to increase the genetic diversity of the individual populations. Juveniles/sub-adults, from different parentage within the ex-situ captive breeding programme alongside the introduction of individuals from Dorset, like that undertaken for *Vipera berus* in Smygehuk, Sweden by Madsen *et al*. (1999) is recommended. The genetic diversity of the Ynyslas population, having received a mixed introduction including Dorset individuals, was observed to be higher than the other release sites not documented to have received individuals of the Dorset race. This method of introducing individuals from a different geographical region goes against those recommendations provided by Beebee & Rowe (2001), where for conservation purposes, populations in Dorset, Surrey and Merseyside should be considered as distinct clades, worthy of protection in their own right. No example of *L. agilis* genetic reference exists from Wales prior to their 1960s extinction which could counfirm that Dorset or Surrey individuals were not present within populations within North Wales or that Merseyside populations should be considered as a distinct clade, however. The introduction of new genetic material is critical in preserving genetic variability as a way of increasing the viability of these wild populations in the absence of natural recruitment. The translocation of additional juveniles/sub-adults from the original source population (Merseyside) is not however, recommended as this population has declined at an alarming rate in recent years (NMARG, 2013). Although attributed to habitat fragmentation and degradation (NMARG, 2013), the removal of individuals from the population may have a significant detrimental effect to its survival and year-on-year recruitment of new individuals.

To ensure supplementary individuals are as genetically distinct from individuals present at any release site, to provide an effective supplementation of genetic material into a population, supplementary releases should only be undertaken upon the establishment and implementation of a breed registry (detailed in Section on ‘Sampling’) for the species.

## 1.5 Sampling

### 1.5.1 Species

Current species survey guidelines are predominantly based on ‘surveyor experience’. Although this has its place a scientifically standardised approach is required for consistency. This research presents scientifically standardised environmental parameters associated with sand and viviparous lizard observations on dune systems in North-West Wales (detailed in Chapter 2). Findings indicate that although sand and viviparous lizards can be observed readily between the months of March and October, some months are more favourable than others, optimising surveyor effort. Adult sand lizards are more readily observed during the month of April, whereas hatchling sand lizards are more readily observed during the months of August, September and October, peaking in October. The total number of observations, including all life stages, indicates that April and August are the most favourable months. Like sand lizards, some months are also more favourable than others for viviparous lizards. Adult and sub-adult viviparous lizards are more readily observed during the month of April, July, August and September, whereas neonate viviparous lizards are more readily observed during the months of August, September and October, peaking in September. The total number of observations, including all life stages indicates that April, August and September are the combined, most favourable months for observation. Furthermore, the thesis identified surface temperature, relative humidity and UV as having the greatest influence on sand lizard activity. Similar environmental variables, surface temperature and UV, were also identified for viviparous lizards.

Biodiversity monitoring is an important factor in identifying conservation needs and testing the efficiency of species management (Kèry & Schmidt, 2008; Kèry *et al*., 2009; Lindenmayer *et al*., 2013), with the estimation of the abundance and distribution of a species becoming a fundamental cornerstone of conservation.

Species detection has long been a concern to practitioners across many fields. The likes of O’Connell *et al.* (2006) have investigated detection probability parameters on meso- and large mammals in a coastal ecosystem, Diefenbach *et al.* (2003) on the variability in grassland bird counts, Kroll *et al.* (2008) of stream-associated amphibians, Bailey *et al.* (2004) for terrestrial salamanders. Detailed Species detection evaluations have been further investigated by the likes of MacKenzie *et al.* (2003), Ribeiro-Júnior *et al.* (2008), Roloff *et al.* (2011) and McDiarmid (2012). Modifications to traditional sampling methods have addressed the most frequently encountered problems in sampling natural populations of viviparous and sand lizards in the UK (Thompson, 1990, 1991 & 2002; Thompson *et al*., 1992; Thompson & Seber, 1996; Foster & Gent, 1996; Fearnley, 2009; Blanke & Fearnley, 2015). These survey methods, collectively referred to as ‘adaptive sampling’ have the potential to greatly increase the efficiency and return of useful information (measured in terms of animals detected per unit effort) and greater information on the ecology of the target species, as well as increase in the precision and decrease in the bias associated with estimates of population parameters (Noon, 2006; Sewell *et al*., 2012).

Together with those environmental parameters identified for heathland sites, it is recommended that environmental parameters associated with sand and viviparous lizard observation identified through this research are included within revised survey guidelines for each species through inclusion with revised issues of a) Natural England's 'Reptile Mitigation Guidelines for Developers', b) JNCC's 'Herpetofauna Workers Manual') and/or c) any further species survey specific literature produced by the likes of NRW and ARC.

Through inclusion within such documents/survey guides, standardised surveys would be undertaken which would improve sand lizard reporting to the EU under Article 17 of the Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (92/43/EEC), future scientific studies, habitat and conservation monitoring.

### 1.5.2 Genetics

The findings of this thesis highlight the importance of establishing the genetic health of reintroduced populations. This can only be established through collaborative working with all parties involved within the programme. While it is understood that there is reluctance for captive breeders to permit others to handle and potentially cause disturbance to the individuals in whom they invest so much of their valuable personal time rearing, there is also a requirement for sampling and research to be undertaken. The comprehensive swabbing of all individuals would allow for a more robust sampling of populations and better evaluation of their genetic health. To facilitate long-term studies into the genetic health of populations and health screening, sampling should be extended to include all individuals held in captivity by the various captive breeders (both public and private). Sampling of individuals should be undertaken, even if undertaken by captive-breeders themselves: upon receiving appropriate training. Furthermore, the sampling of all release individuals (each year) should be undertaken to facilitate the production of a 'breed registry', also known as a 'studbook' in animal husbandry, along with future genetic studies.

Breed registries have been created for endangered reptiles, amphibians and Chelonian species, such as the Chinese newt *Tylototriton shanjing*, armadillo lizard *Ouroborus cataphractus*, Hinduran Paleate spiny-tailed Iguana *Ctenosaura melanosterna*, monkey tailed skink *Corucia zebrata*, Central Asian tortoise *Agrionemys horsfieldii*, radiated tortoise *Astrochelys radiata*, and spiny turtle *Heosemys spinosa* (ESF, 2017). The 'breed registry' should facilitate targeted movement of individuals between captive breeders within the programme to assist in providing a greater genetic structure for subsequently bred and released individuals, along with a comprehensive list of animals within the programme where the parents are known. If supplementation is required (see Section on ‘Reintroduction Strategy’) guidance and support for ‘studbook’ creation is provided by the European Studbook Foundation (ESF, 2017)

As detailed in Chapter 4, buccal swabbing is a widely-accepted methodology for obtaining genetic material from adult reptiles, with the least disturbance, distress and harm to an individual. The segregation of hatchlings [once hatched] and collection of faecal matter would facilitate sampling of these individuals; where buccal swabbing is impractical due to gape size.

# Opportunities for Further Research

This thesis has expanded the current knowledge on both dune system sand and viviparous lizard populations, providing optimal environmental parameters associated with observation for enhanced monitoring guidelines for both species. It provides an indication of future habitat and reintroduction management processes required to enhance the reintroduction programme to ensure the favourable conservation status of both species at these dynamic sites. As with any research undertaken on a rare and elusive species; it poses as many, if not more, questions than it answers. It identifies several areas where additional research would further develop our understanding to improve conservation practices and prescriptions.

*Climate Change*

Investigations into the impacts of future climate change on sites where *L. agailis* reintroductions have been made and are further proposed should be uundertaken. Global climate change is predicted to have a mixed effect on Europe's reptile populations, however many species, particularly those with a northern distribution, are predicted to benefit from warmer conditions (Araujo *et al.*, 2006; Russell, 2012). Recent climate projections (based on a medium increase in $CO_2$ emissions) for Great Britain and more specifically Wales predict an increase in sea-level rise. The median likely ranges are 0.7 - 1.2 m by AD 2100 and 2.0 - 3.0 m by AD 2300, calling into question the future survival of some coastal cities (Horton *et al.*, 2014; Welsh Government, 2014). The status of sand lizards in Great Britain is currently considered stable (UK, 2012), climate change may however have some significant implications to this 'conservation' status. In the south of England, Dorset and Surrey, Thomas *et al*. (1999) predicted that an increase in temperature could result in a sizable increase in suitable habitat for sand lizards. This is in agreement with the current distribution of sand lizards in continental Europe, where temperatures are typically higher. Therefore, Russell (2012) states that in a more favourable climate, the potential exists for British sand lizards to expand beyond its heathland habitat (in the south of England) as the availability of suitable habitat increases and warmer temperatures enable colonisation of other, previously unoccupied habitat types. A study into viviparous lizard dispersal by Massot *et al.* (2008) indicated that an increase in temperature may lead to reduced dispersal behaviour for this species.

On the North-Wales coastline, climate change is anticipated to have an adverse effect on dispersal and survival. With an anticipated sea-level increase of 0.7m – 3.0m

(Horton *et al*., 2014; Welsh Government, 2014), many of the coastal dune system sites, found at a height of 0m to 10m asl, may be significantly eroded or lost. With all the sites lacking connectivity to further areas of 'suitable habitat' for sand lizards, the availability of this species to escape a sea-level increase will be limited. It is recommended that sea-level increase modelling is undertaken for existing reintroduction sites as well as sites proposed to accept reintroductions in the future. Modelling will identify sites which may be susceptible to such a sea-level increase and a sites ability to withstand such an increase. Modelling should include areas of 'favourable habitat' identified in Chapter 3, and any impact on these existing and available areas when assessing impact(s). It is not anticipated that modelling for viviparous lizards is required as they utilise a wider variety of habitats and therefore should be more adapted to any sea-level increase.

Furthermore, an increase in both summer and winter mean temperatures of 3.9 °C and 2.8 °C respectively (central estimate) is predicted. It is predicted that annual mean precipitation will remain similar to current levels, however, there will be a significant shift to wetter winters and drier summers (Murphy *et al*., 2009; Russell, 2012). Beebee & Griffiths (2000) indicate that an increase in weather conditions can however be catastrophic, with hot dry summers being surprisingly as problematic as cold wet ones. The former increase mortality from desiccation while the later delays hatching and probably increases the chance of fungal infections. Investigation into a mean increase in temperature rise similar to predicted values would benefit the release programme to establish future recruitment at site.

### *DNA Extraction*

The development of a more refined method of extracting high-yield sand lizard DNA from faecal samples of hatchlings, where buccal swabbing is impractical due to gape size would facilitate the creation of a comprehensive 'breed registry'. This method of sampling has been successfully implemented for species including the white-headed langur *Trachypithecus leucocephalus* (Wang *et al*., 2016), a wild pig *Sus scrofa* (Kierepka *et al*., 2016), a proboscis monkey *Nasalis larvatus* (Inoue *et al*., 2016), the European slow worm *Anguis fragilis* (Brown *et al*., 2012), smooth snake *Coronella austriaca*, grass snake *Natrix natrix* and European adder *Vipera berus* (Jones *et al*., 2008). A major caveat to this process, however, is that faecal samples often yield low quality DNA that is prone to genotyping errors, potentially leading to biases in population parameter estimation (Kierepka *et al*., 2016). Furthermore, as faecal material contains a range of micro-organisms and is particularly prone to deterioration by endogenous nucleases the highest quality DNA is found in freshly collected faeces (Taberlet *et al*., 1999; Wehausen *et al*., 2004; Jones *et al*., 2008).

*Emerging Diseases*

Fungal infections have been identified in lizards such as *Pogona vitticeps* (Bowman *et al*., 2007) and several species of chameleons (Paré *et al*., 1997; Tetzlaff *et al*., 2015). Wildlife diseases continue to emerge as a consequence of globalization. As with *B. dendrobatidis*, the International spread of fungal infections is largely facilitated by the commercial trade in live reptiles UK (Wilkinson, 2017; Franklinos *et al*., 2017) and greater effort is urgently needed to protect global health (Skerratt *et al*., 2007; Kolby & Daszak, 2016), especially with the number of exotic naturalised species increasing in the wild in the UK (common wall lizard *Podarcis muralis,* green lizard *Lacerta bilineata*, formerly *Lacerta viridis;* Michaelides *et al*., 2015*).*

A ‘disease risk management and post-release health surveillance programme’ (ARC, 2011b) is available for captive breeders and conservation practitioners. The programme details disease risk and its requirement, management and isolation procedures, post-release health surveillance, quarantine guidelines and post-mortem guidelines. With the potential for captive breeders, who may also monitor populations of sand lizards, to transmit disease, a detailed study into emerging diseases in the UK (including endo and eco-parasite), similar to those undertaken in the EU and by Franklinos *et al*., 2017 in wild grass snakes in the UK, should be undertaken to inform ARCs ‘disease risk management and post-release health surveillance programme’.

# Conclusions

The sand lizard in Great Britain can be considered a ‘physical’ conservation success story thanks to the monumental efforts of conservation organisations, the dedicated captive breeders, volunteers and project sponsors, as the dramatic declines in the 19th and 20th Centuries have been halted (Russell, 2012) and to an extent reversed. Although the viviparous lizard is listed by the IUCN (2010) as being of ‘least concern’, the scale is different, with a qualitative decline of the species noted by surveyors across the UK. Dune systems are therefore perceived as a refuge for both species against current and future anthropogenic changes, with sites currently protected under UK and EU legislation. With no [reptile] species specific habitat management and Britain now exiting the European Union, many uncertainties exist as to the future protection of these sites, along with their constituent species, leaving them vulnerable to deterioration and future policy change.

The establishment of a process for ensuring genetic integrity is maintained and restored to sites is required. On a site level this may include the supplementation of genetically distinct individuals. Climate change and/or the spread of global reptile diseases may, however, present the largest challenges for small isolated populations in the future. This research will assist surveyors, conservation practitioners and site managers in the monitoring (including genetic health) of existing and future sand and viviparous lizard populations, enabling them to target and maximise efforts in times when future funding is always a concern.

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# Appendix A

## Environmental Conditions and Detection Supporting Information

**Table A.1:** Example of record sheet utilised during field survey.

| | | | | | |
|---|---|---|---|---|---|
| **Species** | | **Date** | | **Time (24Hrs)** | |
| | | | | | |
| **Life stage** | | **Sex** | M F | **Weight (g)** | |
| **SVL (mm)** | | **VTL (mm)** | | **Temperature of individual (ºC)** | |
| **Body condition** | | | | **Humidity (%)** | |
| **Image No(s).** | | **Recaptured individual** | Y N | **Surface temp (ºC)** | |
| **Location (GPS)** | | **UV (microW / cm$^2$)** | | **Air temp (ºC)** | |
| **Microhabitat** | | | | **Wind direction** | |
| | | | | **Wind speed (kph)** | |
| | | | | **Cloud cover (%)** | |
| | | | | **Gravid**<br>**Y N** | |
| **slope** | | **Aspect** | | | |
| | | **BURROW** | | | |
| **Number of burrows** | | **microhabitat** | | **Surface temperature at entrance (ºC)** | |
| **Distance from vegetation (mm)** | | | | **Air temperature (ºC)** | |
| **UV (microW / cm$^2$)** | | **Image No(s).** | | **Slope** | |
| **Location (GPS)** | | | | **Aspect** | |

**Table A.2:** List of field equipment including description and accuracies (where appropriate).

| Item | Description | Accuracy |
|---|---|---|
| **Camera** | Nikon D80 10 megpic, lens; AF-S Dx zoom- / Nikkor 18-135mm f/3.5-5.6g 1F-ED | N/A |
| **Compass** | Silvia expedition 4 compass | N/A |
| **Digital callipers** | LCD callipers with a fine adjustment roller | Accurate to 0.01mm. External, Internal, Depth or Set measurement modes. 150mm long – resolution 0.01mm, 0-100mm accurate to +/-0.02mm, 100-150mm accurate to +/-0.04mm. |
| **GPS unit** | Garmin GPSmap 60CSx | +/- 2-5m |
| **Holding bag** | Cotton fabric bag with circular bottom, string tie and cord lock. Size; 20cm x 30cm. | N/A |
| **Noose** | Carbon fibre pole with nylon line | N/A |
| **Infrared thermometer** | OS542 laser sighting thermometer, Field of view 12:1, Temperature range; 20-500ºC (-4 to 932ºF). | Accuracy ±2ºC (4ºF) or ±2% of reading. |
| **Ultraviolet (UVA) meter** | Tecpel UVA-830**:** - 3 1/2 digit liquid crystal display (LCD) with a maximum reading of 1999, 0 degC to 50degC, at <70% relative humidity operating environment | Stated accuracy at 23degC +/- 5degC, <75% relative humidity |
| **Quadrat** | Collapsible quadrat made from white plastic. Size; 2mx2m. | N/A |
| **Scales** | PESOLA Micro-Line 100g & 60g | +/- 0.3% of load |
| **Air temp/Humidity/Wind speed** | SKYWATCH atmos, | ±0.5ºC at 25ºC and ±1.5ºC in the measuring range between -20 and 80ºC / ±3.5% / ±4% |
| **Slope** | Silva Clino Master Clinometer CM 360% PA | Precision sighting +/-0.25° from true angle. Direct reading (at lubber line from top of housing) +/-1° |

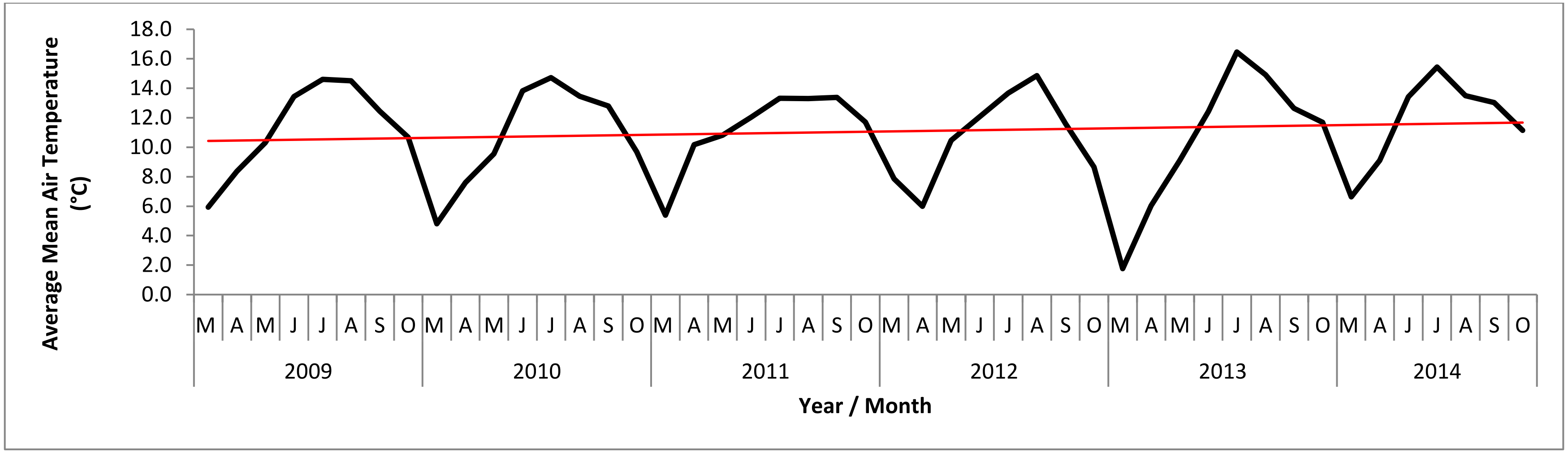

**Figure A2.1:** Average mean monthly temperature values recorded across the Cardigan Bay area between March 2010 and October 2014 (including).

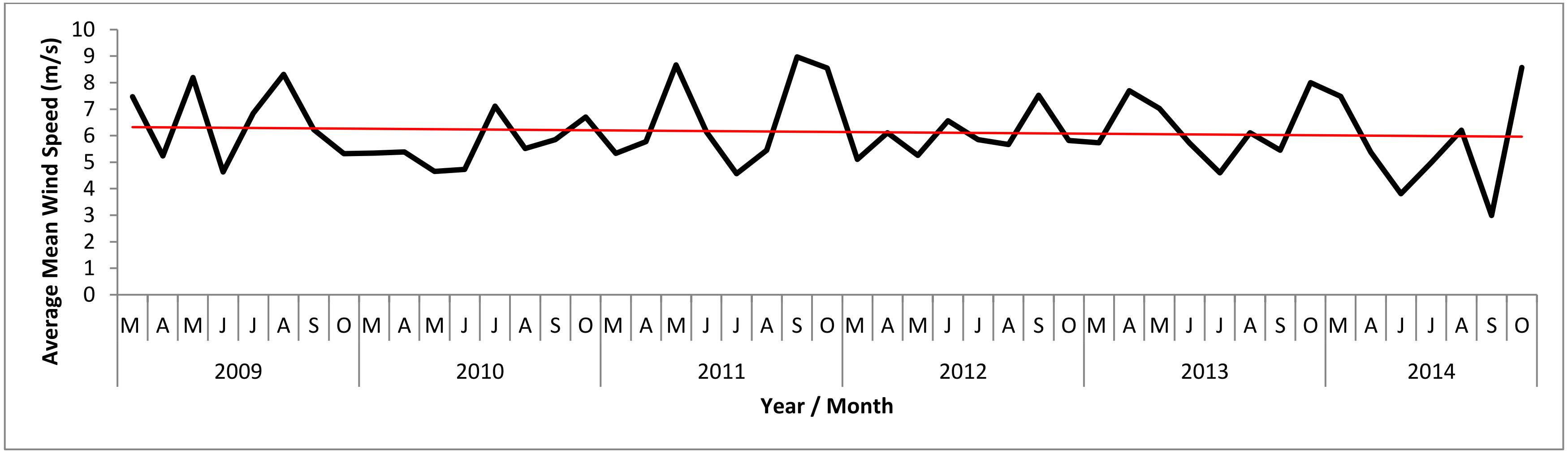

**Figure A2.2:** Average mean monthly wind speed values recorded across the Cardigan Bay area between March 2010 and October 2014 (including).

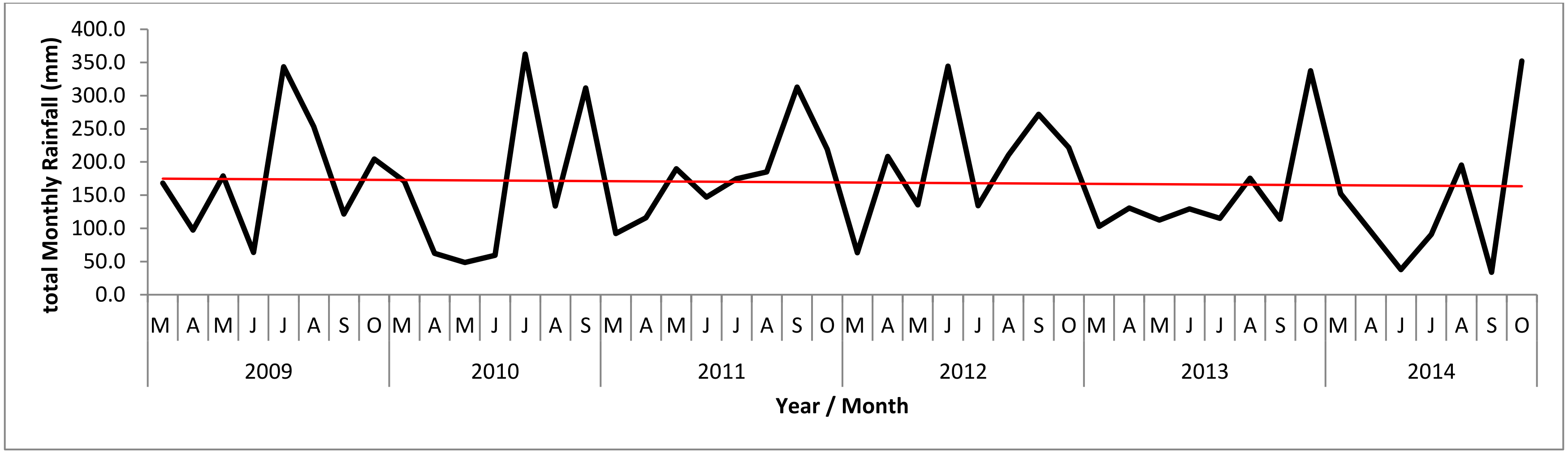

**Figure A2.3:** Average mean monthly rainfall values recorded across the Cardigan Bay area between March 2010 and October 2014 (including).

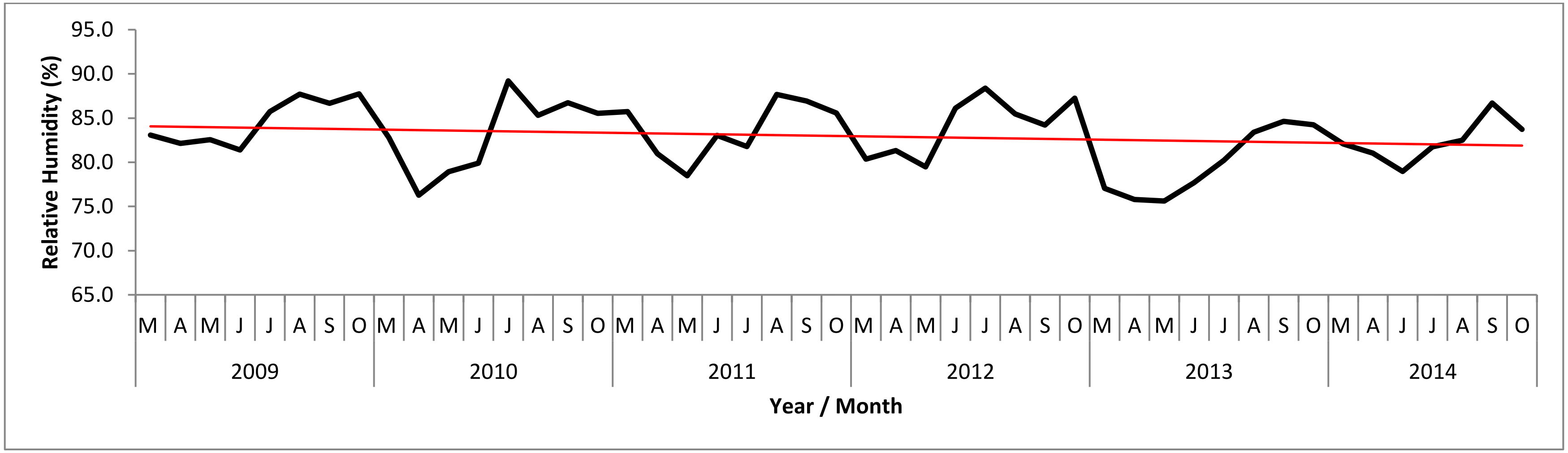

**Figure A2.4:** Average mean monthly relative humidity recorded across the Cardigan Bay area between March 2010 and October 2014 (including).

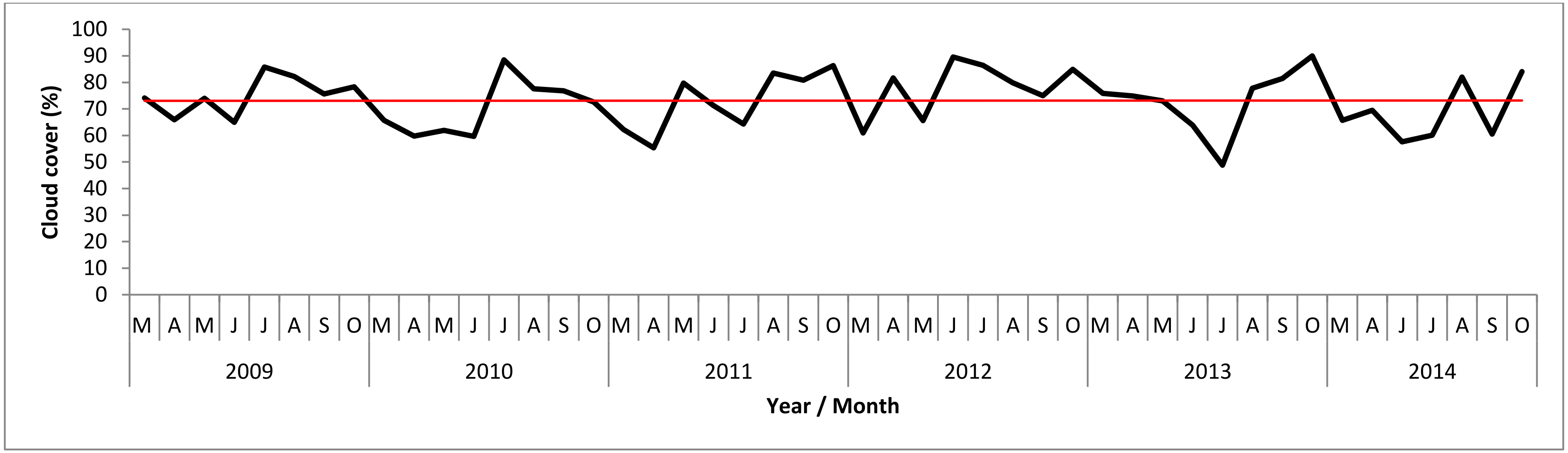

**Figure A2.5:** Average mean monthly cloud cover recorded across the Cardigan Bay area between March 2010 and October 2014 (including).

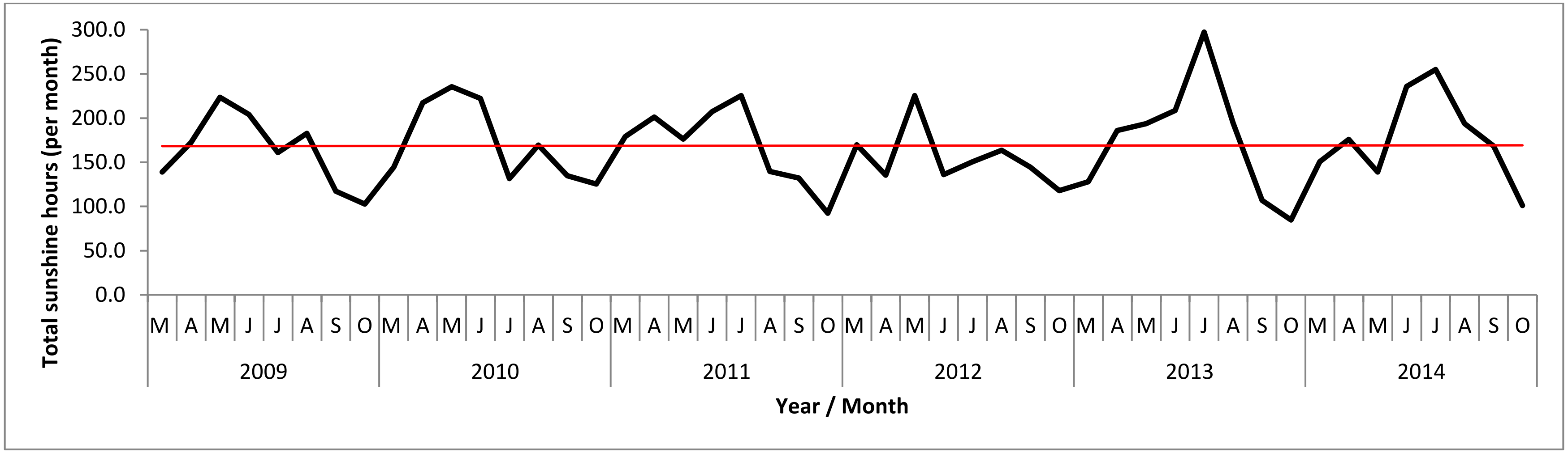

**Figure A2.6:** Average mean monthly total sunshine hours recorded across the Cardigan Bay area between March 2010 and October 2014 (including).

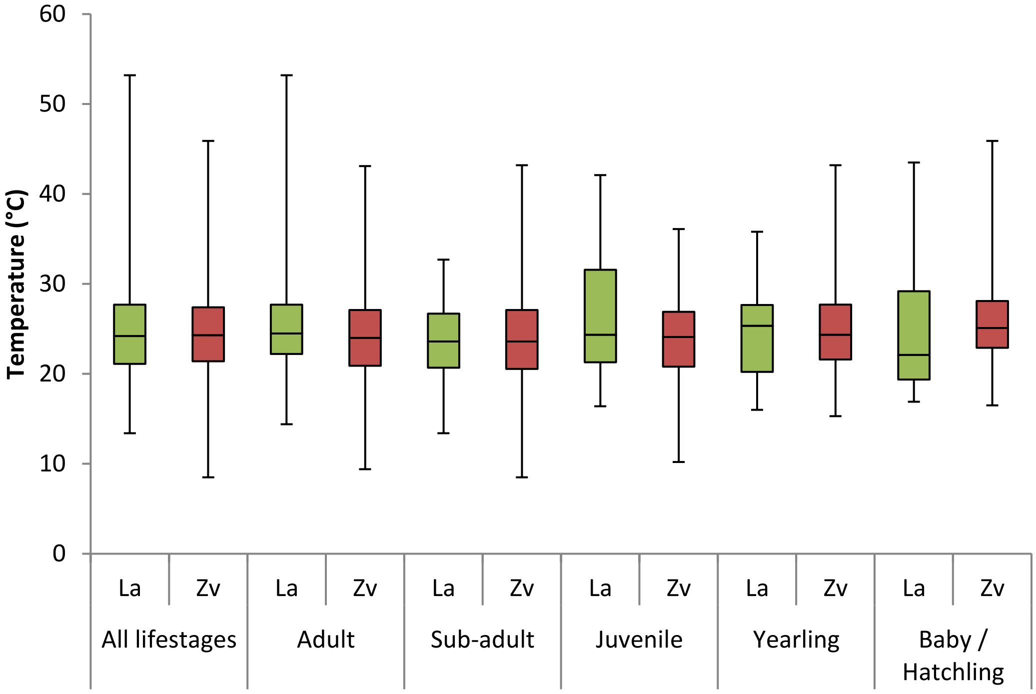

**Figure A2.7a:** Basking surface temperature associated with lizard observations across the three research sites between March 2010 and October 2014 (La – *Lacerta agailis*, Zv – *Zootoca vivipara*).

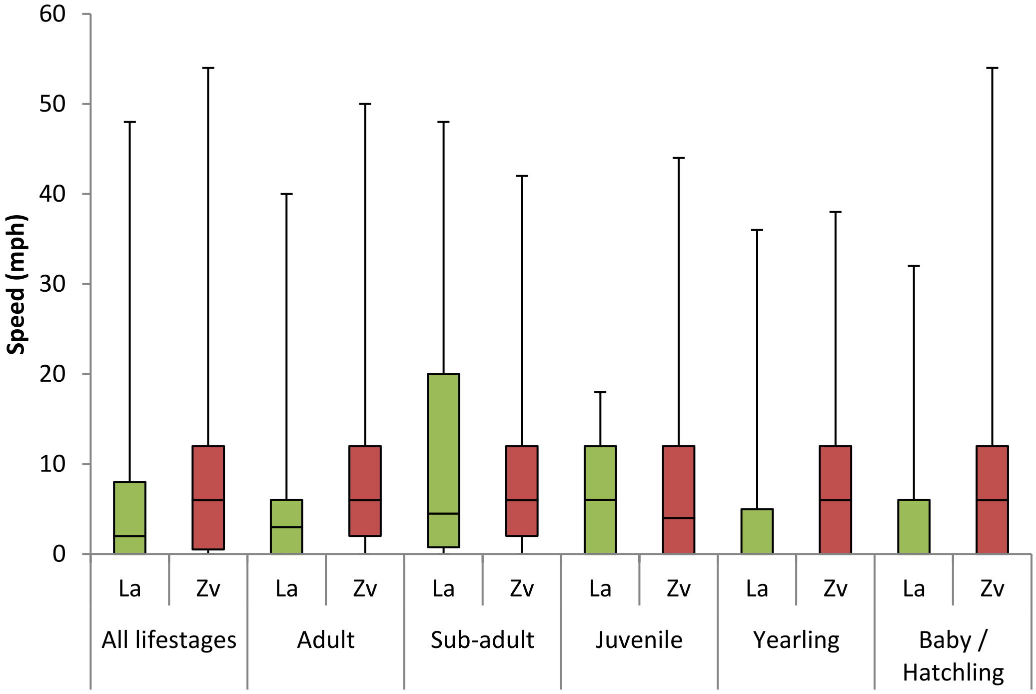

**Figure A2.7b:** Experienced wind speed associated with lizard observations across the three research sites between March 2010 and October 2014 (La – *Lacerta agailis*, Zv – *Zootoca vivipara*).

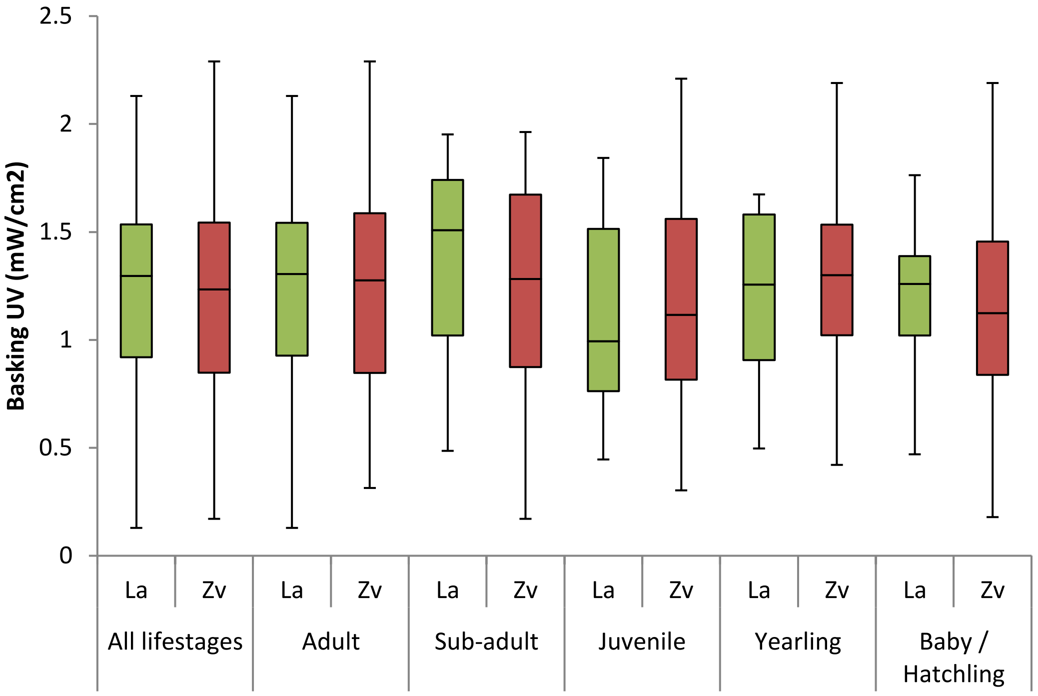

**Figure A2.7c:** Basking UV associated with lizard observations across the three research sites between March 2010 and October 2014 (La – *Lacerta agailis*, Zv – *Zootoca vivipara*).

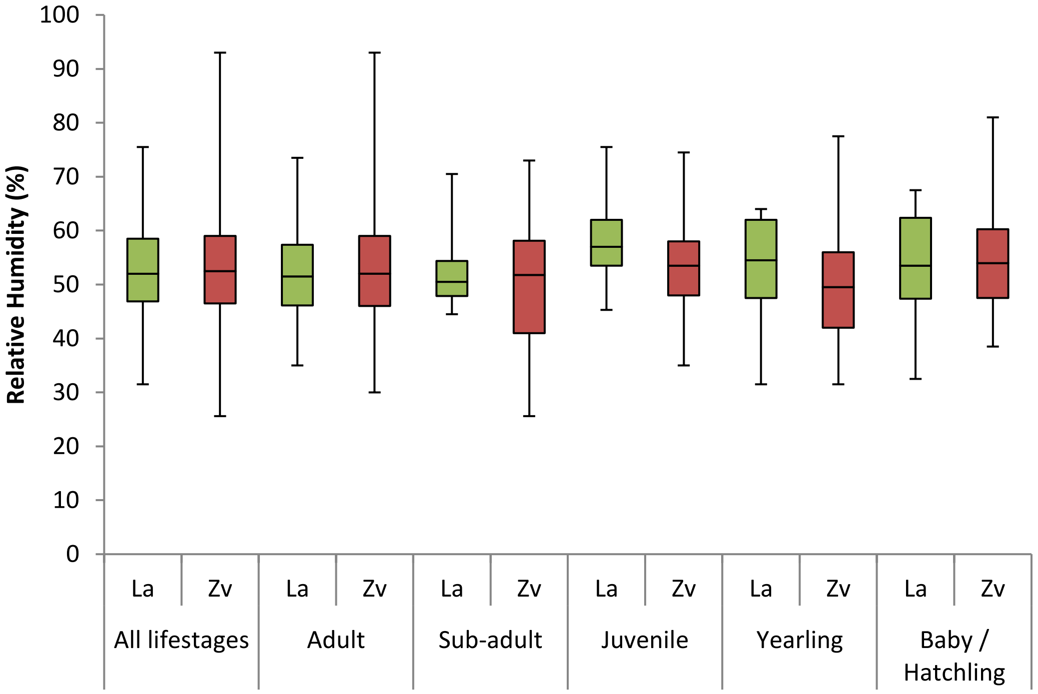

**Figure A2.7d:** Experienced humidity associated with lizard observations across the three research sites between March 2010 and October 2014 (La – *Lacerta agailis*, Zv – *Zootoca vivipara*).

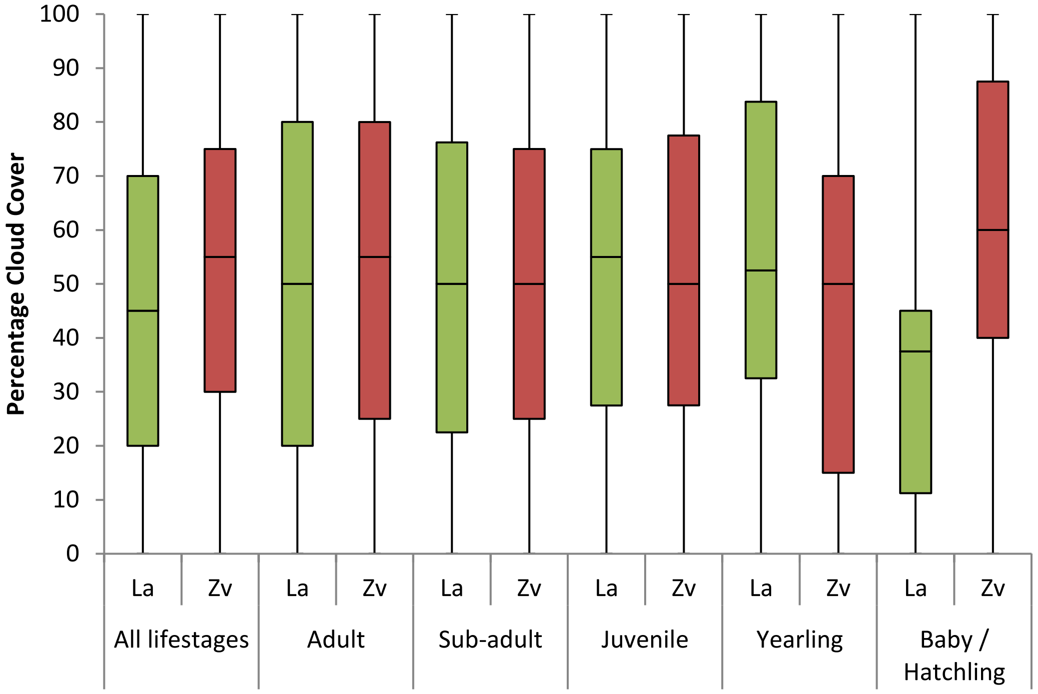

**Figure A2.7e:** Experienced cloud cover associated with lizard observations across the three research sites between March 2010 and October 2014 (La – *Lacerta agailis*, Zv – *Zootoca vivipara*).

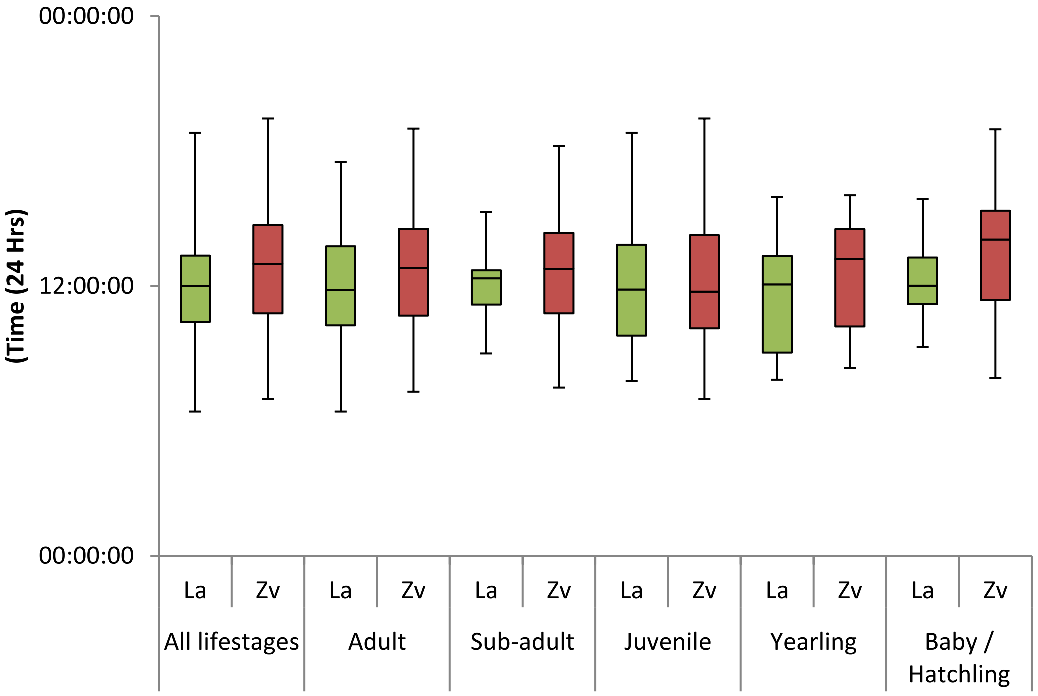

**Figure A2.7f:** Time of lizard observations across the three research sites between March 2010 and October 2014 (La – *Lacerta agailis*, Zv – *Zootoca vivipara*).

**Table A.3a:** PCA results table summarising environmental conditions associated with detection of sand lizards at Morfa Harlech between March 2010 and October 2012. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentages generated for each environmental variable (– indicates an absence of results generated during analysis).

| HARLECH | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| Air temperature | 0.482 / 18.4% | 0.748 / 21.9% | 0.717 | - | - | 0.330 | 0.826 / 24.6% | 0.309 |
| Surface temperature | 0.823 / 31.9% | 0.844 / 32.8% | -0.793 | - | - | 0.667 | 0.567 | 0.929 / 35.5% |
| Humidity | 0.196 | 0.357 | 0.568 | - | - | 0.775 / 38.5% | 0.159 | 0.290 |
| UV | 0.191 | 0.783 | 0.823 / 24.8% | - | - | 0.838 / 24.6% | 0.755 | 0.820 |
| Wind speed | 0.466 | 0.886 / 17.5% | 0.496 | - | - | 0.619 | 0.727 / 20.6% | 0.910 / 17.3% |
| Cloud cover | 0.807 / 20.8% | 0.477 | 0.952 / 43.6% | - | - | 0.651 / 17.7% | 0.757 / 27.9% | 0.829 / 21.3% |
| **Total variance explained** | 71.1 | 72.2 | 68.4 | - | - | 80.8 | 73.1 | 74.1 |

**Table A.3b:** PCA results table summarising environmental conditions associated with detection of viviparous lizards at Morfa Harlech between March 2010 and October 2012. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentages generated for each environmental variable.

| HARLECH | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| Air temperature | 0.712 / 21.8% | 0.549 | 0.894 / 23.5% | 0.779 / 22.8% | 0.549 | 0.730 | 0.750 / 22.8% | 0.809 / 20.3% |
| Surface temperature | 0.709 / 27.0% | 0.751 / 26.4% | -0.632 | -0.686 | 0.706 / 31.5% | 0.767 / 28.5% | 0.737 / 27.0% | 0.770 / 25.4% |
| Humidity | -0.565 | -0.490 | 0.701 / 32.9% | 0.765 / 33.7% | 0.702 / 19.1% | -0.549 | -0.536 | -0.374 |
| UV | 0.576 | 0.724 / 19.6% | -0.129 | -0.561 | 0.544 | 0.534 | 0.511 | 0.763 |
| Wind speed | 0.761 / 19.0% | -0.247 | 0.785 / 18.1% | 0.674 | 0.688 / 24.6% | 0.854 / 20.6% | 0.850 / 19.8% | 0.783 / 21.8% |
| Cloud cover | -0.470 | 0.667 / 22.1% | 0.623 | 0.781 / 17.5% | -0.638 | 0.872 / 22.7% | -0.482 | -0.342 |
| **Total variance explained** | 67.8 | 68.1 | 74.5 | 74 | 75.2 | 71.8 | 69.6 | 67.5 |

**Table A.4a:** PCA results table summarising environmental conditions associated with detection of sand lizards at Aberdyfi between March 2013 and October 2014. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentages generated for each environmental variable (– indicates an absence of results generated during analysis).

| ABERDYFI | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| Air temperature | 0.720 | 0.445 | - | - | - | - | 0.665 | - |
| Surface temperature | 0.881 / 34.3% | -0.798 | - | - | - | - | 0.889 / 35.0% | - |
| Humidity | -0.725 | 0.706 / 36.6% | - | - | - | - | -0.701 | - |
| UV | 0.896 / 18% | 0.925 / 30.7% | - | - | - | - | 0.903 / 17.7% | - |
| Wind speed | 0.705 / 25.6% | 0.581 | - | - | - | - | 0.349 | - |
| Cloud cover | 0.061 | 0.328 | - | - | - | - | 0.726 / 29.2% | - |
| **Total variance explained** | 43.6 | 67.3 | - | - | - | - | 81.9 | - |

**Table A.4b:** PCA results table summarising environmental conditions associated with detection of viviparous lizards at Aberdyfi between March 2013 and October 2014. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentages generated for each environmental variable.

| ABERDYFI | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| Air temperature | -0.489 | 0.806 | 0.774 / 19.4% | 0.892 / 39.4% | -0.333 | 0.738 | -0.486 | 0.581 |
| Surface temperature | 0.826 / 33.5% | 0.867 / 36.1% | 0.730 | 0.810 | 0.946 / 38.4% | 0.781 / 31.8% | 0.909 / 35.5% | 0.933 / 45.0% |
| Humidity | 0.272 | 0.613 | -0.778 | -0.686 | 0.811 | -0.662 | 0.843 | 0.245 |
| UV | -0.209 | 0.614 / 17.7% | 0.792 / 37.8% | 0.571 | 0.960 / 36.5% | -0.007 | 0.784 / 19.9% | 0.896 |
| Wind speed | 0.503 | 0.748 / 18.4% | 0.189 | 0.093 | 0.751 | -0.162 | 0.573 | -0.694 |
| Cloud cover | 0.715 / 20.9% | -0.214 | 0.715 / 31.1% | 0.819 / 28.5% | -0.265 | 0.578 / 23.3% | 0.671 / 20.3% | -0.846 / 27.1% |
| **Total variance explained** | 54.4 | 72.2 | 88.3 | 67.9 | 74.9 | 55.1 | 75.7 | 72.1 |

**Table A.5a:** PCA results table summarising environmental conditions associated with detection of sand lizards at Ynyslas between March 2013 and October 2014. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentages generated for each environmental variable.

| YNYSLAS | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| Air temperature | 0.734 | 0.556 | 0.391 | 0.052 | 0.947 / 50.8% | 0.682 | 0.845 / 43.7% | 0.741 |
| Surface temperature | 0.811 / 37.6% | 0.740 | 0.975 / 56.1% | 0.848 | 0.549 | 0.745 / 29.5% | 0.789 | 0.734 |
| Humidity | -0.516 | 0.836 / 22.4% | 0.926 / 43.8% | -0.437 | -0.900 | -0.318 | 0.883 / 17.4% | 0.521 / 26.5% |
| UV | 0.801 | 0.895 / 40.4% | 0.490 | 0.980 / 55.8% | 0.858 / 40.7% | 0.934 / 51.9% | 0.840 | 0.754 / 40.0% |
| Wind speed | 0.680 / 25.1% | -0.674 | -0.495 | 0.788 / 23.3% | -0.699 | 0.851 | -0.619 | -0.105 |
| Cloud cover | -0.237 | 0.696 / 19.3% | 0.972 | -0.561 | 0.560 | -0.930 | 0.765 / 19.6% | 0.819 / 17.2% |
| **Total variance explained** | 62.7 | 82.1 | 99.9 | 79.1 | 91.5 | 81.4 | 80.7 | 83.7 |

**Table A.5b:** PCA results table summarising environmental conditions associated with detection of viviparous lizards at Ynyslas between March 2013 and October 2014. Note: the darker the colour, the greater the percentage of variance explained by the environmental parameter and numbers in the individual boxes indicate the individual component matrix values and percentages generated for each environmental variable.

| YNYSLAS | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| Air temperature | 0.253 | 0.827 / 30.6% | 0.331 | 0.902 | 0.778 / 28.9% | 0.893 / 40.2% | -0.341 | 0.802 |
| Surface temperature | 0.827 / 30.9% | 0.723 | 0.743 / 36.7% | 0.953 / 35.8% | -0.310 | 0.723 | -0.653 | 0.271 |
| Humidity | -0.536 | -0.344 | -0.589 | -0.012 | 0.860 / 39.8% | -0.665 | 0.601 | 0.031 |
| UV | 0.855 / 26.8% | 0.854 / 26.7% | 0.901 | -0.635 | 0.858 | 0.831 / 27.7% | 0.822 / 28.8% | 0.592 |
| Wind speed | 0.946 / 18.4% | 0.971 / 17.3% | 0.640 / 17.2% | 0.868 / 25.4% | -0.324 | 0.946 / 21.8% | 0.869 / 17.9% | 0.616 / 26.9% |
| Cloud cover | 0.416 | 0.674 | 0.793 / 25.8% | 0.835 / 20.1% | 0.810 | 0.675 | 0.788 / 25.0% | 0.890 / 31.5% |
| **Total variance explained** | 76.1 | 74.6 | 79.7 | 81.3 | 68.7 | 89.7 | 71.7 | 58.4 |

**Table A.6a:** BIC results table summarising environmental conditions, identified during the 'test' and 'validation' analysis, associated with detection of sand lizards at Morfa Harlech between March 2010 and October 2012. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model (– indicates an absence of results generated during analysis).

| HARLECH | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| Air temperature | 209.7 / 203.2 | 133.7 / 134.6 | 26.6 / 14.1 | 5.2 / 2.0 | 5.2 / 2.0 | 52.0 / 57.1 | 87.5 / 91.6 | 71.4 / 85.8 |
| Surface temperature | 373.3 / 363.3 | 217.7 / 210.0 | 23.1 / 17.3 | 5.2 / 2.0 | 5.2 / 2.0 | 60.4 / 57.7 | 126.6 / 129.8 | 122.1 / 128.3 |
| Humidity | 239.7 / 240.1 | 152.1 / 150.6 | 21.8 / 17.3 | 2.0 / - | 5.7 / 2.0 | 52.3 / 56.4 | 103.7 / 83.8 | 95.0 / 96.4 |
| UV | 443.1 / 437.4 | 245.0 / 251.8 | 26.6 / 17.3 | 5.2 / 2.0 | 5.2 / 2.0 | 64.8 / 66.2 | 140.7 / 134.5 | 128.3 / 128.3 |
| Wind speed | 110.5 / 87.4 | 70.9 / 76.6 | 19.6 / 11.6 | 2.7 / 2.0 | 2.7 / - | 30.4 / 25.1 | 58.0 / 52.2 | 56.4 / 45.0 |
| Cloud cover | 144.9 / 143.1 | 114.0 / 114.3 | 19.6 / 21.8 | 5.2 / 2.0 | 5.2 / 2.0 | 45.3 / 44.9 | 74.5 / 83.8 | 81.6 / 77.4 |

**Table A.6b:** BIC results table summarising environmental conditions, identified during the 'test' and 'validation' analysis, associated with detection of viviparous lizards at Morfa Harlech between March 2010 and October 2012. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model (– indicates an absence of results generated during analysis).

| HARLECH | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| Air temperature | 451.7 / 467.6 | 319.8 / 326.2 | 96.8 / 106.0 | 97.8 / 80.0 | 73.2 / 71.8 | 172.6 / 173.2 | 308.3 / 293.6 | 150.1 / 178.4 |
| Surface temperature | 1284.6 / 1267.5 | 775.5 / 774.7 | 137.0 / 145.2 | 126.6 / 119.0 | 77.5 / 80.3 | 435.8 / 412.4 | 632.7 / 631.1 | 330.9 / 307.2 |
| Humidity | 672.8 / 689.1 | 511.2 / 523.0 | 141.8 / 135.6 | 96.4 / 93.5 | 53.7 / 55.1 | 314.2 / 283.0 | 445.3 / 457.5 | 240.7 / 244.5 |
| UV | 2212.8 / 2231.4 | 1129.5 / 1083.6 | 161.2 / 159.7 | 134.5 / 116.0 | 81.8 / 74.6 | 525.3 / 549.0 | 824.9 / 804.7 | 364.8 / 391.1 |
| Wind speed | 232.2 / 236.5 | 179.5 / 180.3 | 65.5 / 53.7 | 66.1 / 60.9 | 46.0 / 44.9 | 112.0 / 103.4 | 145.9 / 154.0 | 117.5 / 114.8 |
| Cloud cover | 218.3 / 218.4 | 188.4 / 182.0 | 96.7 / 96.0 | 73.0 / 82.0 | 46.4 / 49.6 | 141.2 / 141.8 | 177.6 / 170.6 | 125.2 / 131.3 |

**Table A.7a:** BIC results table summarising environmental conditions, identified during the ‘test’ and ‘validation’ analysis, associated with detection of sand lizards at Aberdyfi between March 2013 and October 2014. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model (– indicates an absence of results generated during analysis).

| ABERDYFI | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| Air temperature | 63.2 / 57.5 | 34.1 / 34.1 | - / - | 2.0 / 2.0 | - / - | 5.2 / 2.0 | 34.1 / 30.3 | 5.2 / 5.2 |
| Surface temperature | 80.3 / 68.9 | 41.7 / 41.7 | - / - | 2.0 / 2.0 | - / - | 5.2 / 2.0 | 41.7 / 41.7 | 5.2 / 2.0 |
| Humidity | 61.9 / 70.4 | 37.9 / 37.9 | - / - | 2.0 / 2.0 | - / - | 5.2 / 2.0 | 37.9 / 41.7 | 5.2 / 2.0 |
| UV | 80.3 / 74.6 | 41.7 / 41.7 | - / - | 2.0 / 2.0 | - / - | 5.2 / 2.0 | 41.7 / 41.7 | 5.2 / 5.2 |
| Wind speed | 26.5 / 24.1 | 18.5 / 9.9 | - / - | 2.0 / 2.0 | - / - | - / - | 15.2 / 16.2 | - / - |
| Cloud cover | 58.7 / 48.3 | 34.1 / 33.9 | - / - | 2.0 / 2.0 | - / - | 5.2 / 2.0 | 37.9 / 37.9 | - / 5.2 |

**Table A.7b:** BIC results table summarising environmental conditions, identified during the ‘test’ and ‘validation’ analysis, associated with detection of viviparous lizards at Aberdyfi between March 2013 and October 2014. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model.

| ABERDYFI | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| Air temperature | 247.9 / 246.5 | 178.2 / 155.9 | 26.6 / 23.1 | 37.9 / 37.9 | 12.9 / 17.3 | 53.0 / 53.5 | 144.0 / 143.5 | 27.9 / 23.1 |
| Surface temperature | 443.2 / 460.6 | 234.8 / 251.2 | 18.5 / 26.6 | 41.7 / 41.7 | 17.3 / 17.3 | 70.4 / 70.4 | 178.5 / 183.5 | 27.9 / 21.8 |
| Humidity | 310.0 / 302.1 | 208.3 / 191.4 | 23.1 / 26.6 | 37.9 / 37.9 | 12.9 / 17.3 | 61.9 / 57.7 | 147.0 / 163.3 | 27.6 / 26.6 |
| UV | 551.9 / 544.7 | 273.7 / 268.5 | 26.6 / 26.6 | 36.5 / 41.7 | 17.3 / 17.3 | 74.6 / 70.4 | 193.5 / 200.0 | 26.6 / 26.6 |
| Wind speed | 119.9 / 132.7 | 86.0 / 108.0 | 15.9 / 19.4 | 26.3 / 17.6 | 9.9 / 14.1 | 48.6 / 39.7 | 73.2 / 73.5 | 20.5 / 19.6 |
| Cloud cover | 141.6 / 141.3 | 90.6 / 101.9 | 23.1 / 26.6 | 26.5 / 37.9 | 6.9 / 14.1 | 44.4 / 53.3 | 90.7 / 84.8 | 24.3 / 15.9 |

**Table A.8a:** BIC results table summarising environmental conditions, identified during the ‘test’ and ‘validation’ analysis, associated with detection of sand lizards at Ynyslas between March 2013 and October 2014. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model. – indicates an absence of results generated during analysis.

| YNYSLAS | Sand lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Hatchling | Male | Female |
| Air temperature | 45.3 / 67.3 | 23.1 / 14.9 | 2.0 / - | 2.7 / 5.2 | 8.8 / 5.2 | 27.2 / 5.2 | 23.1 / 19.6 | 6.1 / 8.8 |
| Surface temperature | 80.3 / 80.3 | 26.6 / 21.8 | 2.0 / - | 5.2 / 5.2 | 8.8 / 5.2 | 5.2 / 5.2 | 26.6 / 26.6 | 8.8 / 8.8 |
| Humidity | 74.6 / 63.2 | 21.8 / 21.8 | 2.0 / - | 5.2 / 5.2 | 8.8 / 5.2 | 5.2 / 5.2 | 26.6 / 21.8 | 8.8 / 8.8 |
| UV | 80.3 / 80.3 | 26.6 / 21.8 | 2.0 / - | 5.2 / 5.2 | 8.8 / 5.2 | 5.2 / 5.2 | 26.6 / 26.6 | 8.8 / 8.8 |
| Wind speed | 44.9 / 29.9 | 15.9 / 11.2 | 2.0 / - | 5.2 / 2.7 | 3.1 / 5.2 | 5.2 / 5.2 | 7.8 / 19.6 | 6.1 / 6.1 |
| Cloud cover | 50.2 / 49.6 | 16.2 / 18.5 | 2.0 / - | 5.2 / 5.2 | 8.8 / 5.2 | 5.2 / 5.2 | 15.9 / 26.6 | 8.8 / 8.8 |

**Table A.8b:** BIC results table summarising environmental conditions, identified during the ‘test’ and ‘validation’ analysis, associated with detection of viviparous lizards at Ynyslas between March 2013 and October 2014. Note: the darker the colour, the greater the significance of the environmental parameter identified within the model.

| YNYSLAS | Viviparous lizard | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable / Life stage | All life stages | Adult | Sub adult | Juvenile | Yearling | Neonate | Male | Female |
| Air temperature | 228.7 / 211.7 | 143.7 / 173.7 | 29.1 / 32.8 | 32.8 / 41.7 | 26.6 / 27.9 | 36.5 / 41.2 | 119.7 / 92.1 | 51.0 / 42.6 |
| Surface temperature | 457.0 / 461.1 | 254.1 / 256.5 | 32.8 / 36.5 | 36.5 / 41.7 | 23.1 / 31.5 | 52.3 / 53.7 | 170.6 / 143.2 | 63.3 / 55.1 |
| Humidity | 345.5 / 313.3 | 194.8 / 213.2 | 32.8 / 32.8 | 36.5 / 37.9 | 23.1 / 27.9 | 40.2 / 57.8 | 135.5 / 132.9 | 51.0 / 59.2 |
| UV | 607.3 / 586.3 | 285.9 / 294.3 | 36.5 / 36.5 | 36.5 / 41.7 | 26.6 / 31.5 | 52.3 / 57.8 | 185.4 / 172.5 | 63.3 / 63.3 |
| Wind speed | 116.3 / 114.3 | 80.6 / 86.5 | 21.2 / 32.8 | 21.3 / 34.1 | 15.9 / 13.1 | 32.3 / 41.7 | 71.8 / 70.9 | 33.8 / 33.9 |
| Cloud cover | 149.1 / 145.6 | 102.3 / 115.3 | 24.3 / 27.9 | 32.8 / 24.3 | 23.1 / 27.9 | 18.8 / 28.4 | 102.7 / 85.5 | 40.2 / 33.1 |

# Appendix B

## Habitat Utilisation Mapping, Modelling and Supporting Information

**Table B.3.1:** Standard Phase 1 recording card (adapted from JNCC, 2003).

| | | |
|---|---|---|
| Survey title | | |
| Map sheet no. | 10 km sq. | Orig. ref. no. |
| Surveyor(s) | | Survey date(s) |
| County (counties) | | |
| L.A. district(s) | | |
| Area (ha) surveyed | | |
| Total area (ha) surveyed | Location of records | |
| Notes (include e.g. use of aerial photos, supplementary sources of information, level of detail, habitats and areas omitted) | | |

**Habitat measurements**

| Habitat name | Or standard Phase 1 Alphanumeric code | Area (ha) | Length (m) |
|---|---|---|---|
| | | | |

**Table B.3.2:** Phase 1 and NVC study field equipment.

| ITEM | DESCRIPTION | ACCURACY |
|---|---|---|
| University Identity card | N/A | |
| Copy of Risk Assessment and Method Statement | Site specific | |
| Copy of species and site Licences | Site specific | |
| First aid kit | Lifesystems Mountain First Aid kit | |
| Mobile phone | Apple IPhone 5 & Nokia 3310 | |
| Waterproof jacket and over trousers | N/A | |
| Walking boots | N/A | |
| Rucksack | N/A | |
| Paper copy of maps and Phase 1 maps | OS MasterMap® | 1:1,250 scale |
| Field manual(s) | JNCC Handbook for Phase 1 habitat survey/British Plant Communities, Vol 5: Maritime Communities and Vegetation of Open Habitats | |
| Coloured pencils; Berol Verithin series | Berol Verithin series;<br>VT 01 Black, VT 05 Indigo blue, VT 08 Sky blue, VT 25 Flesh, VT 31 Green, VT 32 True green, VT 45 Magenta, VT 46 Orange, VT 49 Pink, VT 51 Purple, VT 55 Scarlet red, VT 66 Terra cotta, VT 80 Canary yellow, VT 89 Yellow ochre | |
| Lead pencils | Tesco mechanical pencils | |
| Weather writer | A3/A4 WeatherWriter® PRO Waterproof Clipboard | |
| Waterproof spiral Notebook / ruled A4 notepad | Rite in the Rain All-Weather notebook No. 135/A4 ruled notepad | |
| Large plastic bags | N/A | |
| Botanical field guides | Collins British Wildlife (Paul Sterry, 2008), Field Study Council; Plants, common on sand dunes, Moorland plants, Saltmarsh plants of Britain, Common water plants, Guide to grassland plants 1, Guide to grassland plants 2, Playing field plants, Grasses, New Flora of the British Isles (Stace, 1997 & 2010) | |
| Hand lens | Opticron 23mm x10 & Opticron 23mm x15 magnification | |
| GPS unit | Garmin GPSmap 60CSx | +/- 2-5m |
| Quadrat | (4x plastic/metal peg, Tape measure, string) | |
| Camera | Nikon D80 10 megpic, lens; AF-S Dx zoom / Nikkor 18-135mm f/3.5-5.6g 1F-ED | |

**Table B.3.3**: JNCC alpha-numeric codes, description and letter codes.

| ALPHANUMERIC CODE | DESCRIPTION | LETTERED CODE |
|---|---|---|
| **A Woodland and scrub** | | |
| A1.1.1 | Broadleaved semi-natural woodland | BW |
| A1.1.2 | Broadleaved plantation woodland | PBW |
| A1.2.1 | Coniferous semi-natural woodland | CW |
| A1.2.2 | Coniferous plantation woodland | PCW |
| A1.3.1 | Mixed semi-natural woodland | MW |
| A1.3.2 | Mixed plantation woodland | PMW |
| A2.1 | Dense/continuous scrub | DS |
| A2.2 | Scattered scrub | SS |
| A3.1 | Broad-leaved parkland/scattered trees | SBW |
| A3.2 | Coniferous parkland/scattered trees | SCW |
| A3.3 | Mixed parkland/scattered trees | SMW |
| A4.1 | Broad-leaved recently-felled woodland | FB |
| A4.2 | Coniferous recently-felled woodland | FC |
| A4.3 | Mixed recently-felled woodland | FM |
| **B Grassland and marsh** | | |
| B1.1 | Unimproved acid grassland | AG |
| B1.2 | Semi-improved acid grassland | SAG |
| B2.1 | Unimproved neutral grassland | NG |
| B2.2 | Semi-improved neutral grassland | SNG |
| B3.1 | Unimproved calcareous grassland | CG |
| B3.2 | Semi-improved calcareous grassland | SCG |
| B4 | Improved grassland | I |
| B5 | Marsh/marshy grassland | MG |
| B6 | Poor semi-improved | SI |
| **C Tall herb and fern** | | |
| C1.1 | Continuous bracken | CB |
| C1.2 | Scattered bracken | SB |

**Table B.3.3:** Continued over page.

**Table B.3.3:** Cont.

| ALPHANUMERIC CODE | DESCRIPTION | LETTERED CODE |
|---|---|---|
| **C Tall herb and fern** | | |
| C2 | Upland species-rich ledges | Target note |
| C3.1 | Tall ruderal other | TR |
| C3.2 | Non-ruderal other | NR |
| **D Heathland** | | |
| D1.1 | Acid dry dwarf shrub heath | ADH |
| D1.2 | Basic dry dwarf shrub heath | BDH |
| D2 | Wet dwarf shrub heath | WH |
| D3 | Lichen/bryophyte heath | LH |
| D4 | Montane heath/dwarf herb | MH |
| D5 | Dry heath/acid grassland mosaic | DGM |
| D6 | Wet heath/acid grassland mosaic | WGM |
| **E Mire** | | |
| E1.6.1 | Blanket bog | BB |
| E1.6.2 | Raised bog | RB |
| E1.7 | Wet modified bog | WB |
| E1.8 | Dry modified bog | DB |
| E2.1 | Acid/neutral flush/spring | AF |
| E2.2 | Basic flush/spring | BF |
| E2.3 | Bryophyte dom. flush/spring | Target note |
| E3.1 | Valley mire fen | VM |
| E3.2 | Basin mire fen | BM |
| E3.3 | Flood-plain fen | FPM |
| E4 | Bare peat | P |
| **F Swamp, marginal and inundation** | | |
| F1 | F1 | F1 |
| F2.1 | F2.1 | F2.1 |
| F2.2 | F2.2 | F2.2 |

**Table B.3.3:** Continued over page.

**Table B.3.3:** Cont.

| ALPHANUMERIC CODE | DESCRIPTION | LETTERED CODE |
|---|---|---|
| **G Open water** | | |
| G1.1 | Eutrophic standing water | SWE |
| G1.2 | Mesotrophic standing water | SWM |
| G1.3 | Oligotrophic standing water | SWO |
| G1.4 | Dystrophic standing water | SWD |
| G1.5 | Marl standing water | SWC |
| G1.6 | Brackish standing water | SWB |
| G2.1 | Eutrophic running water | RWE |
| G2.2 | Mesotrophic running water | RWM |
| G2.3 | Oligotrophic running water | RWO |
| G2.4 | Dystrophic running water | RWD |
| G2.5 | Marl running water | RWC |
| G2.6 | Brackish running water | RWB |
| **H Coastal** | | |
| H1.1 | Intertidal mud/sand | O.S. symbol |
| H1.2 | Intertidal shingle/cobbles | O.S. symbol |
| H1.3 | Intertidal boulders/rocks | O.S. symbol |
| H1.(1-2).1 | Intertidal *Zostera* beds | Zo |
| H1.(1-3).2 | Intertidal green algal beds | Ga |
| H1.(1-3).3 | Intertidal brown algal beds | Ba |
| H2.3 | Saltmarsh/dune interface | Target note |
| H2.4 | Saltmarsh scattered plants | SSM |
| H2.6 | Saltmarsh dense/continuous | DSM |
| H3 | Shingle above high tide mark | O.S. symbol |
| H4 | Boulders/rocks above high tide mark | O.S. symbol |
| H5 | Strandline vegetation | Target note |
| H6.4 | Sand dune – dune slack | DW |
| H6.5 | Sand dune – dune grassland | DG |

**Table B.3.3:** Continued over page.

**Table B.3.3:** Cont.

| ALPHANUMERIC CODE | DESCRIPTION | LETTERED CODE |
|---|---|---|
| H6.6 | Sand dune – dune heath | DH |
| H6.7 | Sand dune – dune scrub | DX |
| H6.8 | Sand dune – open dune | OD |
| H8.1 | Hard maritime cliff and slope | HC |
| H8.2 | Soft maritime cliff and slope | SC |
| H8.3 | Crevice/ledge maritime cliff and slope vegetation | Target note |
| H8.4 | Coastal maritime cliff and slope grassland | SG + target note |
| H8.5 | Coastal maritime cliff and slope heathland | SH + target note |
| **I Rock exposure and waste** | | |
| I1.1.1 | Acid/neutral inland cliff | AC |
| I1.1.2 | Basic inland cliff | BC |
| I1.2.1 | Acid/neutral scree | AS |
| I1.2.2 | Basic scree | BS |
| I1.3 | Limestone pavement | LP |
| I1.4.1 | Other exposure – acid/neutral | AR |
| I1.4.2 | Other exposure – basic | BR |
| I1.5 | Cave | CA |
| I2.1 | Artificial quarry | Q |
| I2.2 | Artificial spoil | S |
| I2.3 | Artificial mine | MI |
| I2.4 | Artificial refuse-tip | R |
| **J Miscellaneous** | | |
| J1.1 | Cultivated/disturbed land – arable | A |
| J1.2 | Cultivated/disturbed land – amenity grassland | AM |
| J1.3 | Cultivated/disturbed land – ephemeral/short perennial | ESP |
| J1.4 | Cultivated/disturbed land – introduced shrub | IS |
| J2.1.1 | Species-rich intact hedge | RH |
| J2.1.2 | Species-poor intact hedge | PH |

**Table B.3.3:** Continued over page.

**Table B.3.3:** Cont.

| ALPHANUMERIC CODE | DESCRIPTION | LETTERED CODE |
|---|---|---|
| J2.2.1 | Specie-rich defunct hedge | RH- |
| J2.2.2 | Species-poor defunct hedge | PH- |
| J2.3.1 | Species-rich hedge with trees | RHT |
| J2.3.2 | Species-poor hedge with trees | PHT |
| J2.4 | Fence | F |
| J2.5 | Wall | W |
| J2.6 | Dry ditch | DD |
| J2.7 | Boundary removed | X |
| J2.8 | Earth bank | EB |
| J3.4 | Caravan site | CS |
| J3.5 | Sea wall | SWALL |
| J3.6 | Buildings | Shade black |
| J4 | Bare ground | BG |
| J5 | Other habitat | Target note |

**Table B.3.4:** List of National Vegetation Classification types commonly found on sand dunes (grouped by habitat).

| **ALPHANUMERIC CODE** | **DESCRIPTION** |
|---|---|
| **Strandline** | |
| SD1a | *Rumex crispus-Glaucium flavum* shingle community, typical sub-community. |
| SD2 | *Honkenya peploides-Cakile maritime* strandline community. |
| SD3 | *Matricaria maritime-Galium aparine* strandline community. |
| **Mobile dunes** | |
| SD4 | *Elymus farctus* ssp. *Boreali-atlanticus* foredune community. |
| SD5a | *Leymus arenarius* mobile dune, species-poor sub-community. |
| SD5c | *Leymus arenarius* mobile dune, *Elymus farctus* sub-community. |
| SD6a | Ammophila arenaria mobile dune, *Elymus farctus* sub-community. |
| SD6b | *Ammophila arenaria* mobile dune, *Elymus farctus-Leymus arenarius* sub-community. |
| SD6c | *Ammophila arenaria* mobile dune, *Leymus arenarius* sub-community. |
| SD6d | *Ammophila arenaria* mobile dune, typical sub-community. |
| SD6e | *Ammophila arenaria* mobile dune, *Festuca rubra* sub-community. |
| SD6f | *Ammophila arenaria* mobile dune, *Poa pratensis* sub-community. |
| SD6g | *Ammophila arenaria* mobile dune, *Carex arenaria* sub-community. |
| **Semi-fixed dunes** | |
| SD7a | *Ammophila arenaria-Festuca rubra* semi-fixed dune, typical sub-community. |
| SD7b | *Ammophila arenaria-Festuca rubra* semi-fixed dune, *Hypnum cupressiforme* sub-community. |
| SD7c | *Ammophila arenaria-Festuca rubra* semi-fixed dune, *Ononis repens* sub-community. |
| SD7d | *Ammophila arenaria-Festuca rubra* semi-fixed dune, *Tortula ruralis* ssp. *ruraliformis* sub-community. |
| SD7e | *Ammophila arenaria-Festuca rubra* semi-fixed dune, *Elymus pycnanthus* sub-community. |
| SD7f | *Ammophila arenaria-Festuca rubra* semi-fixed dune, provisional *Galium verum* sub-community. |
| SD7g | *Ammophila arenaria-Festuca rubra* semi-fixed dune, provisional *Heracleum sphondylium* sub-community. |
| **Dune grassland** | |
| SD8a | *Festuca rubra-Galium verum* fixed dune grassland, typical sub-community. |
| SD8b | *Festuca rubra-Galium verum* fixed dune grassland, *Luzula campestris* sub-community. |
| SD8c | *Festuca rubra-Galium verum* fixed dune grassland, *Tortula ruralis* ssp. *ruraliformis* sub-community. |
| SD8d | *Festuca rubra-Galium verum* fixed dune grassland, *Bellis perennis-Ranunculus acris* sub-community. |
| SD8e | *Festuca rubra-Galium verum* fixed dune grassland, *Prunella vulgaris* sub-community. |

**Table B.3.4:** Continued over page.

**Table B.3.4:** Cont.

| **ALPHANUMERIC CODE** | **DESCRIPTION** |
|---|---|
| SD9a | *Ammophila arenaria-Arrhenatherum elatius* dune grassland, typical sub-community. |
| SD9b | *Ammophila arenaria-Arrhenatherum elatius* dune grassland, *Geranium sanguineum* sub-community. |
| SD12a | *Carex arenaria-Festuca ovina-Agrostis capillaris* grassland, *Anthoxanthum oderatum* sub-community. |
| SD12b | *Carex arenaria-Festuca ovina-Agrostis capillaris* dune grassland, *Holcus lanatus* sub-community. |
| **Neutral grassland** | |
| MG1a | *Arrhenatherum elatius* coarse grassland, *Festuca rubra* sub-community. |
| MG1b | *Arrhenatherum elatius* coarse grassland, *Urtica dioica* sub-community. |
| MG1d | *Arrhenatherum elatius* coarse grassland, *Pastinaca sativa* sub-community. |
| MG1e | *Arrhenatherum elatius* coarse grassland, *Centaurea nigra* sub-community. |
| MG2 | *Filipendula ulmaria-Arrhenatherum elatius* tall-herb grassland. |
| MG5a | *Cynosurus cristatus-Centaurea nigra* meadow, *Lathyrus pratensis* sub-community. |
| MG5b | *Cynosurus cristatus-Centaurea nigra* meadow, *Galium verum* sub-community. |
| MG6a | *Lolium perenne-Cynosurus cristatus* pasture, Typical sub-community. |
| MG6b | *Lolium perenne-Cynosurus cristatus* pasture, *Anthoxanthum odoratum* sub-community. |
| MG7a | *Lolium perenne* leys, *Lolium perenne-Trifolium repens* leys. |
| MG7e | *Lolium perenne* leys, *Plantago lanceolata* sub-community. |
| MG9a | *Holcus lanatus-Deschampsia cespitosa* coarse grassland, *Arrhenatherum elatius* sub-community. |
| MG10a | *Holcus lanatus-Juncus effuses* rush pasture, Typical sub-community. |
| MG10b | *Holcus lanatus-Juncus effuses* rush pasture, *Juncus inflexus* sub-community. |
| MG10c | *Holcus lanatus-Juncus effuses* rush pasture, *Iris pseudacorus* sub-community. |
| MG11a | *Festuca rubra-Agrostis stolonifera-Potentilla anserine* inundation grassland *Lolium perenne* sub-community |
| MG12a | *Festuca arundinacea* coarse grassland, *Lolium perenne-Holcus lanatus* sub-community. |
| MG12b | *Festuca arundinacea* coarse grassland, *Oenanthe lachenalii* sub-community. |
| **Calcicolous grassland** | |
| CG6a | Avenula pubescens grassland. |
| CG7b | *Festuca ovina-Hieracium pilosella-Thymus praecox* grassland, *Cladonia* spp. Sub-community. |
| CG7c | *Festuca ovina-Hieracium pilosella-Thymus praecox* grassland, *Fragaria vesca-Rumex acetosa* Sub-community. |
| **Acid grassland** | |
| U1c | *Festuca ovina-Agrostis capillaris-Rumex acetosella* grassland, *Erodium cicutarium-Teesdalia nudicaulis* sub-community. |

**Table B.3.4:** Continued over page.

**Table B.3.4:** Cont.

| ALPHANUMERIC CODE | DESCRIPTION |
|---|---|
| U4a | *Festuca ovina-Agrostis capillaris-Galium saxatile* grassland, Typical sub-community. |
| U4b | *Festuca ovina-Agrostis capillaris-Galium saxatile* grassland, *Holcus lanatus-Trifolium repens* sub-community. |
| U5 | *Nardus stricta-Galium saxatile* grassland, undifferentiated. |
| U6 | *Juncus squarrosus-festuca ovina* grassland, undifferentiated. |
| U20 | *Pteridium aquilinum-Galium saxatile* community, undifferentiated. |
| **Sand sedge and 'grey' dunes** | |
| SD10a | *Carex arenaria* dune, *Festuca rubra* sub-community. |
| SD10b | *Carex arenaria* dune, *Festuca ovina* sub-community. |
| SD11 | *Carex arenaria-Cornicularia aculeate* community, undifferentiated. |
| **Heath** | |
| H1 | *Calluna vulgaris-festuca ovina* heath, undifferentiated. |
| H7e | *Calluna vulgaris-Scilla verna* heath, *Calluna vulgaris* sub-community. |
| H8 | *Calluna vulgaris-Ulex gallii* heath, undifferentiated. |
| H10a | *Calluna vulgaris-Erica cinerea* heath, Typical sub-community. |
| H11a | *Calluna vulgaris-Carex arenaria* dune heath, *Erica cinerea* sub-community. |
| H11a | *Calluna vulgaris-Carex arenaria* dune heath, *Hypnum cupressiforme* sub-community. |
| **Wet heaths and mires** | |
| M5 | *Carex rostrata-Sphagnum squarrosum* mire. |
| M10c | Carex dioica-Pinguicula vulgaris mire, *Gymnostomum recurvirostrum* sub-community. |
| M11 | *Carex demissa-Saxifraga aizoides* mire, undifferentiated. |
| M23 | *Juncus effuses/acutiflorus-Galium palustre* rush pasture, undifferentiated. |
| M25b | *Molinia caerulea-Potentilla erecta* mire, *Anthoxanthum odoratum* sub-community. |
| M27b | *Filipendula ulmaria-Angelica sylvestris* mire, *Urtica dioica-Vicia cracca* sub-community. |
| M28a | *Iris pseudacorus-Filipendula ulmaria* mire, *Juncus* spp. sub-community. |
| M28b | *Iris pseudacorus-Filipendula ulmaria* mire, *Urtica dioica-Galium aparine* sub-community. |
| **Dune slacks** | |
| SD13b | *Salix repens-Bryum pseudotriquetrum* dune slack, *Holcus lanatus-festuca rubra* sub-community. |
| SD14a | *Salix repens-Campylium stellatum* dune slack, *Carex serotina-Drepanocladus sendtneri* sub-community. |
| SD14b | *Salix repens-Campylium stellatum* dune slack, *Rubus caesius-Galium palustre* sub-community. |

**Table B.3.4:** Continued over page.

## **Table B.3.4:** Cont.

| **ALPHANUMERIC CODE** | **DESCRIPTION** |
|---|---|
| SD14c | *Salix repens-Campylium stellatum* dune slack, *Bryum pseudotriquetrum-Aneura pinguis* sub-community. |
| SD14d | *Salix repens-Campylium stellatum* dune slack, *Festuca rubra* sub-community. |
| SD15a | *Salix repens-Calliergon cuspidatum* dune slack, *Carex nigra* sub-community. |
| SD15b | *Salix repens-Calliergon cuspidatum* dune slack, *Carex nigra* sub-community. |
| SD15c | *Salix repens-Calliergon cuspidatum* dune slack, *Carex flacca-Pulicaria dysenterica* sub-community. |
| SD15d | *Salix repens-Calliergon cuspidatum* dune slack, *Holcus lanatus-Angelica sylvestris* sub-community. |
| SD16a | *Salix repens-Holcus lanatus* dune slack, *Ononis repens* sub-community. |
| SD16b | *Salix repens-Holcus lanatus* dune slack, *Rubus caesius* sub-community. |
| SD16c | *Salix repens-Holcus lanatus* dune slack, *Prunella vulgaris-Equisetum variegatum* sub-community. |
| SD16d | *Salix repens-Holcus lanatus* dune slack, *Agrostis stolonifera* sub-community. |
| SD17a | *Potentilla anserine-Carex nigra* dune slack, *Festuca rubra-Ranunculus repens* sub-community. |
| SD17b | *Potentilla anserine-Carex nigra* dune slack, *Carex flacca* sub-community. |
| SD17c | *Potentilla anserine-Carex nigra* dune slack, *Caltha palustris* sub-community. |
| SD17d | *Potentilla anserine-Carex nigra* dune slack, *Hydrocotyle vulgaris-Ranunculus flammula* sub-community. |
| **Swamp and tall-herb fens** | |
| S4a | *Phragmites australis* swamp, *Phragmites australis* sub-community. |
| S4d | *Phragmites australis* swamp, *Atriplex hastata* sub-community. |
| S5 | *Glyceria maxima* swamp. |
| S6 | *Carex riparia* swamp. |
| S7 | *Carex acutiformis* swamp. |
| S8a | *Scirpus lacustris* ssp. *lacustris* swamp, *S. lacustris* spp. *Lacustris* sub-community. |
| S10 | *Equisetum fluviatile* swamp, undifferentiated. |
| S12b | *Typha latifolia* swamp, *Mentha aquatic* sub-community. |
| S14 | *Sparganium erectum* swamp, undifferentiated. |
| S18a | *Carex otrubae* swamp, *Carex otrubae* sub-community. |
| S19a | *Eleocharis palustris* swamp, *Eleocharis palustris* sub-community. |
| S19c | *Eleocharis palustris* swamp, *Agrostis stolonifera* sub-community. |
| S20b | *Scirpus lacustris* ssp. *tabernaemontani* swamp, *Agrostis stolonifera* sub-community. |
| S21a | *Scirpus maritimus* swamp, *Scirpus maritimus* sub-community |
| S21c | *Scirpus maritimus* swamp, *Potentilla anserina* sub-community. |

**Table B.3.4:** Continued over page.

## **Table B.3.4:** Cont.

| **ALPHANUMERIC CODE** | **DESCRIPTION** |
|---|---|
| S25 | *Phragmites australis-Eupatorium cannabinum* tall-herb fen. |
| S25D | *Phragmites australis-Urtica dioica* tall-herb fen, *Epilobium hirsutum* sub-community. |
| S26c | *Phalaris arundinacea* tall-herb fen, *Elymus repens-Holcus lanatus* sub-community. |
| **Scrub and woodland** | |
| SD18a | *Hippophae rhamnoides* scrub, *Festuca rubra* sub-community. |
| SD18b | *Hippophae rhamnoides* scrub, *Urtica dioica-Arrhenatherum elatius* sub-community. |
| W1 | *Salix cinerea-Galium palustre* woodland, undifferentiated. |
| W2 | *Salix cinerea-Betula pubescens-Phragmites australis* woodland, undifferentiated. |
| W4 | *Betula pubescens-Molinia caerulea* woodland, undifferentiated. |
| W6 | *Alnus glutinosa-Urtica dioica* woodland, undifferentiated. |
| W8 | *Fraxinus excelsior-Acer campestre-Mercurialis perennis* woodland, undifferentiated. |
| W10c | *Quercus robur-Pteridium aquilinum-Rubus fruticosus* agg. Woodland, *Herdera helix* sub-community. |
| W21a | *Cratargus monogyna-Hedera helix* scrub, *Hedera helix-Urtica dioica* sub-community. |
| W21b | *Cratargus monogyna-Hedera helix* scrub, *Mercurialis perennis* sub-community. |
| W22a | *Prunus spinosa-Rubus fruticosus* agg. Scrub, *Hedera helix-Silene dioica* sub-community. |
| W22b | *Prunus spinosa-Rubus fruticosus* agg. Scrub, *Viola riviniana-Veronica chamaedrys* sub-community. |
| W22c | *Prunus spinosa-Rubus fruticosus* agg. Scrub, *Dactylis glomerata* sub-community. |
| W23a | *Ulex europaeus-Rubus fruticosus* agg. Scrub, *Anthoxanthum odoratum* sub-community. |
| W23b | *Ulex europaeus-Rubus fruticosus* agg. Scrub, *Rumex acetosella* sub-community. |
| W23c | *Ulex europaeus-Rubus fruticosus* agg. Scrub, *Teucrium scorodonia* sub-community. |
| W24a | *Rubus fruticosus agg.-Holcus lanatus* underscrub, *Cirsium arvense-Cirsium vulgare* sub-community, |
| W24b | *Rubus fruticosus agg.-Holcus lanatus* underscrub, *Arrhenatherum elatius-Heracleum sphondylium* sub-community, |
| W25 | *Pteridium aquilinum-Rubus fruticosus* agg. Underscrub, undifferentiated. |

**Table B.3.4:** Continued over page.

**Table B.3.4:** Cont.

| ALPHANUMERIC CODE | DESCRIPTION |
|---|---|
| **Transitions to other habitats** | |
| MC5b | *Armeria maritime-Ceratium diffusum* spp. *diffusum* maritime therophyte community, *Anthyllis vulneraria* sub-community. |
| MC5d | *Armeria maritime-Ceratium diffusum* spp. *diffusum* maritime therophyte community, *Arenaria serpyllifolia* sub-community. |
| MC8a | *Festuca rubra-Armeria maritime* maritime grassland, Typical sub-community. |
| MC8e | *Festuca rubra-Armeria maritime* maritime grassland, *Plantago coronopus* sub-community. |
| **Maritime cliff (from SD8 dune grassland)** | |
| MC8f | *Festuca rubra-Armeria maritima* maritime grassland, *Anthyllis vulneraria* sub-community. |
| MC9b | *Festuca rubra-Holcus lanatus* maritime grassland, *Dactylis glomerata* sub-community. |
| MC10 | *Festuca rubra-Plantago* spp. maritime grassland, undifferentiated. |
| MC12a | *Festuca rubra-Hyacinthoides non-scripta* bluebell community, *Silene vulgaris* ssp. *maritima* sub-community. |
| **Saltmarsh (from various strandline, dune grassland, dune slack and swamp types – lower saltmarsh types mapped in Wales as part of the dune survey are not included** | |
| SM15 | *Juncus maritimus-Triglochin maritima* saltmarsh, undifferentiated. |
| SM16a | *Festuca rubra* saltmarsh, *Puccinellia maritima* sub-community. |
| SM16b | *Festuca rubra* saltmarsh, sub-community with *Juncus gerardi* dominant. |
| SM16c | *Festuca rubra* saltmarsh, *Festuca rubra-Glaux maritima* sub-community. |
| SM16d | *Festuca rubra* saltmarsh, sub-community with tall *Festuca rubra* dominant. |
| SM18a | *Juncus maritimus* saltmarsh, *Plantago maritima* sub-community. |
| SM18b | *Juncus maritimus* saltmarsh, *Oenanthe lachenalii* sub-community. |
| SM20 | *Eleocharis uniglumis* saltmarch. |
| SM24 | Elymus *pycnanthus* saltmarsh. |
| SM28 | Elymus *repens* saltmarsh. |

**Table B.3.5:** Species present within each habitat element associated with sand and viviparous lizard observations across the three research sites between March 2010 and October 2014.

| Feature | Feature/species |
|---|---|
| Sand | Sand |
| Stone | Stones |
| Man-made | Wooden fence post, Rubbish/litter, Other (underwear, clothes etc). |
| Tall ruderal | Yarrow *Achillea millefolium,* Cow parsley *Anthriscus sylvestris,* Rosebay willowherb *Chamerion angustifolium,* Creeping thistle *Cirsium arvense,* Wild carrot *Daucus carota,* Foxglove *Digitalis* spp., American willowherb *Epilobium ciliatum,* Hogweed *Heracleum sphondylium,* Evening primrose *Oenothera* spp., Burnet saxifrage *Pimpinella saxifrage,* Ragwort *Senecio jacobaea* |
| Shrub | Sycamore *Acer pseudoplatanus,* Pyramidal Orchid *Anacamptis pyramidalis,* Scarlet pimpanel *Anagallis arvensis,* Deadly nightshade *Solanum nigrum,* Daisy *Belis perennis,* Sea bindweed *Calystegia soldanella,* Harebell *Campanula rotundifolia,* Common mouse-ear *Cerastium fontanum,* Clematis *Clematis flammula var. maritima,* Leyland cypress *Cupressus × leylandii,* Sea holly *Eryngium maritimum,* Sea spurge *Euphorbia paralias,* Eyebright *Euphrasia* spp., Mare tail *Equisetum arvense,* Fungi spp., Ladies bedstraw *Galium verum,* Ivy *Hedera helix,* Cat's-ear *Hypochaeris radicata,* Sheep's bit scabious *Jasione montana,* Narrow leaved everlasting pea *Lathyrus sylvestris,* Rough hawkbit *Leontodon hispidus,* Lesser hawkbit *Leontodon taraxacoides,* Pineappleweed *Matricaria discoidea,* Creeping forget me not *Myosotis secunda,* Common restharrow *Ononis repens,* Bee Orchid *Ophrys apifera,* Royal Fern *Osmunda regalis,* Butterbur *Petasites* spp., Mouse-eared hawkbit *Pilosella officinarum,* Ribwort plantain *Plantago lanceolate,* Blackthorn *Prunus spinose,* Creeping buttercup *Ranunculus repens,* Dewberry *Rubus caesius,* Bramble *Rubus fruticosus,* Common sorrel *Rumex acetosa,* Broad-leaved dock *Rumex obtusifolius,* Saltwort Salsola *kali* subsp, Groundsel *Senecio vulgaris,* Charlock *Sinapis arvensis,* Common sowthistle *Sonchus oleraceus,* Betony *Stachys officinalis,* Common chickweed *Stellaria media,* Devil bit scabious *Succisa pratensis,* Dandelion *Taraxacum* spp., Screw moss *Tortula muralis,* Red clover *Trifolium pratense,* Scentless mayweed *Tripleurospermum inodorum,* Sea mayweed *Tripleurospermum maritimum,* Germander speedwell *Veronica chamaedrys,* Vetch *Vicia* spp., Heath dog violet *Viola canina* |

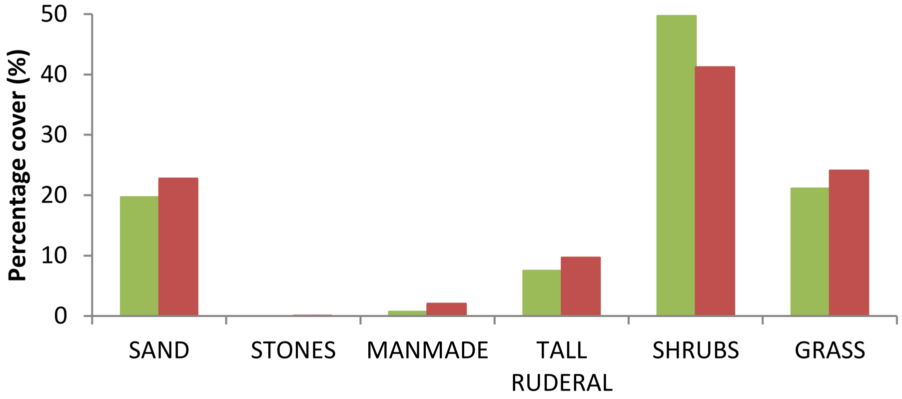

**Figure B.1:** Percentage usage of different habitat elements associated with sand and viviparous lizard observations across Morfa Harlech between March 2010 and October 2012. n = 625 (green indicates sand lizard, red indicates viviparous lizard).

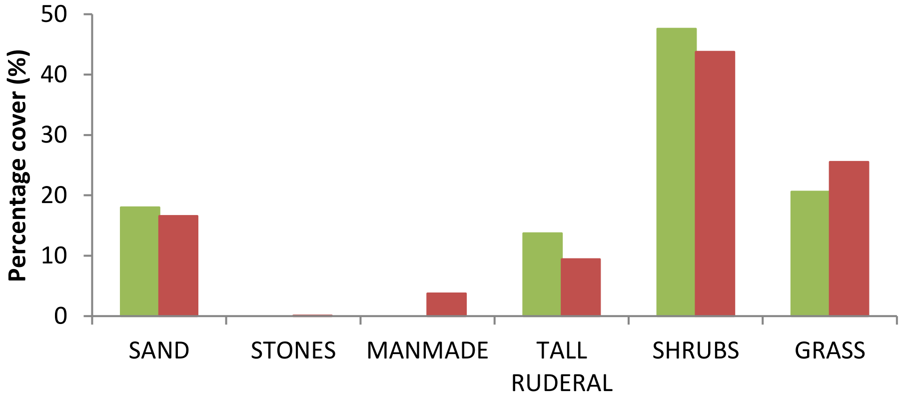

**Figure B.2:** Percentage usage of different habitat elements associated with sand and viviparous lizard observations across Aberdyfi between March 2013 and October 2014. n = 218 (green indicates sand lizard, red indicates viviparous lizard).

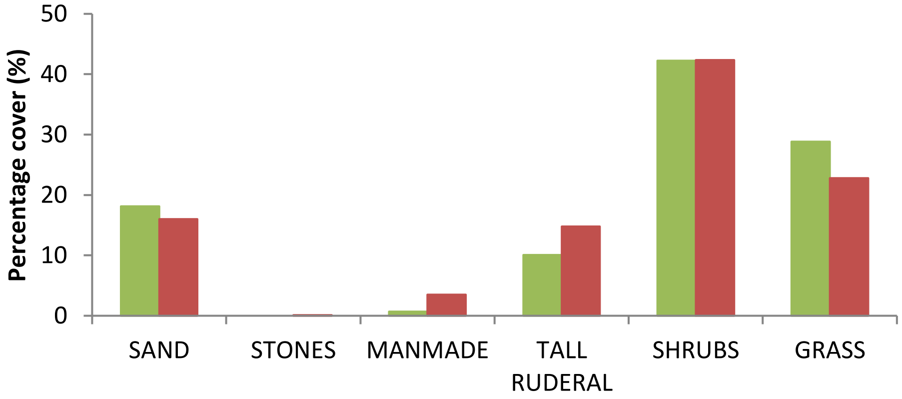

**Figure B.3:** Percentage usage of different habitat elements associated with sand and viviparous lizard observations across Ynyslas March 2013 and October 2014. n = 248 (green indicates sand lizard, red indicates viviparous lizard).

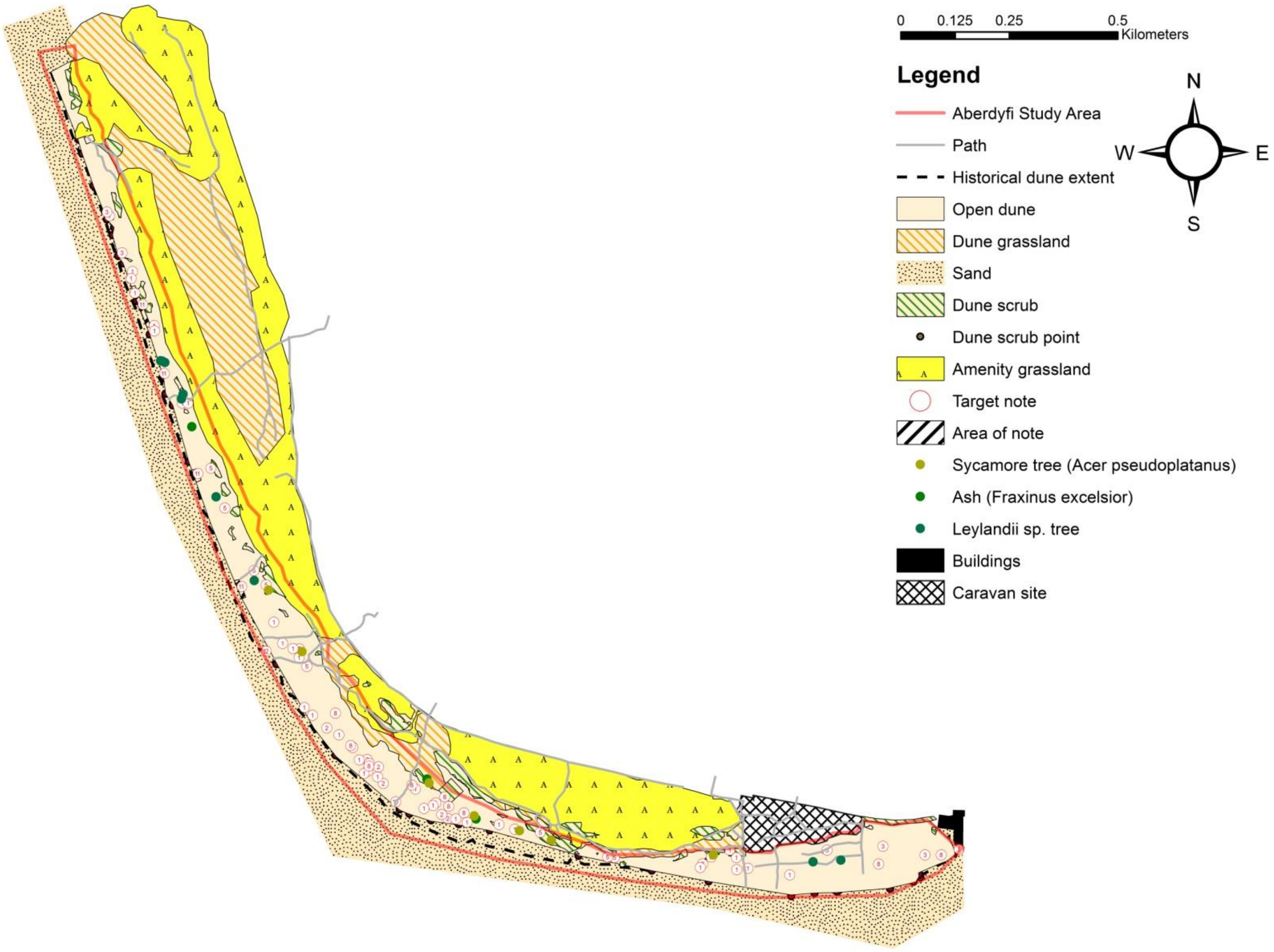

**Figure B.4:** Aberdyfi Phase 1 study map.

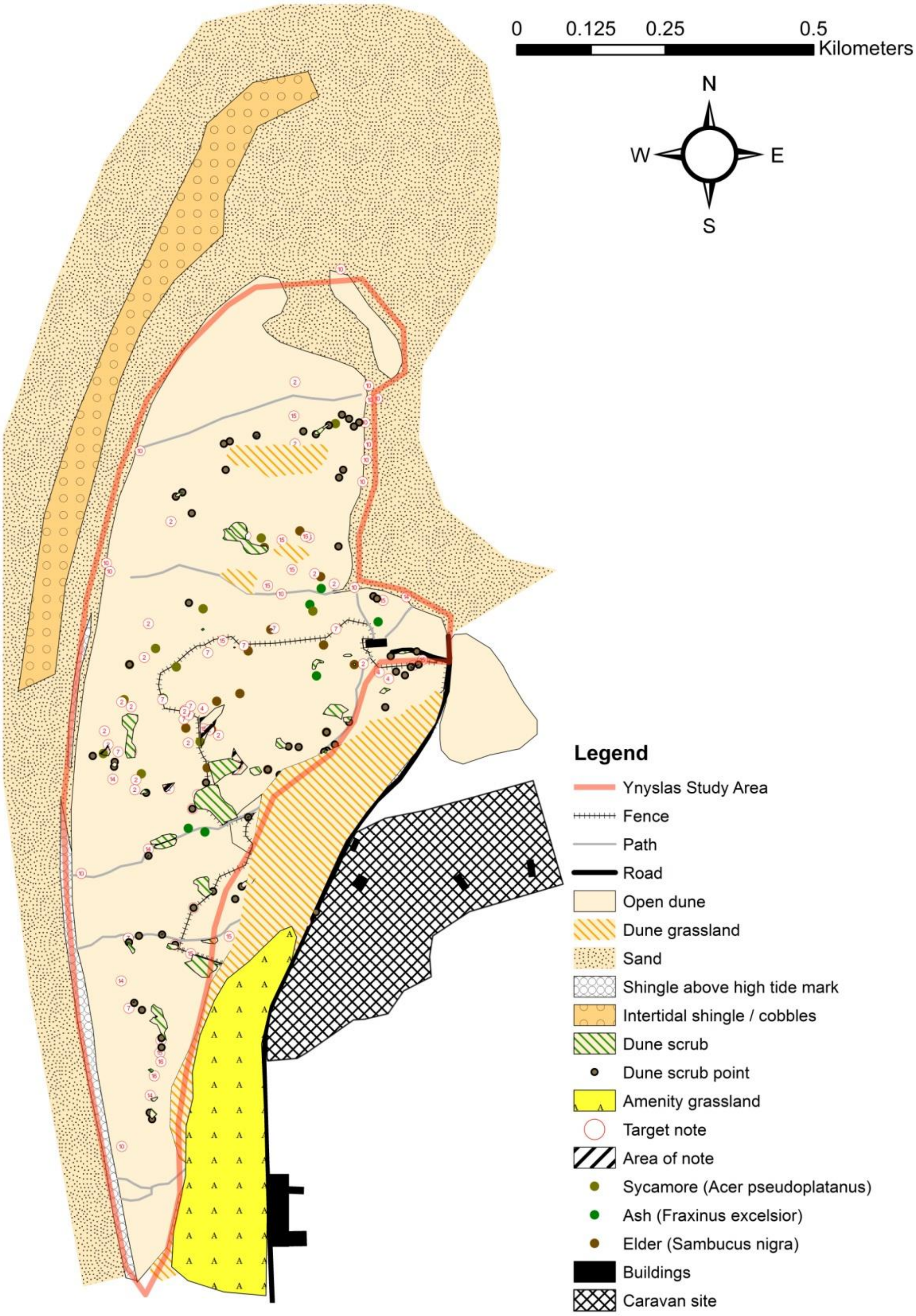

**Figure B.5:** Ynyslas Phase 1 study map.

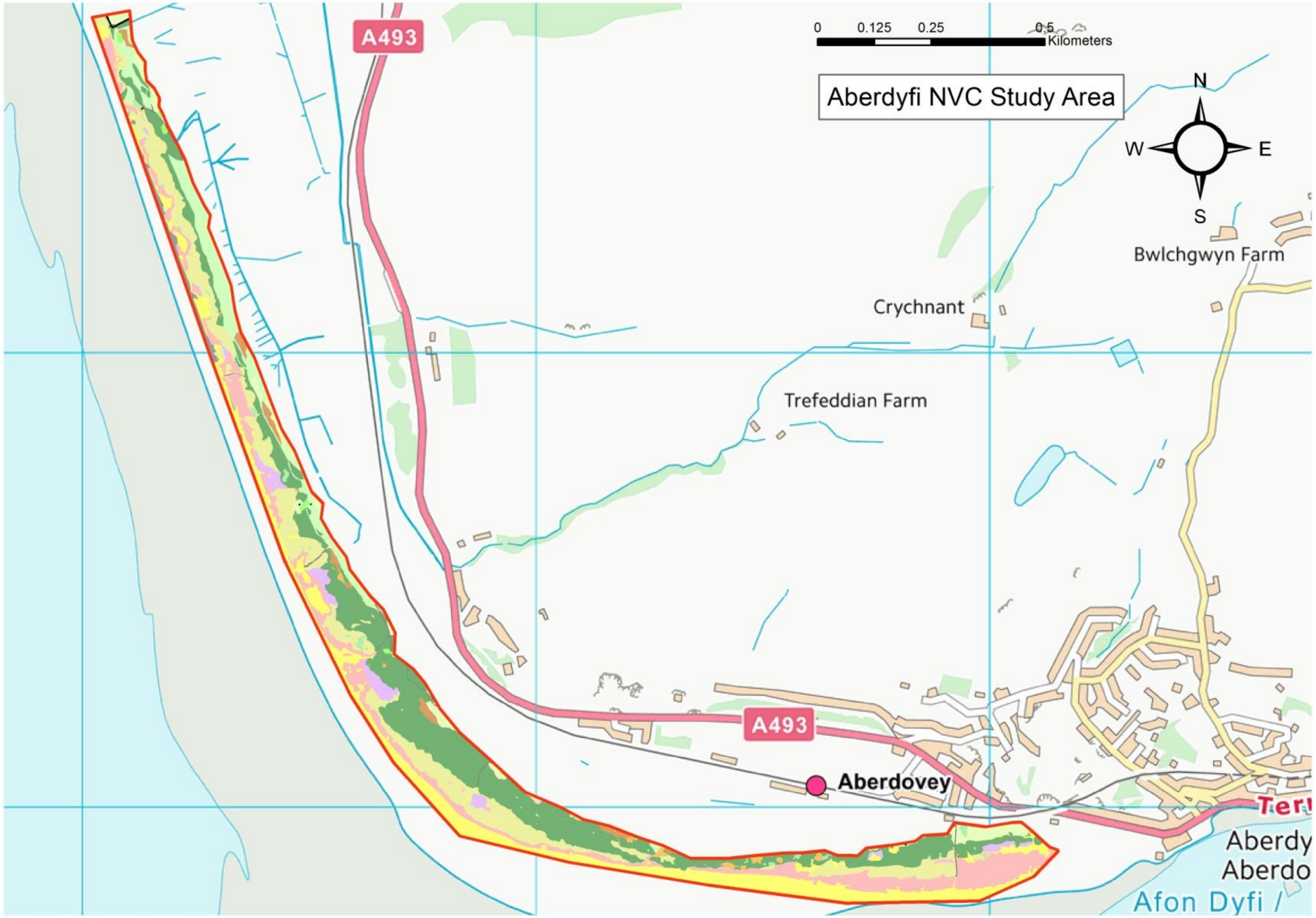

**Figure B.6a:** Aberdyfi National Vegetation Classification study map. To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

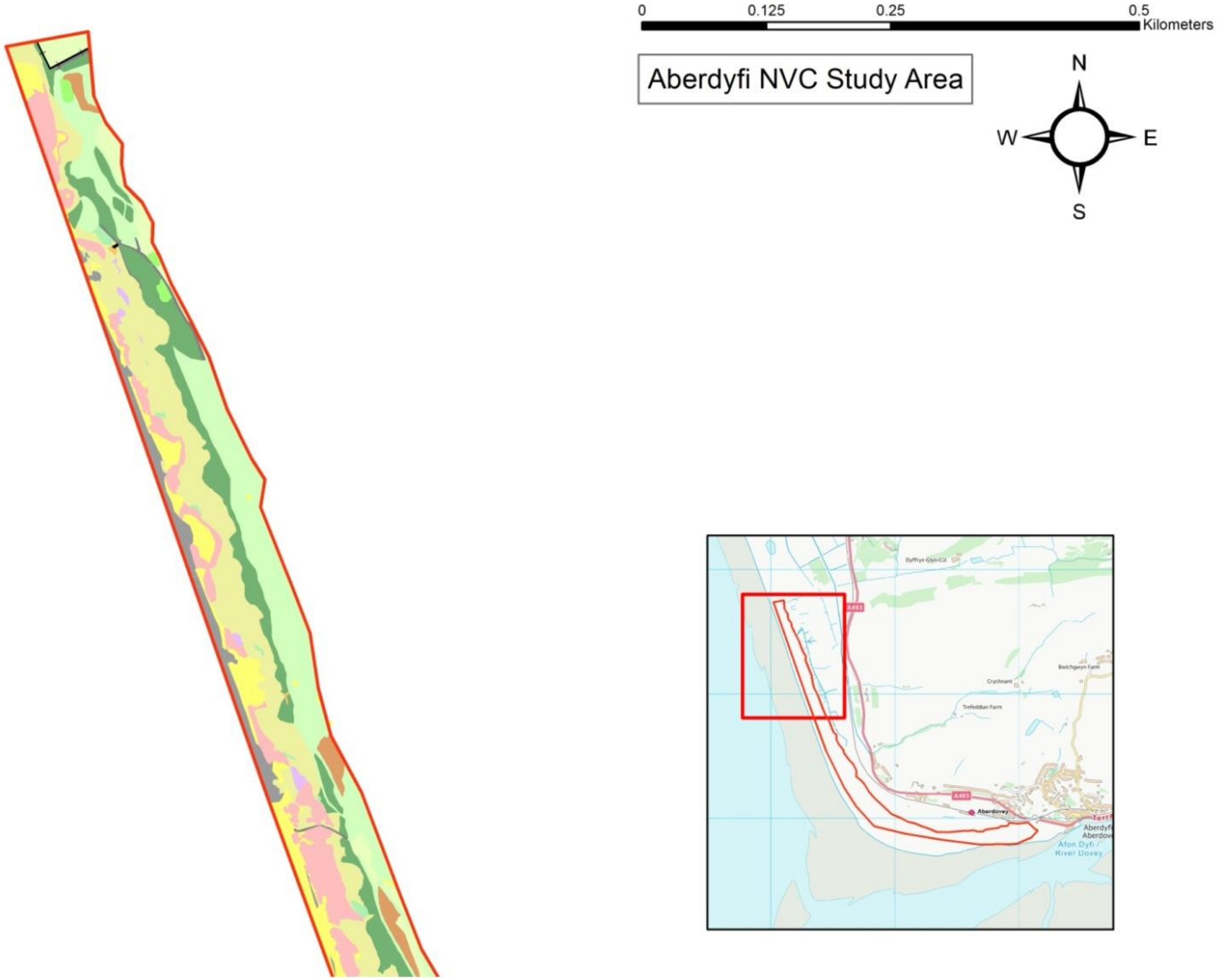

**Figure B.6b:** Detailed section of Aberdyfi National Vegetation Classification study map (Northern section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

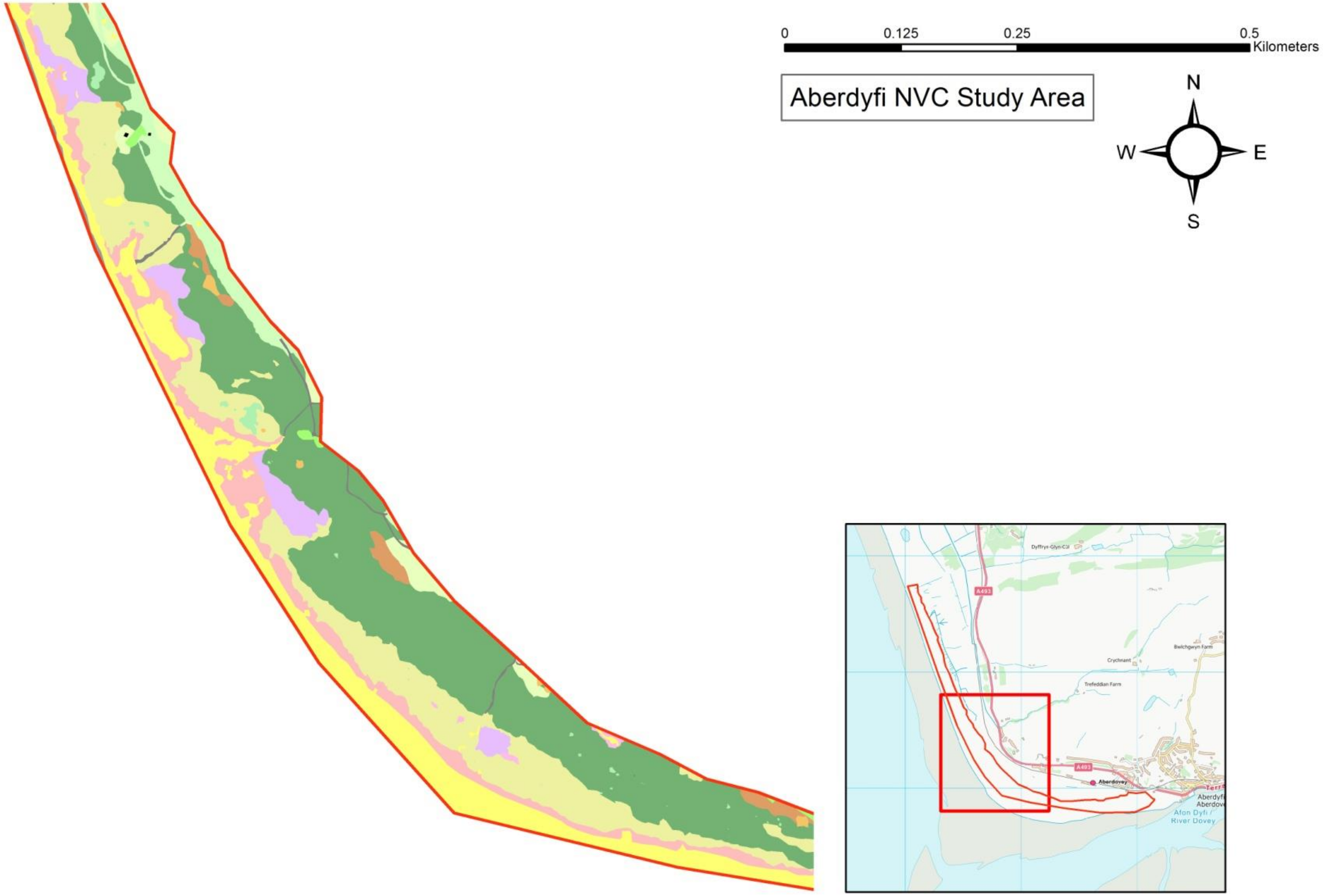

**Figure B.6c:** Detailed section of Aberdyfi National Vegetation Classification study map (mid-section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

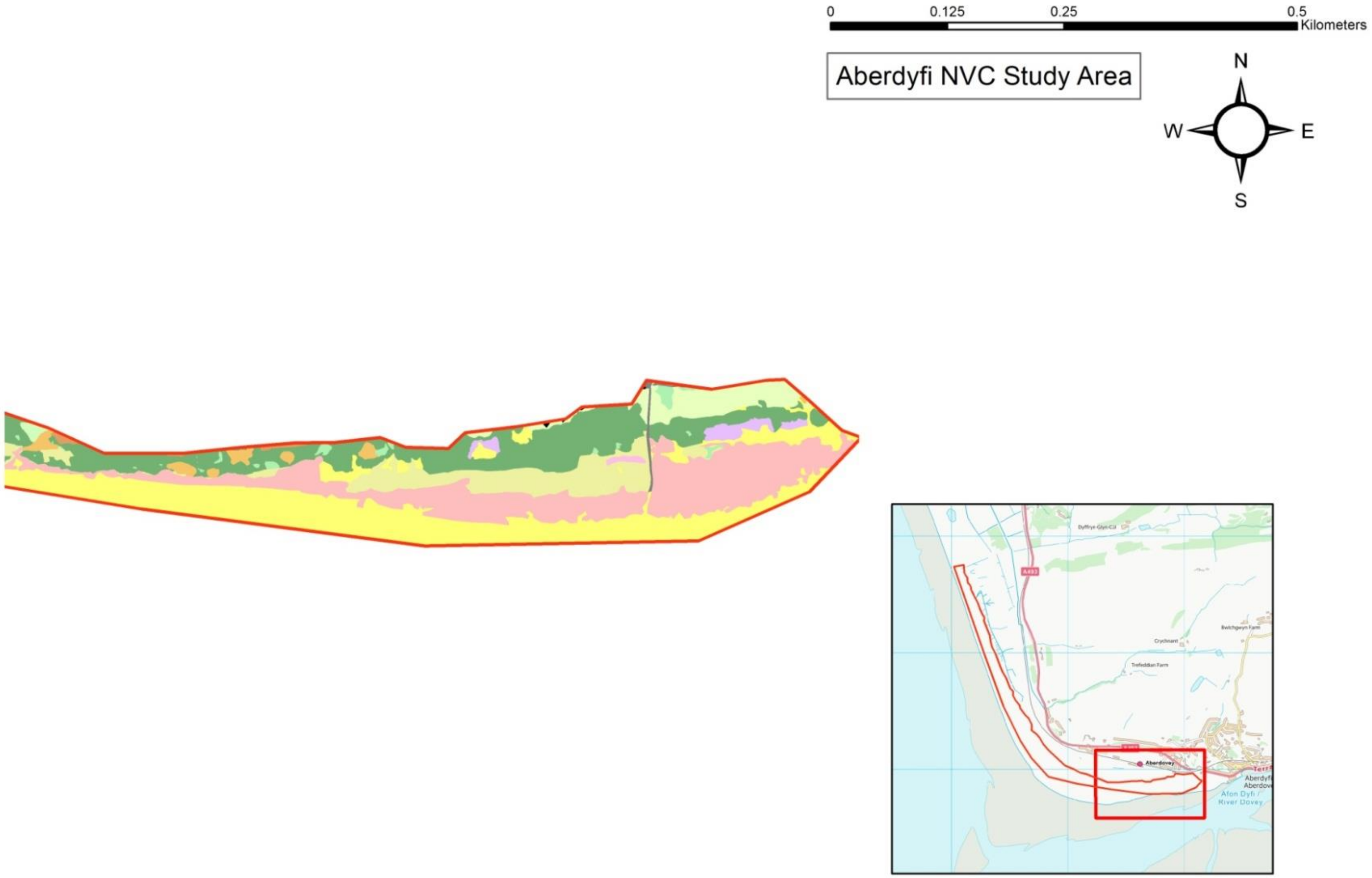

**Figure B.6d:** Detailed section of Aberdyfi National Vegetation Classification study map (Southern section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

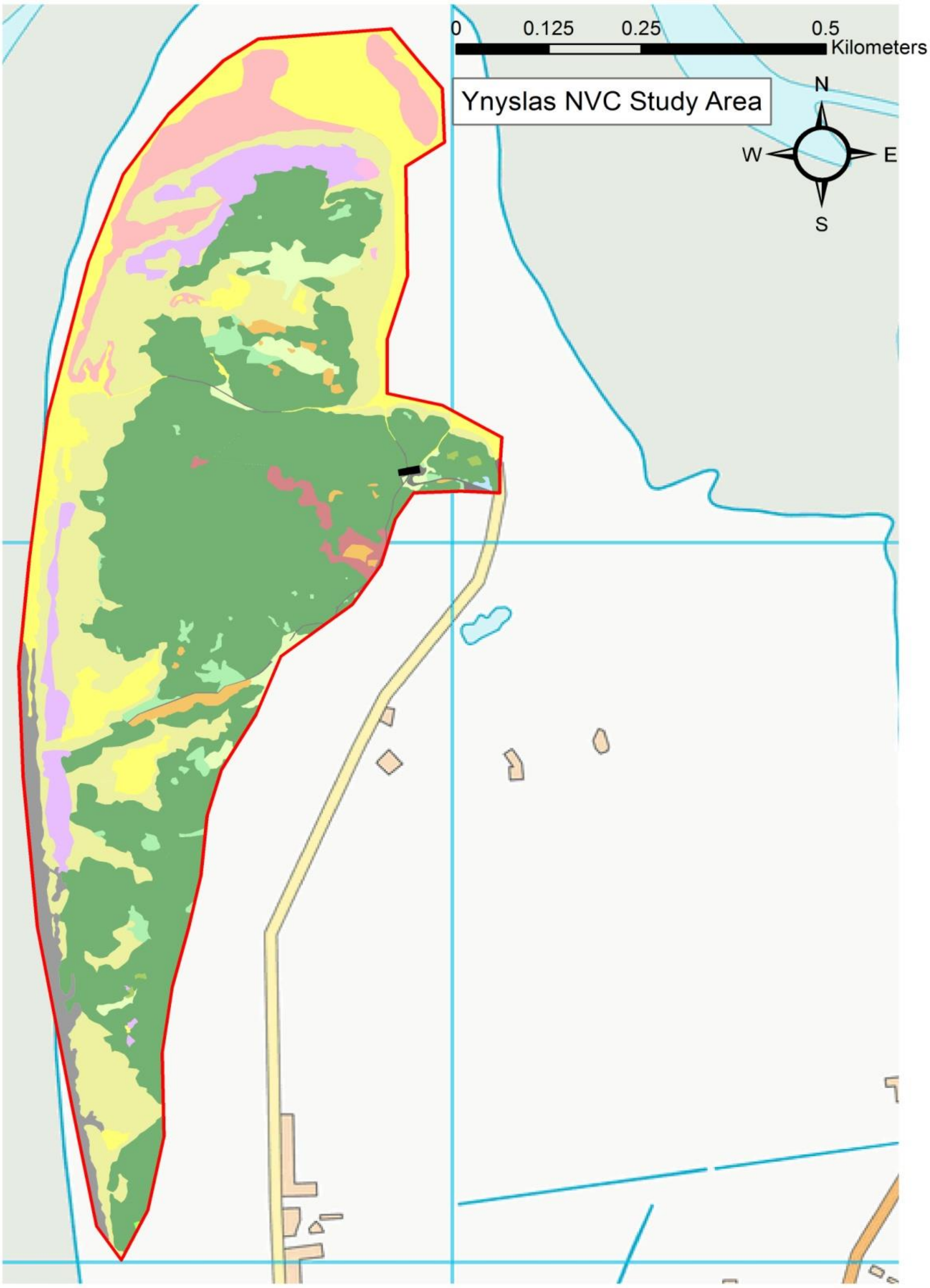

**Figure B.7a:** Ynyslas National Vegetation Classification study map. To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

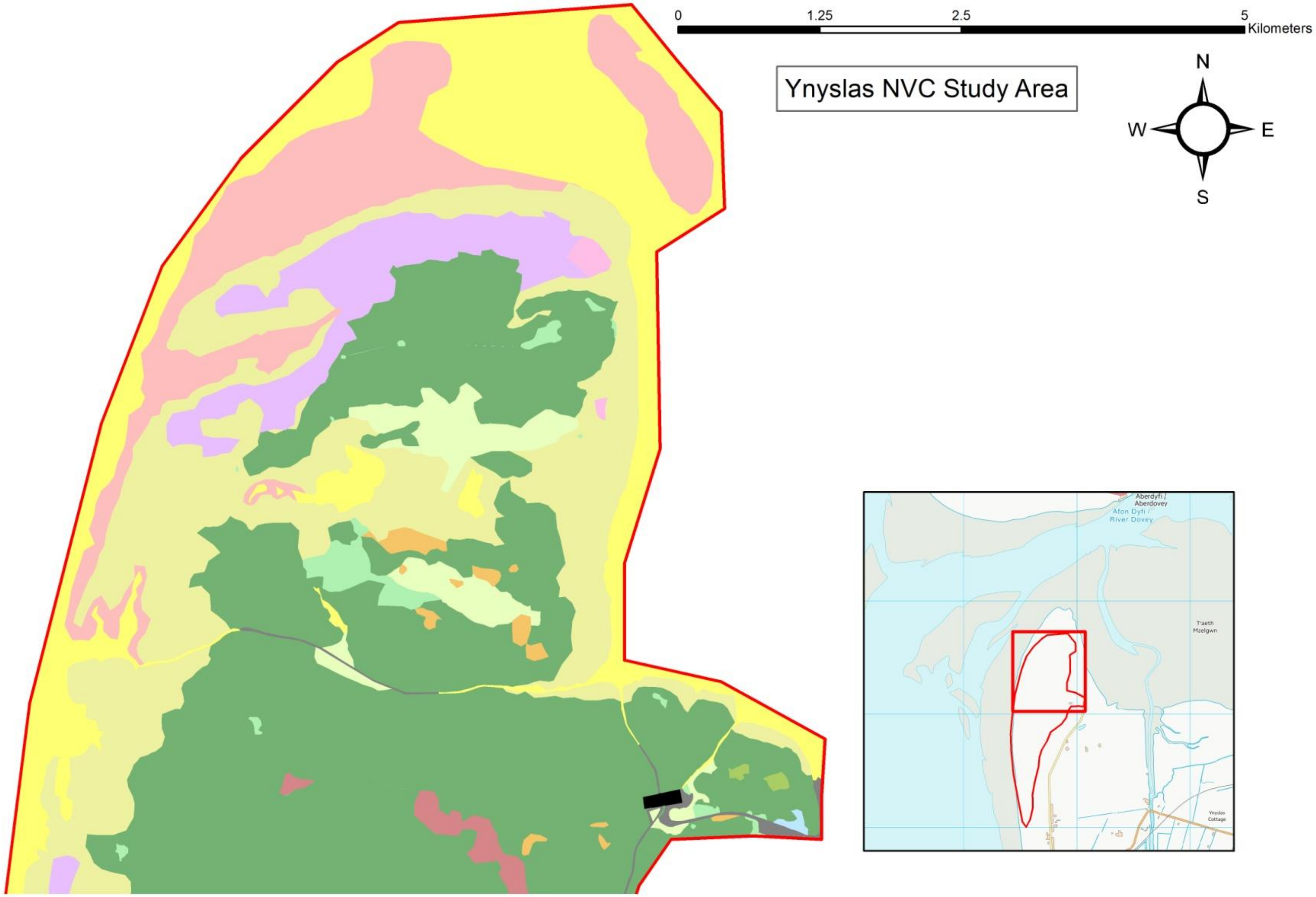

**Figure B.7b:** Detailed section of Ynyslas National Vegetation Classification study map (Northern section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

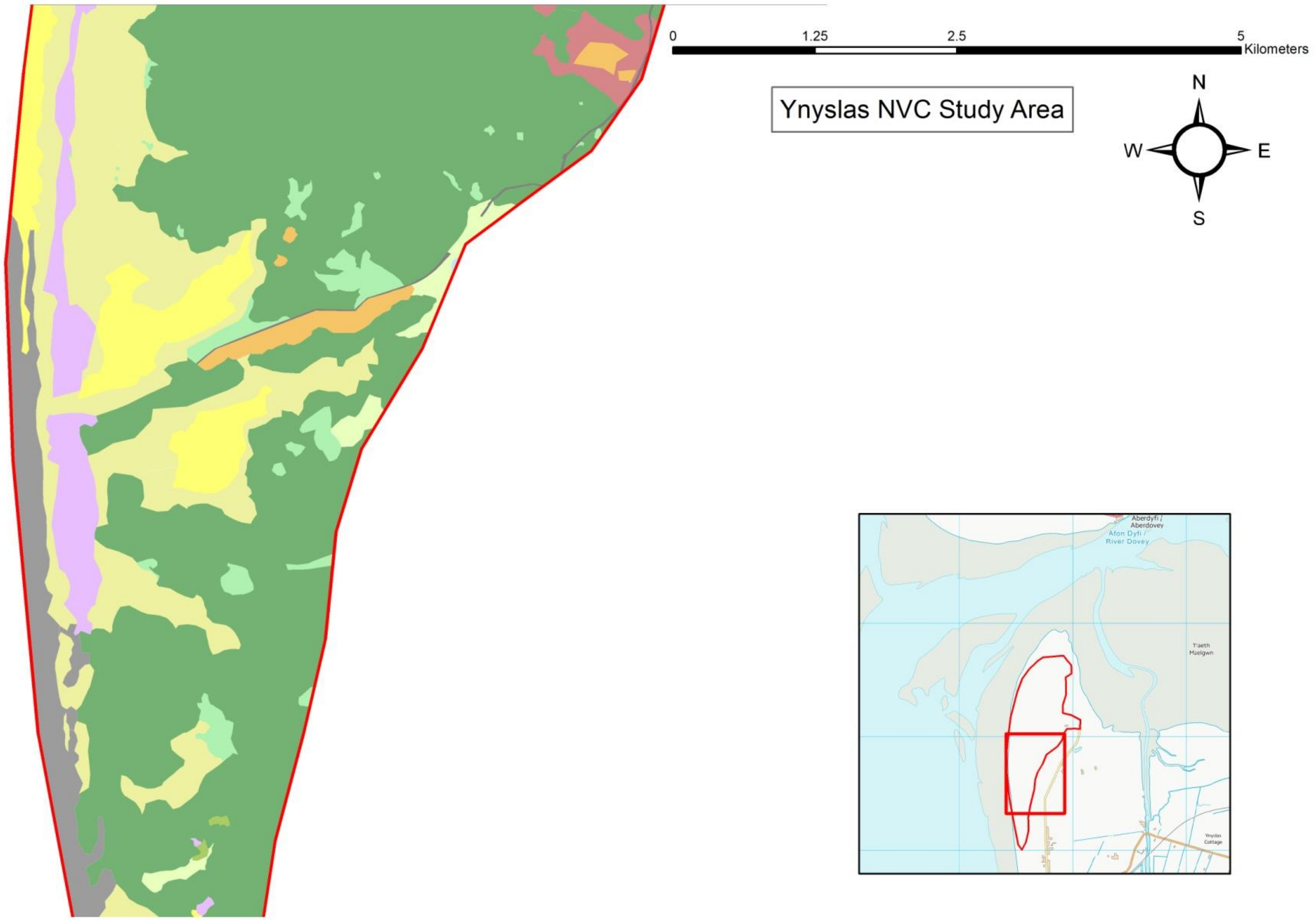

**Figure B.7c:** Detailed section of Ynyslas National Vegetation Classification study map (mid-section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

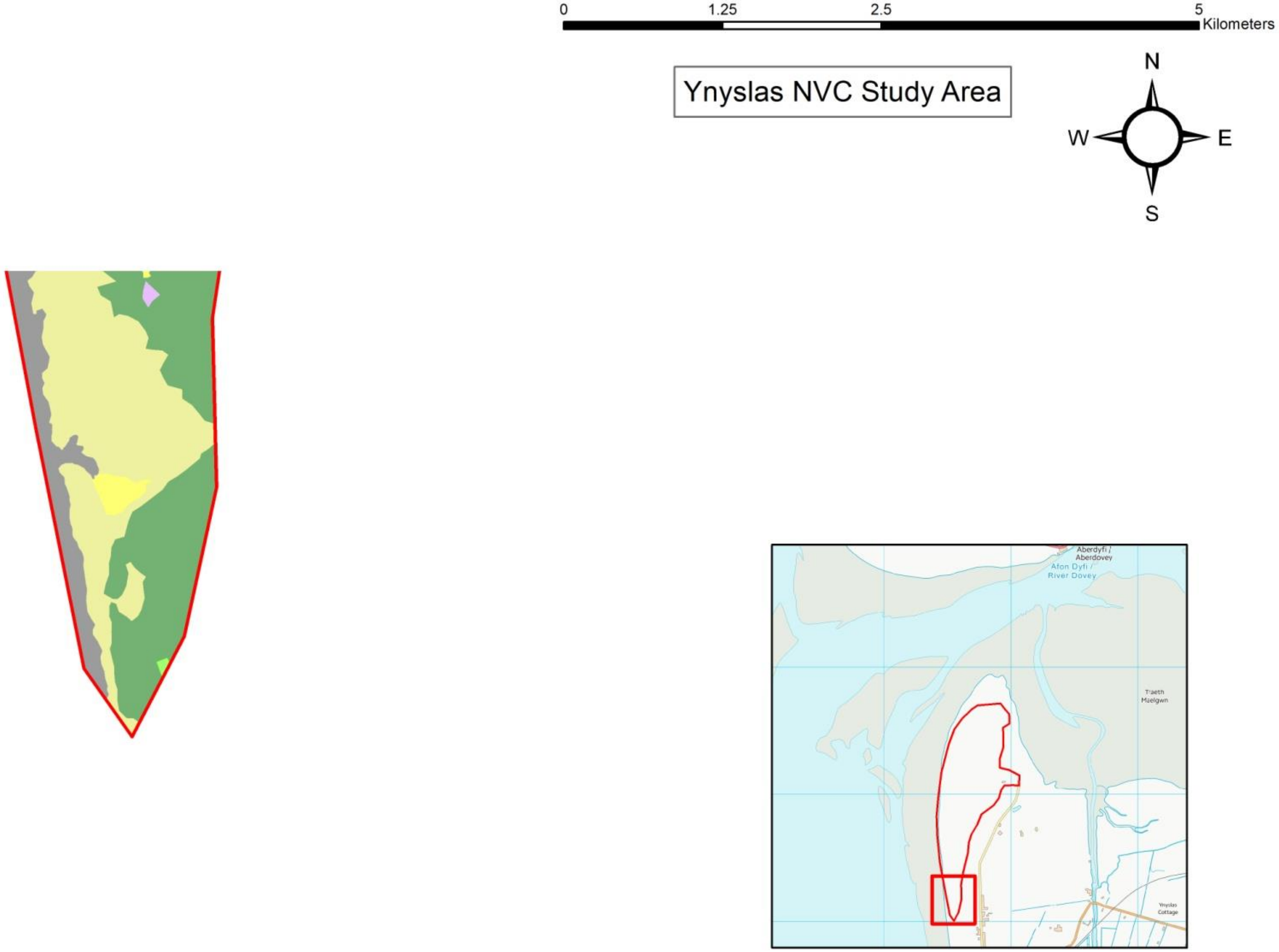

**Figure B.7d:** Detailed section of Ynyslas National Vegetation Classification study map (Southern section). To be read in conjunction with Table 3.2 (National Vegetation Classification legend).

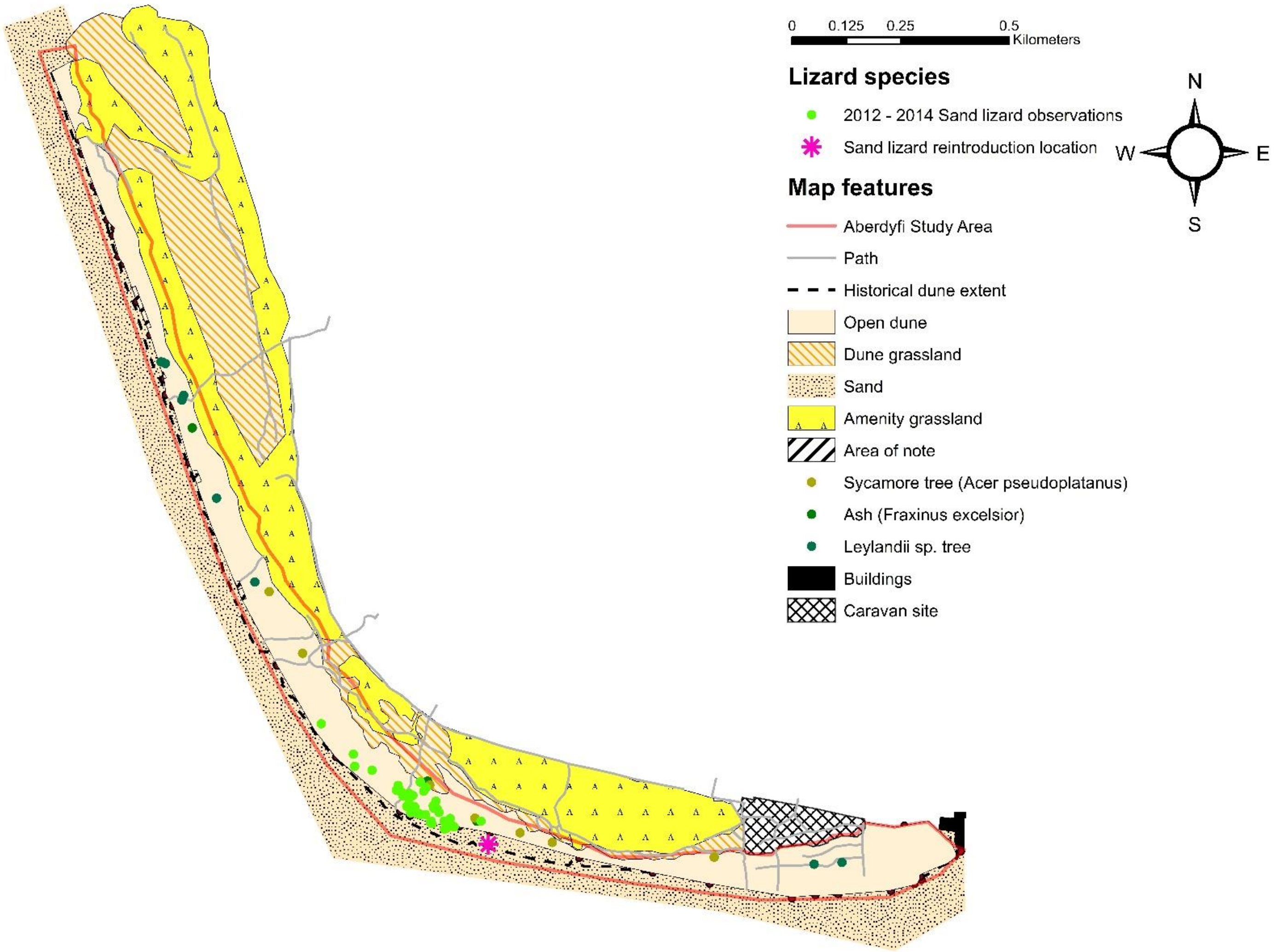

**Figure B.8a:** Visual representation of 2010 – 2014 sand lizard observations across Aberdyfi study area.

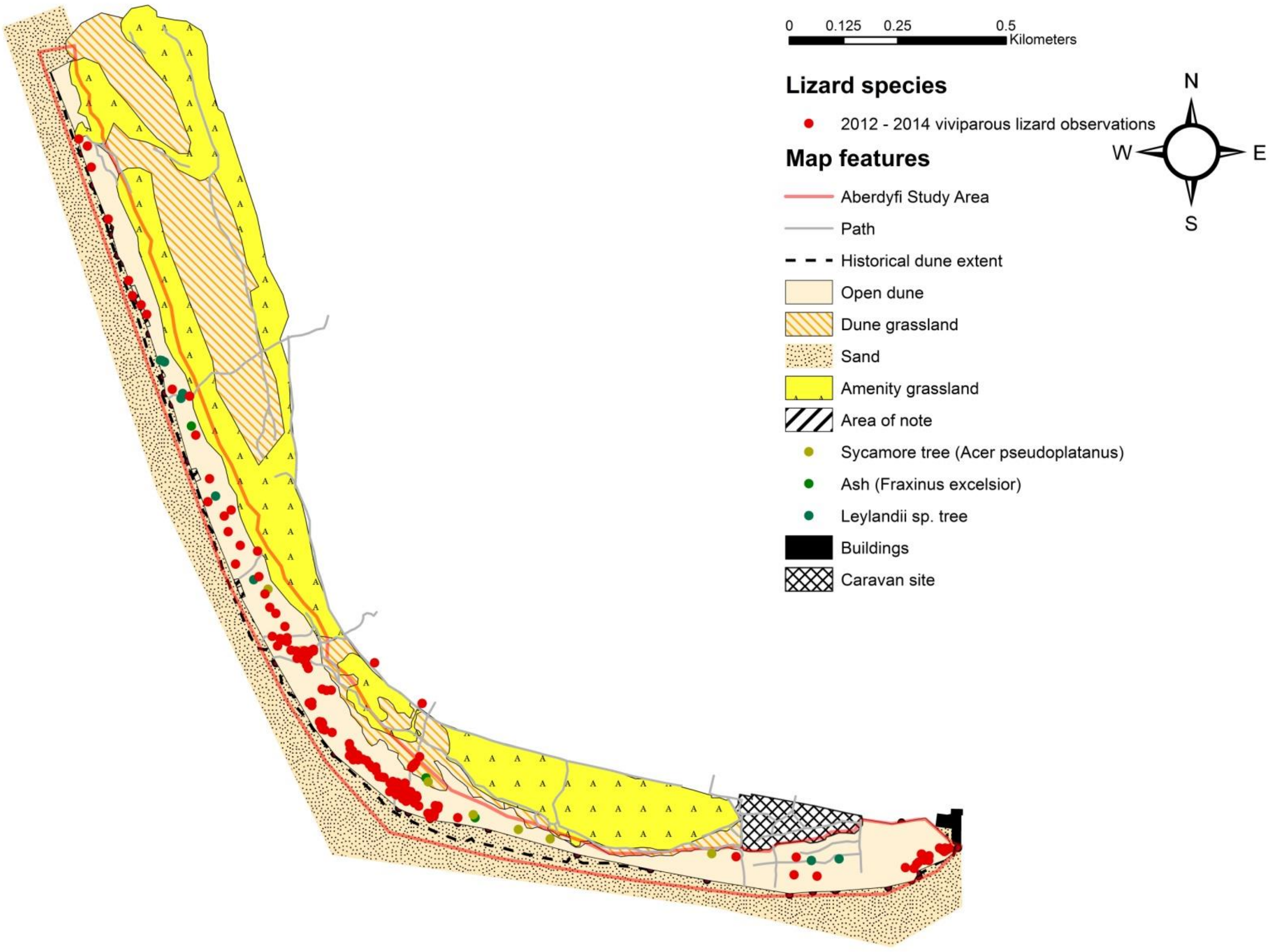

**Figure B.8b:** Visual representation of 2010 – 2014 viviparous lizard observations across Aberdyfi study area.

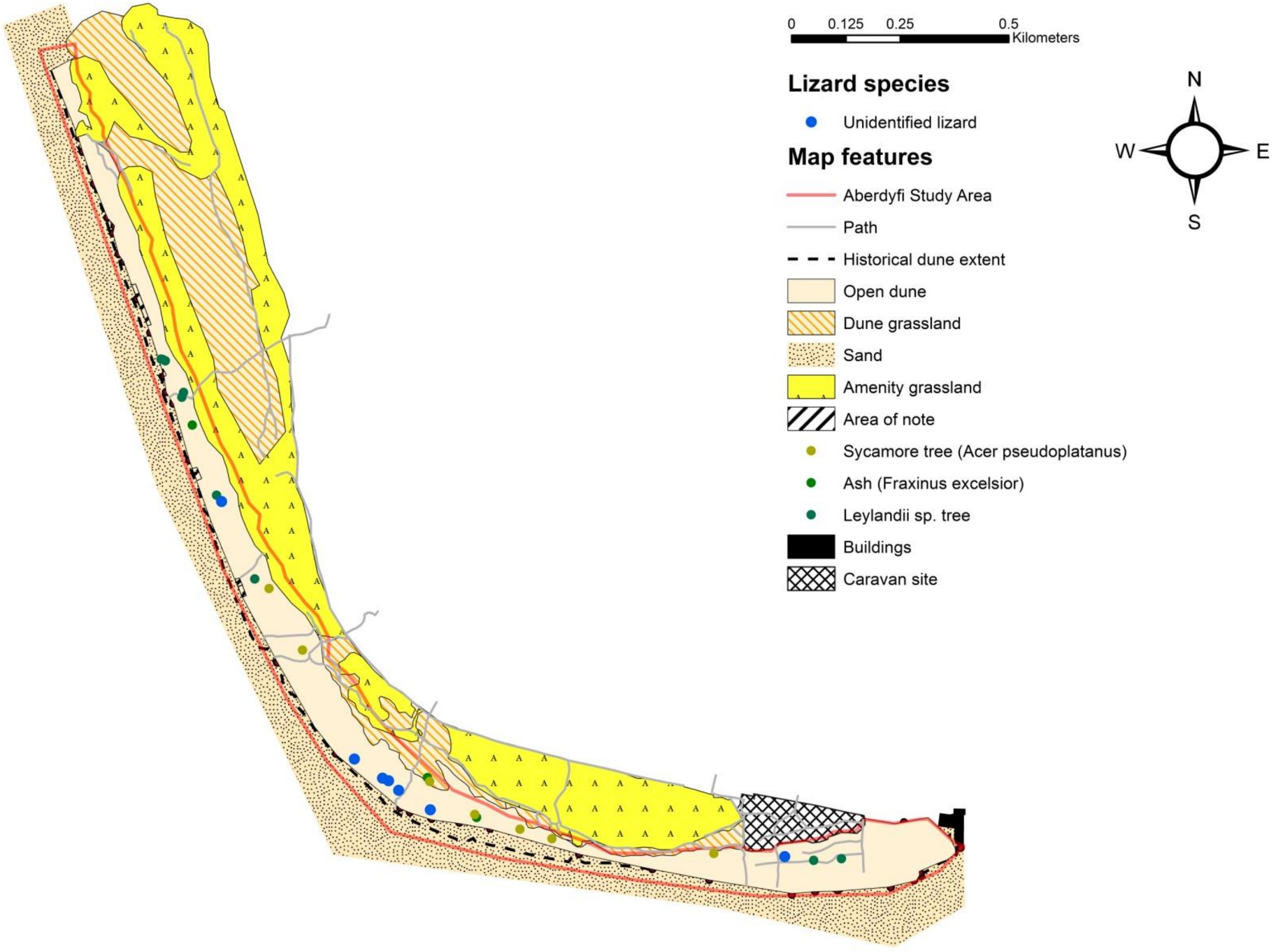

**Figure B.8c:** Visual representation of 2010 – 2014 unidentified lizard observations across Aberdyfi study area.

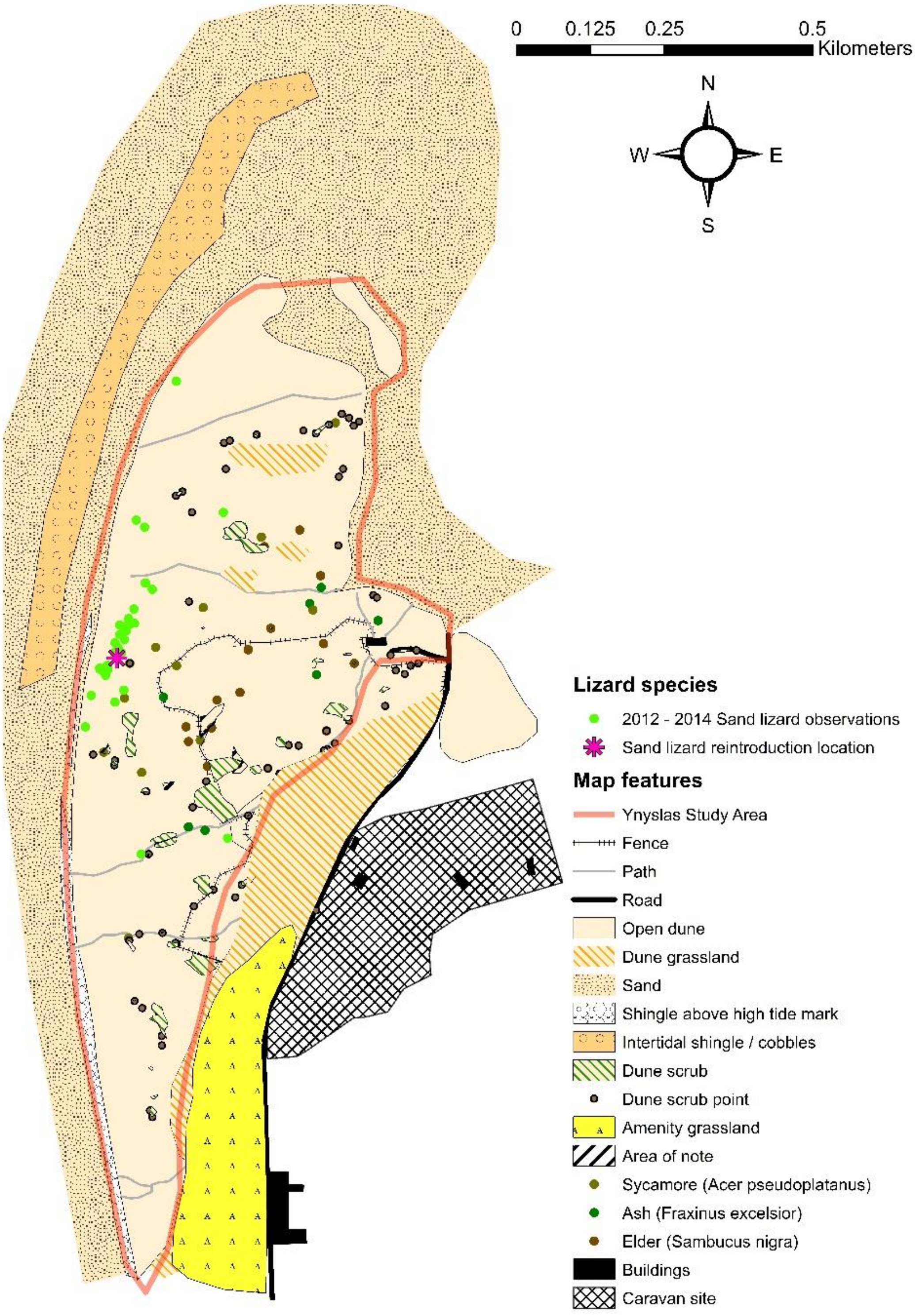

**Figure B.9a:** Visual representation of 2010 – 2014 sand lizard observations across Ynyslas study area.

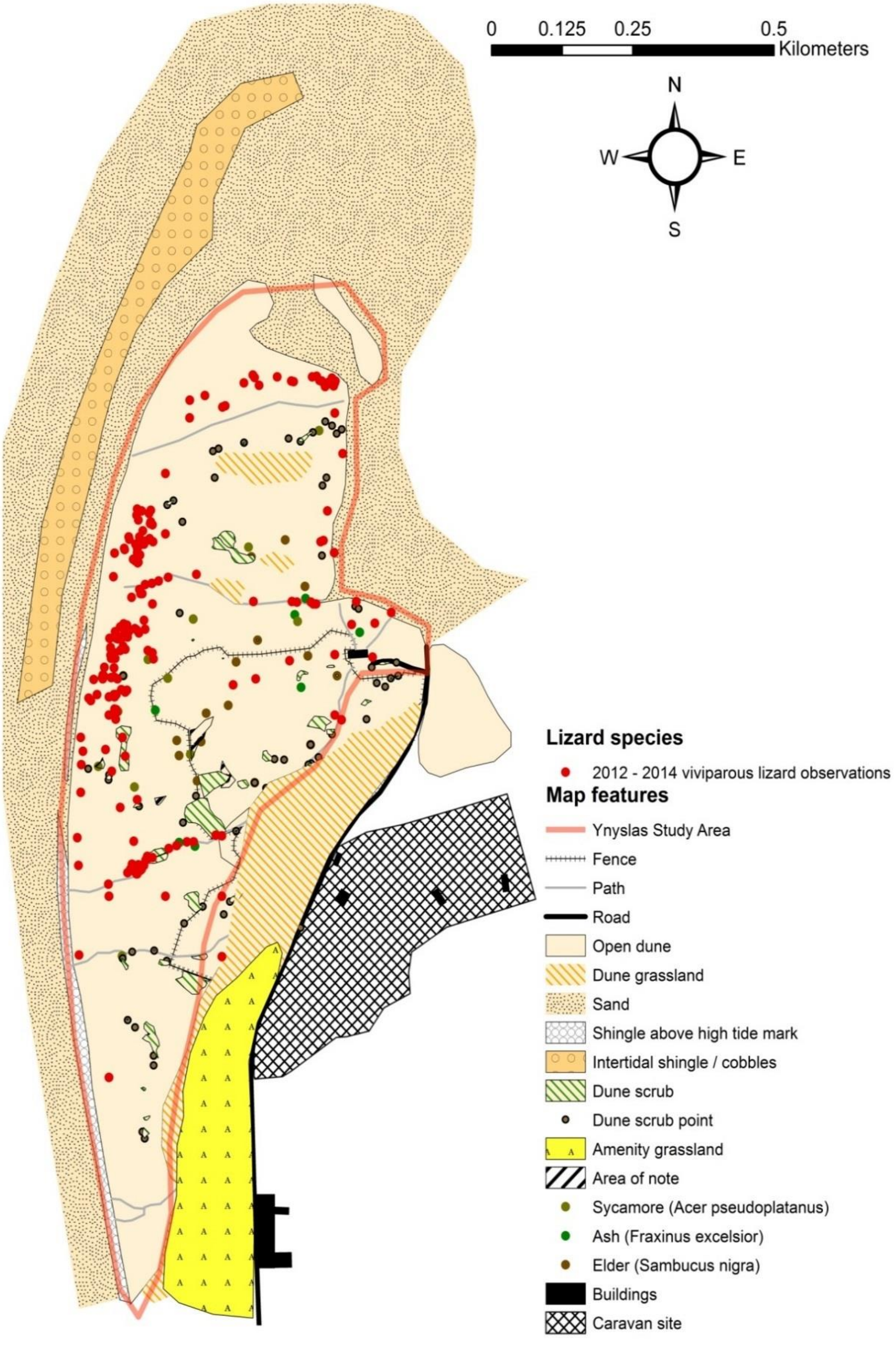

**Figure B.9b:** Visual representation of 2010 – 2014 viviparous lizard observations across Ynyslas study area.

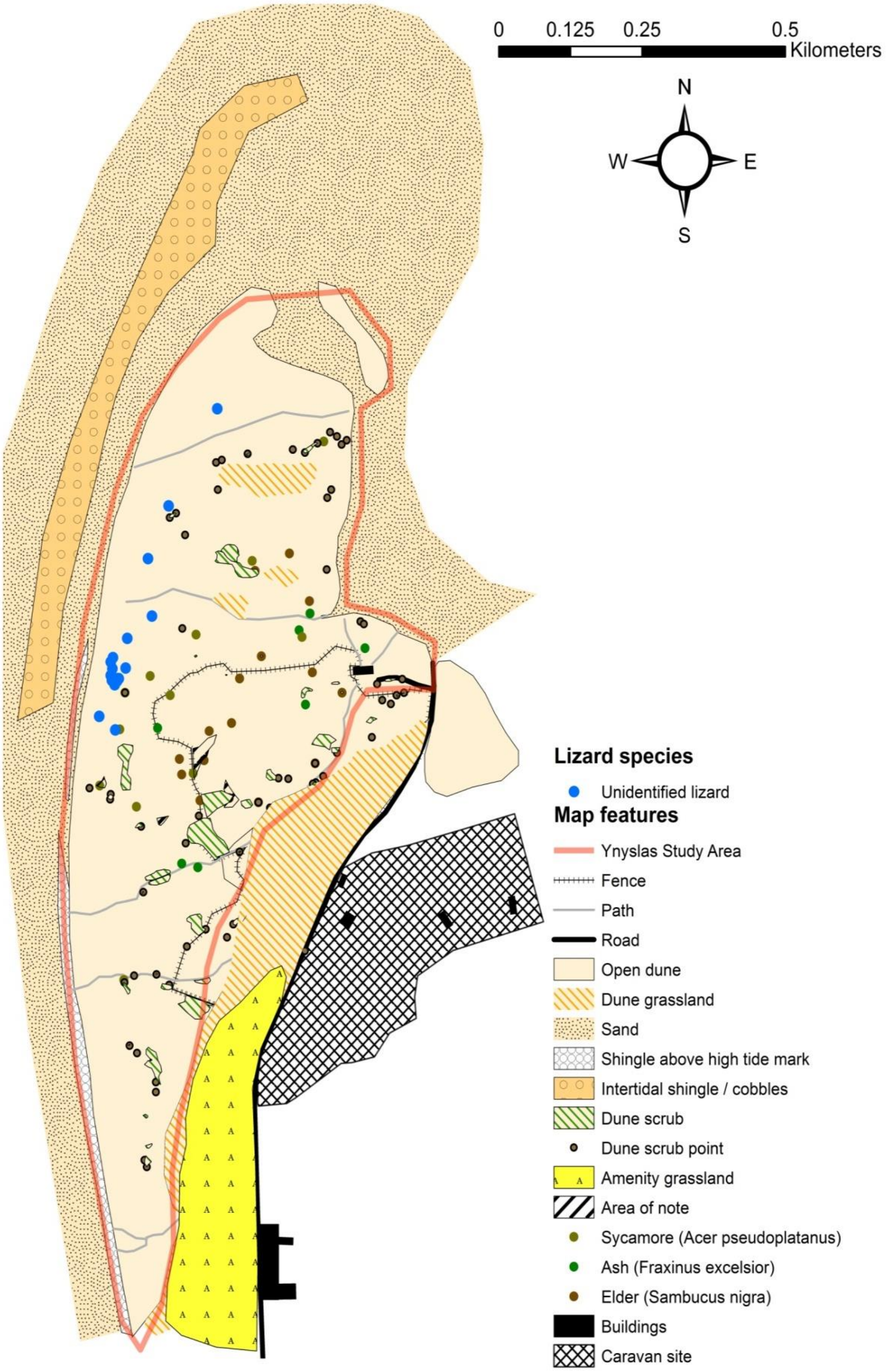

**Figure B.9c:** Visual representation of 2010 – 2014 unidentified lizard observations across Ynyslas study area.

# Appendix C

## Genetic Sampling Information

**Table C.4.1:** List of sampled sand lizards *Lacerta agilis*. Captive Breeder (CB).

| Site | Sample number | Date | Sex | Grid co-ordinate |
|---|---|---|---|---|
| Merseyside | 449 | 29/03/2011 | Male | SD 2963912630 |
| Merseyside | 450 | 29/03/2011 | Female | SD 2988713059 |
| Merseyside | 543 | 23/04/2011 | Male | SD 3001613330 |
| Merseyside | 544 | 23/04/2011 | Male | SD 2989412999 |
| Merseyside | 545 | 23/04/2011 | Female | SD 2992413007 |
| Merseyside | 546 | 23/04/2011 | Male | SD 2994113137 |
| Merseyside | 547 | 23/04/2011 | Male | SD 2994313137 |
| Merseyside | 548 | 23/04/2011 | Female | SD 3003113386 |
| Merseyside | 549 | 23/04/2011 | Male | SD 2991413054 |
| Merseyside | 550 | 24/04/2011 | Male | SD 2961512601 |
| Merseyside | 551 | 24/04/2011 | Male | SD 2964512654 |
| Merseyside | 552 | 24/04/2011 | Male | SD 2958912553 |
| Merseyside | 553 | 24/04/2011 | Female | SD 2996613231 |
| Merseyside | 554 | 24/04/2011 | Female | SD 3000713308 |
| Merseyside | 555 | 25/04/2011 | Female | SD 2959612590 |
| Merseyside | 556 | 25/04/2011 | Female | SD 2962812625 |
| Merseyside | 557 | 25/04/2011 | Male | SD 2960612586 |
| Merseyside | 558 | 25/04/2011 | Male | SD 2965012589 |
| Merseyside | 559 | 25/04/2011 | Female | SD 2989713083 |
| Merseyside | 560 | 25/04/2011 | Female | SD 3003813393 |
| Merseyside | 561 | 25/04/2011 | Female | SD 3002813339 |
| Merseyside | 562 | 29/04/2011 | Male | SD 2975012781 |
| Merseyside | 563 | 29/04/2011 | Male | SD 2978312826 |
| Merseyside | 564 | 29/04/2011 | Male | SD 2978212831 |
| Merseyside | 565 | 29/04/2011 | Female | SD 2978312828 |
| Merseyside | 566 | 29/04/2011 | Male | SD 2971412767 |
| Merseyside | 567 | 29/04/2011 | Male | SD 2978812827 |
| Merseyside | 568 | 29/04/2011 | Male | SD 2975312778 |
| Merseyside | 569 | 29/04/2011 | Male | SD 2989413068 |
| Merseyside | 570 | 29/04/2011 | Male | SD 2990313082 |
| Merseyside | 2 | 20/05/2012 | Male | SD 2989913080 |
| Merseyside | 3 | 20/05/2012 | Male | SD 2990313079 |
| Merseyside | 4 | 20/05/2012 | Male | SD 2981112876 |
| Merseyside | 5 | 20/05/2012 | Male | SD 2981512876 |
| Merseyside | 6 | 10/06/2012 | Female | SD 2980712867 |

**Table C.4.1:** Continued over page.

**Table C.4.1:** Cont.

| Site | Sample number | Date | Sex | Grid co-ordinate |
|---|---|---|---|---|
| Merseyside | 7 | 10/06/2012 | Male | SD 2979912830 |
| Harlech | 8 | 18/03/2012 | Male | SH 5702731507 |
| Harlech | 9 | 31/03/2012 | Male | SH 5699531621 |
| Harlech | 10 | 31/03/2012 | Female | SH 5693931743 |
| Harlech | 11 | 18/03/2012 | Male | SH 5702731507 |
| Harlech | 12 | 31/03/2012 | Male | SH 5699531621 |
| Harlech | 13 | 31/03/2012 | Female | SH 5693931743 |
| Harlech | 14 | 31/03/2012 | Female | SH 5691731738 |
| Harlech | 15 | 31/03/2012 | Female | SH 5691431769 |
| Harlech | 16 | 01/04/2012 | Female | SH 5699631571 |
| Harlech | 17 | 12/05/2012 | Female | SH 5693031734 |
| Harlech | 18 | 13/05/2012 | Male | SH 5691731740 |
| Harlech | 19 | 01/07/2012 | Female | SH 5689731751 |
| Harlech | 20 | 14/07/2012 | Male | SH 5698731620 |
| Harlech | 21 | 14/07/2012 | Female | SH 5738130271 |
| Harlech | 22 | 05/09/2012 | Female | SH 5691231766 |
| Harlech | 23 | 08/09/2012 | Male | SH 5701431569 |
| Harlech | 24 | 14/10/2012 | Female | SH 5694531696 |
| Aberdyfi | 1 | 03/09/2012 | Male | SN 5996995976 |
| Aberdyfi | AB2 | 31/03/2013 | Male | SN 5974996096 |
| Aberdyfi | AB3 | 04/05/2013 | Male | SN 5987096030 |
| Aberdyfi | AB4 | 06/05/2013 | Male | SN 5988796003 |
| Aberdyfi | AB5 | 06/05/2013 | Male | SN 5991295985 |
| Aberdyfi | AB6 | 15/03/2014 | Male | SN 5987296025 |
| CB | CZ01 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ02 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ03 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ04 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ05 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ06 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ07 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ08 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ09 | 23/04/2014 | Male | Chester Zoo |
| CB | CZ10 | 23/04/2014 | Female | Chester Zoo |
| CB | CZ11 | 23/04/2014 | Female | Chester Zoo |

**Table C.4.1:** Continued over page.

**Table C.4.1:** Cont.

| Site | Sample number | Date | Sex | Grid co-ordinate |
|---|---|---|---|---|
| CB | CZ12 | 26/09/2014 | Female | Chester Zoo |
| CB | CZ13 | 26/09/2014 | Female | Chester Zoo |
| CB | CZ14 | 26/09/2014 | Female | Chester Zoo |
| CB | CZ15 | 26/09/2014 | Female | Chester Zoo |
| CB | PH1 | 08-09/2014 | Unknown | Private |
| CB | PH2 | 08-09/2014 | Unknown | Private |
| CB | PH3 | 08-09/2014 | Unknown | Private |
| CB | RL1 | 08-09/2014 | Unknown | Private |
| CB | RL2 | 08-09/2014 | Unknown | Private |
| CB | RL3 | 08-09/2014 | Unknown | Private |
| CB | RL4 | 08-09/2014 | Unknown | Private |
| Ynyslas | Y1 | 01/06/2013 | Male | SN 6050294079 |
| Ynyslas | Y2 | 01/06/2013 | Female | SN 6050094084 |
| Ynyslas | Y3 | 18/08/2013 | Female | SN 6050894130 |
| Ynyslas | Y4 | 18/08/2013 | Male | SN 6053294157 |
| Ynyslas | Y5 | 09/03/2014 | Male | SN 6052094126 |
| Ynyslas | Y6 | 29/03/2014 | Male | SN 6050094080 |
| Ynyslas | Y7 | 01/06/2014 | Female | SN 6055294201 |
| Ynyslas | Y8 | 23/08/2014 | Male | SN 6047594055 |

**Table C.4.2:** Extraction Protocol for buccal swab samples.

Isolation comprised the following steps:

1. 500µl Isohelix$^{TM}$ Lysis buffer (LS) solution was added to tube containing the buccal swab
2. 20µl Isohelix$^{TM}$ Proteinase K solution (PK) added to tube containing the buccal swab and LS solution.
3. Solution was briefly vortexed

At this point the DNA was isolated and stabilised. The extraction of DNA from the isolated tubes followed the following steps:

1. Tubes containing the swab, LS solution and PK solution were left in a 60°C heat block overnight.
2. 400µl of the liquid was transferred into a new sterile 1.5ml centrifuge tube using a sterile pipette tip.
3. Optional step to increase DNA yield was undertaken at this point. This involved inverting the swab head and stick into a new sterile 1.5ml centrifuge tube so that the swab head was uppermost. The tube was spun briefly and a sterile pipette used to recover supernatant; which was subsequently added to 400ul collected previously.
4. 500µl Isohelix$^{TM}$ Capture buffer (CT) solution was added to 1.5ml centrifuge tube from step 3 and briefly vortexed.
5. The 1.5ml centrifuge tube was then spun in a microcentrifuge at 13K rpm for 7 minutes to pellet DNA present (the tube was placed with hinge positioned outwards so liquid could be removed from the opposite side)
6. Supernatant was carefully removed from the 1.5ml centrifuge tube taking care not to disturb the DNA pellet. The tube was briefly re-spun and all remaining supernatant removed.
7. 30µl Isohelix$^{TM}$ Re-hydration buffer (TE) solution was added to the 1.5ml centrifuge tube containing the DNA pellet. The DNA pellet was then left for 15 minutes to re-hydrate.
8. Optional step to increase DNA yield was undertaken at this point. This involved spinning the 1.5ml centrifuge tube at 13K rpm for two minutes to remove any undissolved particulates. Supernatant was removed to a new sterile 1.5ml centrifuge tube.
9. The tube from step 8 was incubated in an 80 °C heat block for 5 minutes prior to being briefly vortexed and spun.

Sample was subsequently stored at 4 °C.

**Table C.4.3:** Extraction Protocol for egg samples.

Isolation comprised the following steps:

1. Eggs (3 – 4) were placed into a sterile mortar, liquid nitrogen added and eggs ground into powder.
2. 600µl Isohelix$^{TM}$ cell lysis buffer was added to the ground tissue in the mortar, mixed and the solution pipetted into a sterile 1.5 ml centrifuge tube using a sterile pipette tip.
3. 5µl (ice-cold) Isohelix$^{TM}$ Proteinase K and 5µl (Room temp) Isohelix$^{TM}$ RNAase enzymes was added to the 1.5ml centrifuge tube from step 2. The sample was mixed in the tube by inverting a few times. Tubes were left in a 55°C heat block overnight.
4. Once out of the heat block, the sample was cooled on ice for 5 minutes before 200µl, ice-cold, 5M ammonium acetate solution was added and the sample mixed by inverting.
5. Samples were centrifuged at 13K rpm for 10 minutes before 600µl of supernatant was removed from the 1.5ml centrifuge tube. 600µl of removed supernatant was replaced by an equal volume of ice-cold isopropanol and mixed by inverting.
6. The sample was frozen overnight in the –20°C freezer.
7. Upon thawing, the 1.5ml centrifuge tube was spun in a microcentrifuge at 13K rpm for 15 minutes to pellet DNA present (the tube was placed with hinge positioned outwards so liquid could be removed from the opposite side).
8. Supernatant was carefully removed from the 1.5ml centrifuge tube taking care not to disturb the DNA pellet. The tube was briefly re-spun and all remaining supernatant removed.
9. 1ml of 70% ethanol was added to the 1.5ml centrifuge tube containing the DNA pellet. The remaining pellet was washed in ethanol prior to being further centrifuged at 13K rpm for 5 minutes.
10. All ethanol was removed and the DNA pell*et al*lowed to dry by inverting the open tube on paper towels for 5 – 10 minutes prior to drying for a further 5 – 10 minutes under vacuum.
11. Once dry, 50µl of Isohelix$^{TM}$ TE buffer (room temperature) was added to the 1.5ml centrifuge tube containing the DNA pellet. The tube was tapped to aid dispersion of the pellet.
12. The DNA pellet was then allowed to rehydrate overnight in the fridge. Sample was subsequently stored at 4 °C.