Swansea University
Prifysgol Abertawe

Short Legs Racing Towards Extinction: The Landscape Genetics of UK Hedgehogs

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Abstract

Biodiversity has been in global declines since the 1940s with industrialised nations including the UK seeing significant declines driven by habitat loss and fragmentation, land use changes and barrier effects, among others. These declines have not only resulted in the loss of species and ecosystem diversity but also genetic diversity, a key component to species survival. The relationship between genetic loss and landscape changes has been demonstrated for a variety of specialist species but is less well established for generalist species, such as the West European Hedgehog (*Erinaceus europaeus*). This species has seen significant declines and changes in distribution across the UK since the 1950s. Although the drivers of these changes are not well understood, anthropogenic changes in the landscape such as modified agricultural practices and increased road traffic have been proposed to play a part. I used microsatellite genetic analysis to investigate the impact of landscape features on the genetic structure of hedgehogs across South Wales. To understand how landscape features might impact on population genetics, I developed landscape resistance mapping for habitats, roads, watercourses and geographic distance, producing surfaces representing the 'resistance' of movement of hedgehogs through South Wales. I then combined these with the genetic data to test for landscape effects on genetic relatedness using circuit theory. I detected weak genetic structure, with four genetic clusters, but many individuals were admixed. The landscape genetic analysis showed no significant effect from any of the resistance variables on genetic relatedness, including geographic distance, suggesting that gene flow within the sample population is not impacted by landscape resistance. I discuss the potential reasons for this along with other possible causes for the genetic structure observed. This study demonstrates the importance of understanding the interactions between a species and landscape to ensure successful conservation management and appropriate consideration within ecological consultancy.

Declarations and Statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed:

Date: 29/09/2023

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed:

Date: 29/09/2023

I hereby give consent for my thesis, if accepted, to be available for electronic sharing

Signed:

Date: 29/09/2023

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

Signed:

Date: 29/09/2023

Statement of Expenditure

Expenditure for the thesis work was approximately as follows:

Qiagen DNA extraction kits £1432

Qiagen multiplex PCR kit £612

Plasticware consumables £185

Textbooks £165

External hard drive for data storage £50

Printing costs £185

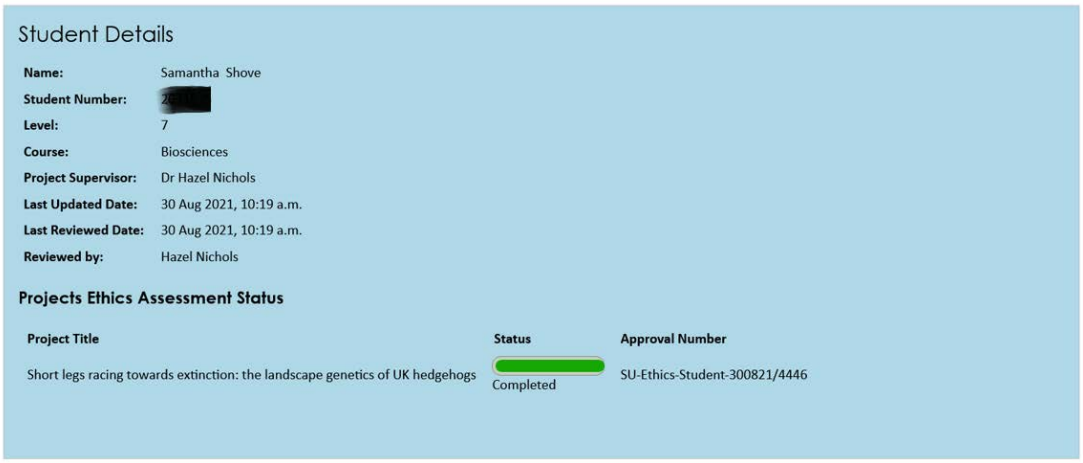
Total expenditure £2,629

Statement of Contributions

Contributor Role	Persons Involved
Conceptualization	Hazel Nichols, Dan Forman, Simon Allen, Samantha Shove
Data Curation	Hazel Nichols, Samantha Shove
Formal Analysis	Samantha Shove
Funding Acquisition	Hazel Nichols, Samantha Shove
Investigation	Samantha Shove, Gabby Howell (lab processing)
Methodology	Hazel Nichols, Samantha Shove, Lilly Zecherle
Project Administration	Hazel Nichols, Samantha Shove
Resources	Hazel Nichols, Samantha Shove, Simon Allen
Software	Samantha Shove, Lilly Zecherle (coding guidance)
Supervision	Hazel Nichols
Validation	Hazel Nichols
Visualisation	Samantha Shove
Writing – original draft preparation	Samantha Shove
Writing – review and editing	Hazel Nichols, Samantha Shove

This study was started in 2021 during which Covid-19 restrictions were in place to varying degrees, which had implications for undertaking lab work as well as availability of lab resources. I also continued to work full-time as an ecological consultant while progressing this study part-time, which reduced my ability to process all of the lab samples and transport samples to other labs for more detailed analysis. For this I relied on Hazel Nichols and Gabby Howell who very kindly supported these aspects of the study where I was unable to.

Ethics Approval



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Health and Safety / Risk Assessments

These are provided in full in Appendix C, as follows:

- Generic risk assessment covid 19 in lab W131A HN 15-09-21
- Biological-Risk-Assessment-Form with amendments 17-01-22
- DNA extraction risk assessment
- Gels risk assessment
- PCR risk assessment

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Definitions of Abbreviations

Abbreviations	Definitions
dbRDA	Distance-based Redundancy Analysis
DNA	Deoxyribonucleic Acid
GBH	Gower Bird Hospital
GIS	Geographic Information System
GPS	Global Positioning System
HW	Hardy-Weinberg
IG	Improved Grassland
IUCN	International Union for Conservation of Nature
JNCC	Joint Nature Conservancy Council
PCo	Principal Co-ordinates
PIC	Polymorphic Information Content

1. Introduction

Biodiversity in its simplest form refers to the species richness of a community (Krebs 2001). However, it has come to refer more widely to the variation of ecosystems, species, populations, and genetic diversity (Frankham, Ballou & Briscoe 2002). The IUCN recognises three key forms of biodiversity, namely species diversity, ecosystem diversity and genetic diversity (Allendorf, Luikart & Aitken 2013). Biodiversity has been in global decline since the 1940s (Robinson & Sutherland 2002) with significant species and ecosystem declines seen across industrialised nations, including the UK, driven by several factors including habitat loss and land use changes (Andrén 1994, Stoate *et al.* 2001, Brooks *et al.* 2002, Crooks *et al.* 2017), habitat fragmentation/isolation (Bright 1993, Yanes, Velasco & Suárez 1995, Fitzgibbon 1997, Clark *et al.* 2001, Van Dyck & Baguette 2005), and overharvesting (Stoate *et al.* 2001, Donald & Evans 2006). Less well known and unintentional factors have also been identified such as pet predation (Baker *et al.* 2003), transport fatalities (Coffin 2007), chemical use (Stoate *et al.* 2001, Robinson & Sutherland 2002), and pollution (Dickman 1987).

Alongside species and ecosystem biodiversity losses, genetic diversity has also been declining (Lacy 1997, Keller & Waller 2002). Such diversity is vital for avoiding inbreeding depression and maintaining genetic resilience within a population making it a key component of species survival (Yanes, Velasco & Suárez 1995, Reed 2004, Coffin 2007, Weeks *et al.* 2011, Allendorf, Luikart & Aitken 2013). As genetic diversity decreases, individuals, groups, populations, and species are less able to respond to deterministic threats such as habitat destruction, climate change etc. or to stochastic threats such as genetic drift (random changes in genetic variation), inbreeding, and natural environmental change ((Frankham, Ballou & Briscoe 2002, Allendorf, Luikart & Aitken 2013). Understanding genetic diversity also provides a retrospective view of the evolution of a species, reveals barriers to movement and gene-flow, and provides a glimpse of the future evolutionary paths populations and species may take (Allendorf, Luikart & Aitken 2013).

Despite the importance of biological and genetic diversity and the significant research that has been conducted to date, there are still notable gaps in our knowledge particularly in relation to the links between habitat resistance (the ease or otherwise with which a species, individual animal, or their genes are able to move through a particular habitat (Spear *et al.* 2010, Balkenhol *et al.* 2016)), barrier effects of man-made features, and genetic diversity (Holderegger & Wagner 2008, Baguette *et al.* 2013).

The influence habitat resistance has on genetic structure has been demonstrated in a variety of specialist species but has been shown to be dependent on how a species interacts with the landscape and the habitats within it (Frankham, Ballou & Briscoe 2002, Spear *et al.* 2010, Baguette *et al.* 2013, Balkenhol *et al.* 2016). The influence of habitat resistance is also known to vary depending on the spatial scale of the model used, which can depend on the availability of data and computational efficiency as well as the species studied (Baguette *et al.* 2013, Balkenhol *et al.* 2016). In other studies, habitat resistance appears to have little influence on the genetic structure of a population despite effects identified for similar or related species (Spear *et al.* 2010, Baguette *et al.* 2013).

Barrier effects have generally been included as part of studies on edge effects, which often focuses more on habitat edge and transition zones rather than separating out or addressing linear features such as roads and watercourses specifically (Akçakaya 2000, Balkenhol *et al.* 2016). Where barriers such as roads and watercourses have been studied independently of habitat edge effects mixed results have also been seen, with several studies identifying no significant effect (De Groot *et al.* 2016, Mateo-Sánchez *et al.* 2015, Kimmig *et al.* 2020) while others have identified significant negative influences of such features (Bergl & Vigilant 2007, Cushman & Lewis 2010, Frantz *et al.* 2012, Draheim *et al.* 2020).

These studies show that there is variability in the influence of habitat resistance and landscape barriers on genetic structure. These could be indicative of insufficient accuracy within resistance models, incorrect model assumptions, limited

understanding of how habitat resistance and landscape barriers affect animal movements, time lags, or other unknown factors not being fully considered within the models, (Spear *et al.* 2010, Landguth *et al.* 2010, Balkenhol *et al.* 2016).

West European Hedgehogs (*Erinaceus europaeus*), referred to as hedgehogs from here on, provide an ideal species to investigate the effects of habitat resistance and movement barriers on genetic structure and diversity. They are generalist nocturnal mammals that feed on a wide range of invertebrates, small vertebrates, and carrion, as well as taking advantage of human provided food in urban and sub-urban environments (Dickman 1987, Reeve 1994, Braaker *et al.* 2014, Morris 2018). They are non-territorial and have home ranges between 10 and 40ha (Braaker *et al.* 2014) and typically range between 0.7 to 2.5km per night (Reeve 1994, Riber 2006), with some individuals ranging up to 10 to 15km per night (Williams, Stafford & Goodenough 2015, Morris 2018), allowing them to take advantage of a variety of habitats and food sources within their home ranges (Driezen *et al.* 2007). Hedgehogs utilise a range of habitats from woodland and scrub to grassland and occasionally heath and have adapted to man-made 'habitats' such as parklands, field margins, and hedgerows (Reeve 1994, Hof & Bright 2010, van de Poel, Dekker & Langevelde 2015, Morris 2018). They are now often more common in urban and sub-urban areas than rural areas (Hof & Bright 2009, Hubert *et al.* 2011, Braaker *et al.* 2014, Williams, Stafford & Goodenough 2015, Pettett *et al.* 2017, Wilson & Wembridge 2018). Their generalist nature means that genetic differences within hedgehog populations are unlikely to be strongly influenced by territoriality, dispersal events, or very specialist species requirements as has been shown in some other species (Baguette *et al.* 2013, Mateo-Sánchez *et al.* 2015, Keeley *et al.* 2017).

Despite the generalist nature of the species, its populations have suffered major declines in recent decades, losing at least 60% of the population since the 1950s (Hof & Bright 2016, Morris 2018, Pettett *et al.* 2018, Finch *et al.* 2020). The drivers behind these declines are unclear and often debated but are likely to include habitat loss and fragmentation and road mortalities (Morris 2018, Moore *et al.* 2020, Taucher *et al.* 2020, Wright *et al.* 2020). In the UK agricultural intensification and land use

changes have resulted in increased field sizes, increased management frequency, and loss of field margins (Stoate *et al.* 2001, Robinson & Sutherland 2002), loss of hedgerows/tree-lines (Kotzageorgis & Mason 1997), and non-agricultural habitat patches (Fitzgibbon 1997), all of which are known to provide connectivity and foraging habitats for hedgehogs (Reeve 1994, Hof & Bright 2010, van de Poel, Dekker & Langevelde 2015, Morris 2018). These habitat changes alter the suitability and resistance across the landscape, affecting the pattern of hedgehog movements as well as the availability and accessibility of the resources needed for survival (Driezen *et al.* 2007, Braaker *et al.* 2014, Wright *et al.* 2020). However, we don't know what effect these changes have had on gene flow and genetic diversity.

The levels of hedgehog mortality on UK roads are also likely to be contributing to these declines with recent studies indicating that approximately 100,000 to 300,000 hedgehogs are killed on UK roads each year (Wright *et al.* 2020). There is some indication that hedgehogs have adapted to certain types and sizes of roads, with minor roads showing positive correlations with hedgehog movement (Hof 2009, Hof & Bright 2010), pattern of vehicle use by becoming active later (Dowding *et al.* 2010) and increasing their movement speed when crossing them (Doncaster, Rondinini & Johnson 2001). However, numerous studies indicate that roads remain a significant factor in population declines, both through direct mortality and as a barrier to movement (Micol, Doncaster & Mackinlay 1994, Huijser & Bergers 2000, Rondinini & Doncaster 2002, Orłowski & Nowak 2004) with larger roads having a greater barrier effect (Orłowski & Nowak 2004, Hof & Bright 2009).

The presence of watercourses can also present a barrier to hedgehog movements and may increase the effect of habitat fragmentation (Morris 2018). However, some studies have shown that hedgehogs can cross such features, including large main rivers, by using bridges and other structures or swimming (Hof & Bright 2009, Barthel *et al.* 2020). As such, the barrier effect of watercourses on gene flow is unclear.

To minimise further declines and encourage recovery of hedgehog populations it is vital to determine to what extent of each these factors have contributed and

continue to contribute to the physical and genetic isolation of hedgehog populations and potentially the declining success of populations. This study uses habitat resistance maps and microsatellite genetic data to investigate the potential barriers to gene flow between populations of hedgehogs across South Wales with the aim of identifying these barriers and proposing potential ways of reducing or removing their influence.

The study focuses on the hedgehog population found within South Wales (referred to as the sample population). The South Wales area has a variety of habitats ranging from grassland and heath to scrub and woodland with urban and developed areas limited in size and restricted to specific locations. South Wales has a robust but varied road network that includes single lane tracks, verged and hedged carriageways and a major motorway. The watercourse network is similarly widespread but varied in size across the South Wales area. Hof & Bright 2012 identified that hedgehog sightings on farmland in Wales was one of the lowest in the UK, with only the South West and London returning lower percentages of sightings, while (Williams *et al.* 2018) showed marked declines in hedgehog numbers in Wales in recent decades. This is similar to data within the State of Britain's Hedgehogs report (Wembridge *et al.* 2022), which indicated strong declines across rural Britain up until 2015, after which the population appeared to be stabilising. These suggest that there are strong drivers of population decline and fragmentation within Wales, and that the influence of these may be observable within the genetic structure of the remaining populations.

This thesis will provide an understanding of land use and habitat fragmentation effects on genetic diversity at a landscape scale.

The objectives of this study are to:

1. Identify genetic subpopulations within the sample population.
2. Determine the effect of habitat resistance, roads, and watercourses on patterns of genetic relatedness within the sample population.

Based on hedgehog ecology and previously published information, I predict that:

1. The genetic structure of the sample population will have spatially distinct subpopulations corresponding to the overall distance between individuals and groups as well as the presence of high resistance habitats and natural and man-made barriers.
2. Areas of low habitat suitability (high habitat resistance) have a significant influence on the genetic structure of the sample population by reducing hedgehog movement and gene flow across the study area.
3. Larger landscape features such as roads and watercourses have a barrier effect on hedgehog movement and therefore gene flow within the sample population.

2. Materials and Methods

2.1. Study Area

This study was conducted in collaboration with the Gower Bird Hospital (GBH), who take in rescued hedgehogs, birds, and other animals from across South Wales. GBH collected DNA samples from rescued hedgehogs along with details of where the hedgehogs were found including post codes and grid references. The study area was defined by mapping these locations using the grid references in ArcGIS Pro 3.0.3 (Esri Inc 2023) using the recorded grid references and a buffer of 10km applied to produce a single merged buffer for all records. This buffer was then amended to remove isolated excluded pockets, to join across slivers of land, and to follow coastlines. Morris 2018 indicates that long range dispersal is possible to 15km, but nightly distances tend to be less than 5km, in line with (Doncaster, Rondinini & Johnson 2001) and similar estimates from (Reeve 1981, Reeve 1994) and (Moorhouse *et al.* 2014). Ultimately a 10km buffer was applied based on the conclusion of (Doncaster, Rondinini & Johnson 2001) that displacement between hedgehog populations rarely exceeds 10km.

The study area covers approximately 5,800km^2 (**Figure 1**) and was dominated by improved grassland habitat with notable areas of broadleaved plantation, dry heath/acid grassland mosaic, and mosaic (largely a combination of acid grassland, marshy grassland, and wet and dry heath). The southern part of the study area included the urban and suburban areas of Cardiff and Swansea and several major roadways including the M4 running east-west. Numerous watercourses were also present, including several large rivers running approximately north-south through the study area, namely River Taff and River Ely to the east, Ogmore River, River Avan, River Neath, and River Tawe to the centre and River Tywi, River Gwili, and River Taf to the west.

Figure 1: The study area in South Wales (shown by the red line) and the location of GBH (shown by the red dot)

2.2. Habitat Resistance Mapping

To assess the impact of land use on the genetic structure of hedgehog populations, a habitat map of the study area was produced and a resistance value allocated to

each habitat type. Open-source phase 1 habitat data was obtained from the LLE Geo-Portal (accessed and downloaded 2021-04-03), imported into ArcGIS and edited to cover the study area only using the clip and edit nodes tools. The accuracy of the habitat mapping was checked against the latest aerial photography available within ArcGIS, with corrections made as necessary. Those areas without a habitat code were also checked against the aerials and adjacent categorised habitats and a visual assessment made to assign a habitat type. These checks were made at a scale of 1:5,000 with all areas less than 1 hectare not checked as they were likely too small to influence habitat resistance given the extent of the study area. These limitations were applied given the number of polygons within the study area and the time constraints of the project.

The data used was collected/recorded at different times with the habitat and roads data dated 2016, the watercourse data dated 2017, the aerial imagery used to update these dated 2020, and the genetic samples collected between 2019 and 2021. The updating of the habitat, roads and watercourse data using the aerial imagery brought the baseline information into chronological line with the genetic samples.

Once this mapping was completed, a habitat resistance field was added to the attribute table within ArcGIS. Each habitat type was assigned a resistance value category between 0 (low resistance) and 99 (high resistance/barrier), following (Zecherle *et al.* 2020). These values were based on species knowledge, such as resource requirements, need for cover, and foraging behaviour, and the identified habitat use patterns from previous studies (detailed within Appendix A), where habitats were categorised based on their resistance or suitability for hedgehogs. The previously published studies used a variety of means to define habitat resistance, varying from simple categories, e.g. (Pettett *et al.* 2017), or positive and negative associations, e.g. (Doncaster, Rondinini & Johnson 2001), to generating resistance values that were then tested across several models, e.g. (Driezen *et al.* 2007). These results were collated and summarised to produce a typical resistance level and value for mapping purposes, as shown in Table 1 below and detailed in full in Appendix A. These values were then entered into the habitat resistance field within ArcGIS as the

mid-point value using the filter and batch edit tools. A similar process as above was followed for roads and watercourses based on the 'type' information provided by the open source GIS data. These values are shown in Table 2 and Table 3 below.

Data wasn't directly available for 49 of the habitat types, so comparable habitats were used as a proxy, the proxies used have been identified within Appendix A. The data and conclusions from published studies was contradictory for 14 of the habitat types where data was directly available, so the most common result was used in combination with species ecology to determine the most appropriate resistance level.

Once added to ArcGIS, the above were then used to create the raster maps required for the Circuitscape analysis discussed below. This was achieved using the feature to raster and taster to ASCII tools to create .asc and .txt file types that could then be imported into the Circuitscape software.

Given the habitat monoculture and intensive management associated with improved grassland habitats, and contradictory research results indicating different use levels (Appendix A), several habitat resistance maps were produced. These altered the resistance input value of improved grassland using each of the different resistance levels providing a set of 7 raster maps for habitat resistance.

Table 1: Summary of habitat resistance values by Phase 1 habitat type (shaded rows are based on estimates from comparable habitats). Habitat codes and types are from the JNCC Phase I Habitat Survey handbook (JNCC 2010)

Habitat Code	Habitat Type	Typical Resistance Level	Typical Resistance Value (0 to 99)	GIS input value
A.1.1.1	Broadleaved semi-natural woodland	Low	2 to 20	11
A.1.1.2	Broadleaved plantation	Low-medium	21 to 40	31
A.1.2.1	Coniferous semi-natural woodland	Medium	41 to 60	51
A.1.2.2	Coniferous plantation	Medium	41 to 60	51
A.1.3.1	Mixed semi-natural woodland	Low-medium	21 to 40	31
A.1.3.2	Mixed plantation	Low-medium	21 to 40	31
A.2.1	Dense / continuous scrub	Negligible	1	1
A.2.2	Scattered scrub	Low	2 to 20	11

Habitat Code	Habitat Type	Typical Resistance Level	Typical Resistance Value (0 to 99)	GIS input value
A.3.1	Broadleaved parkland / scattered trees	Negligible	1	1
A.3.2	Coniferous parkland / scattered trees	Negligible	1	1
A.3.3	Mixed parkland / scattered trees	Negligible	1	1
A.4.1	Broadleaved recently felled woodland	Low	2 to 20	11
A.4.2	Coniferous recently felled woodland	Low-medium	21 to 40	31
A.4.3	Mixed recently felled woodland	Low-medium	21 to 40	31
B.1.1	Unimproved acid grassland	Negligible	1	1
B.1.2	Semi-improved acid grassland	Negligible	1	1
B.2.1	Unimproved neutral grassland	Negligible	1	1
B.2.2	Semi-improved neutral grassland	Negligible	1	1
B.3.1	Unimproved calcareous grassland	Negligible	1	1
B.3.2	Semi-improved calcareous grassland	Negligible	1	1
B.4	Improved grassland (pasture)	Negligible	1	1
B.5	Marsh / marshy grassland	Negligible	1	1
B.6	Poor semi-improved grassland	Negligible	1	1
C.1.1	Continuous bracken	Negligible	1	1
C.1.2	Scattered bracken	Low-medium	21 to 40	31
C.2	Upland species-rich ledges	High	81 to 98	91
C.3.1	Tall ruderal	Negligible	1	1
C.3.2	Non-ruderal	Negligible	1	1
D.1.1	Dry dwarf shrub heath – acid	Low-medium	21 to 40	31
D.1.2	Dry dwarf shrub heath – basic	Low-medium	21 to 40	31
D.2	Wet dwarf shrub heath	Medium-high	61 to 80	71
D.3	Lichen / bryophyte heath	Medium-high	61 to 80	71
D.4	Montane heath / dwarf herb	Medium	41 to 60	51
D.5	Dry heath / acid grassland mosaic	Low-medium	21 to 40	31
D.6	Wet heath / acid grassland mosaic	Medium	41 to 60	51
E.1.6.1	Blanket bog	High	81 to 98	91
E.1.6.2	Raised bog	High	81 to 98	91
E.1.7	Wet modified bog	High	81 to 98	91
E.1.8	Dry modified bog	High	81 to 98	91
E.2.1	Acid / neutral flush / spring	High	81 to 98	91
E.2.2	Basic flush / spring	High	81 to 98	91

Habitat Code	Habitat Type	Typical Resistance Level	Typical Resistance Value (0 to 99)	GIS input value
E.2.3	Bryophyte dominated flush / spring	High	81 to 98	91
E.3.1	Valley mire fen	High	81 to 98	91
E.3.2	Basin mire fen	High	81 to 98	91
E.3.3	Flood plain fen	High	81 to 98	91
E.4	Bare peat	High	81 to 98	91
F.1	Swamp	High	81 to 98	91
F.2.1	Marginal vegetation	High	81 to 98	91
F.2.2	Inundation vegetation	High	81 to 98	91
G.1	Standing water	High	81 to 98	91
G.1.1	Eutrophic	High	81 to 98	91
G.1.2	Mesotrophic	High	81 to 98	91
G.1.3	Oligotrophic	High	81 to 98	91
G.1.4	Dystrophic	High	81 to 98	91
G.1.5	Marl	High	81 to 98	91
G.1.6	Brackish	High	81 to 98	91
G.2	Running water	High	81 to 98	91
G.2.1	Eutrophic	High	81 to 98	91
G.2.2	Mesotrophic	High	81 to 98	91
G.2.3	Oligotrophic	High	81 to 98	91
G.2.4	Dystrophic	High	81 to 98	91
G.2.5	Marl	High	81 to 98	91
G.2.6	Brackish	High	81 to 98	91
H.1.1	Intertidal mud / sand	High	81 to 98	91
H.1.2	Intertidal shingles / cobbles	High	81 to 98	91
H.1.3	Intertidal boulders / rocks	High	81 to 98	91
H.1.(1-2).1	Zostera beds	High	81 to 98	91
H.1.(1-3).2	Green algal beds	High	81 to 98	91
H.1.(1-3).3	Brown algal beds	High	81 to 98	91
H.2.3	Saltmarsh / dune interface	High	81 to 98	91
H.2.4	Scattered saltmarsh plants	High	81 to 98	91
H.2.6	Dense / continuous saltmarsh plants	High	81 to 98	91
H.3	Shingle above high tide mark	High	81 to 98	91
H.4	Boulders / rocks above high tide mark	High	81 to 98	91
H.5	Strandline vegetation	High	81 to 98	91
H.6.4	Sand dune – dune slack	Medium-high	61 to 80	71
H.6.5	Sand dune – dune grassland	Medium-high	61 to 80	71
H.6.6	Sand dune – dune heath	High	81 to 98	91
H.6.7	Sand dune – dune scrub	Medium-high	61 to 80	71
H.6.8	Sand dune – open dune	High	81 to 98	91
H.8.1	Maritime cliff and slope – hard cliff	High	81 to 98	91
H.8.2	Maritime cliff and slope – soft cliff	High	81 to 98	91
H.8.3	Maritime cliff and slope – crevice / ledge vegetation	High	81 to 98	91
H.8.4	Maritime cliff and slope – coastal grassland	Negligible	1	1

Habitat Code	Habitat Type	Typical Resistance Level	Typical Resistance Value (0 to 99)	GIS input value
H.8.5	Maritime cliff and slope – coastal heathland	Low-medium	21 to 40	31
I.1.1.1	Natural inland cliff – acid / neutral	High	81 to 98	91
I.1.1.2	Natural inland cliff – basic	High	81 to 98	91
I.1.2.1	Natural scree – acid / neutral	High	81 to 98	91
I.1.2.2	Natural scree – basic	High	81 to 98	91
I.1.3	Natural limestone pavement	Medium-high	61 to 80	71
I.1.4.1	Natural other exposure – acid / neutral	High	81 to 98	91
I.1.4.2	Natural other exposure – basic	High	81 to 98	91
I.1.5	Natural cave	High	81 to 98	91
I.2.1	Artificial quarry	High	81 to 98	91
I.2.2	Artificial spoil	High	81 to 98	91
I.2.3	Artificial mine	High	81 to 98	91
I.2.4	Artificial refuse tip	High	81 to 98	91
J.1.1	Arable	Medium-high	61 to 80	71
J.1.2	Amenity grassland	Negligible	1	1
J.1.3	Ephemeral / short perennial	Negligible	1	1
J.1.4	Introduced shrub (inc INNS)	Negligible	1	1
J.1.5	Gardens	Low	2 to 20	11
J.2.1.1	Intact species rich hedge	Negligible	1	1
J.2.1.2	Intact species poor hedge	Negligible	1	1
J.2.2.1	Defunct species rich hedge	Negligible	1	1
J.2.2.2	Defunct species poor hedge	Negligible	1	1
J.2.3.1	Species rich hedge with trees	Negligible	1	1
J.2.3.2	Species poor hedge with trees	Negligible	1	1
J.2.4	Fence	Medium	41 to 60	51
J.2.5	Wall	High	81 to 98	91
J.2.6	Dry ditch	Negligible	1	1
J.2.7	Boundary removed	Negligible	1	1
J.2.8	Earth bank	Negligible	1	1
J.3.4	Caravan site	Medium-high	61 to 80	71
J.3.5	Sea wall	Complete	99	99
J.3.6	Buildings	Complete	99	99
J.3.7	Track / road	Medium	41 to 60	51
J.3.7	Road – including all unpaved and asphalt roads, and railroads.	Low	2 to 20	31
J.3.7	Large Road – as above but over 4m wide	High	81 to 98	91
J.4	Bare ground	Low-medium	21 to 40	31
J.5	Other habitat	Low-medium	21 to 40	31
J.5	Solar panel fields	Low	2 to 20	11

Table 2: Summary of road resistance values by type

Road Type	Typical Resistance Level	Typical Resistance Value (0 to 99)	GIS input value
A Road	High	81 to 98	91
B Road	Medium-high	61 to 80	71
Classified Unnumbered	Medium	41 to 60	51
Motorway	Complete	99	99
Not Classified	Low-medium	21 to 40	31
Unclassified	Low-medium	21 to 40	31

Table 3: Summary of watercourse resistance values by type

Watercourse Type	Typical Resistance Level	Typical Resistance Value (0 to 99)	GIS input value
Canal	High	81 to 98	91
Inland River	High	81 to 98	91
Lake	Complete	99	99
Tidal River	Complete	99	99

2.3. Genetic Analysis

Genetic samples were taken from rescued hedgehogs by Simon Allen of the GBH between October 2019 and September 2021. These samples included 147 frozen buccal swabs, and 164 tail tip samples stored in ethanol and frozen at -20^{o}C. Of these samples, 3 individuals had both buccal swabs and tail samples taken. Buccal swabs were only taken while hedgehogs were under anaesthetic for veterinary procedures. Tail and ear samples were taken from hedgehogs that were dead on arrival or died while in the care of GBH. No hedgehogs were killed, handled, or anaesthetised for the purposes of this study. Samples were taken in accordance with the ethics permit, number SU-Ethics-Staff-200721/213.

DNA extraction used the Qiagen® DNeasy Blood and Tissue kit, following the manufacturer's instructions, applying the technique specified for tissue DNA extraction for both tissue and swab samples. Samples were genotyped using 14 fluorescently labelled microsatellites across three multiplex reactions (Table 4). The microsatellites were identified through published literature (Becher & Griffiths 1997, Curto *et al.* 2019, Henderson *et al.* 2000). Microsatellites are small sequences of tandemly repeating DNA no more than 6 bases long that have a high rate of mutation and are easy to extract and genotype in comparison to other genetic approaches (Jarne & Lagoda 1996, Goldstein & Schlotterer 1999). This makes the ideal for

identifying the genetic structure of a given population or sample group, especially when budgets constrain the use of more expensive techniques.

Polymerase chain reactions (PCR) were performed to amplify microsatellite sequences using a Qiagen® Multiplex PCR Kit following the manufacturer's recommendations, except that we used 12 µl reaction volumes to keep the use of reagents to a minimum. The following PCR conditions were used: one cycle of 15 min at 95°C; 35 cycles of 30 s at 94°C, 90 s at 57°C, 60 s at 72°C; and one final cycle of 30 min at 60°C. PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer (Applied Biosystems) and allele sizes were scored using GeneMapper® Software Version 4.0 (Applied Biosystems). To maximise genotype quality, we manually inspected all of the traces and corrected any genotype calls where necessary. To assess error rates for each microsatellite locus, we independently re-genotyped a subset of 49 individuals and compared the resulting genotypes to calculate the error rate per allele.

Summary statistics on the resultant genotypes (information content, deviation from the Hardy-Weinberg equilibrium, and frequency of null alleles) were calculated using Cervus 3.0.7. The Hardy-Weinberg (HW) principle states that the allele/genotype frequency within a population will reach equilibrium after one generation and remain constant from generation to generation in the absence of other influences. This principle uses several assumptions namely random mating, absence of natural selection, a large population, no gene flow (migration), and no mutation (Allendorf, Luikart & Aitken 2013), although these do occur in naturally outbreeding populations (Frankham, Ballou & Briscoe 2002). It is important to determine if the HW principle is violated to show if one or more of the processes above are operating at a level to significantly influence allele and genotype frequencies and therefore the genetic structure of the population (Balkenhol *et al.* 2016) or if there are potential errors in the sampling and extraction process (Van Oosterhout *et al.* 2004, Kwong *et al.* 2021).

Table 4: Multiplex microsatellites used for hedgehog genotyping.

Multiplex	Locus	Allele Size (bp)	Colour
1	EEU12H	91-97	blue (6-FAM)
	EEU1	129-143	green (HEX)
	EEU4	144-170	blue (6-FAM)
	EEU37H	236-280	green (HEX)
	E13	310	blue (6-FAM)
2	EEU5	107-139	green (HEX)
	EEU3	131-181	blue (6-FAM)
	EEU6	145-159	green (HEX)
	EEU2	257-281	blue (6-FAM)
3	W23	114-126	blue (6-FAM)
	EEU43H	156-168	green (HEX)
	W30	177-197	blue (6-FAM)
	W8	244-269	blue (6-FAM)
	E36	319-348	green (HEX)

2.4. Population structure analysis

Structure 2.3.1 was used to assess the genetic subdivision within the sample population, as set out in (Pritchard, Wen & Falush 2010), using the admixture model. This model can be used to determine the most likely number of genetic clusters (K) within a sample population and which individuals most likely belong to which cluster using the Bayesian method. K values ranging from 1 to 10 were subject to five independent runs each with run length and MCMC of 10^5 following a burn-in time of 10^5. Given the difficulty in determining K values below 3 (Janes *et al.* 2017) the following were reviewed together to assess the most likely value of K (1) Average estimated likelihood of K (Ln Pr(X|K)), which estimates the posterior probability of the data, across all runs, (2) estimated likelihood of K (Ln Pr(X|K)) for individual runs, and (3) ΔK, the statistic developed by Evanno et al (2005) based on the second-order rate of change of LN Pr(X|K), as generated by Structure Harvester Web v0.6.94 July 2014, Plot vA.1 November 2012, Core vA.2 July 2014 (Earl & vonHoldt 2012).

2.5. Landscape Genetic Analysis

Circuitscape in Julia (Anantharaman *et al.* 2020) was used to calculate the pairwise resistance distances for the sampled population against the different resistance surfaces. This utilises electronic circuit theory to estimate the resistance to current flow between nodes (sampled individuals) and was run in pairwise mode with nodes

connected to all eight neighbouring cells (Zecherle *et al.* 2020). This provides pairwise resistance distances which were then converted to relatedness coefficients to provide a measure of the resistance between paired sampled individuals. The genetic distance and relatedness coefficients were estimated using the pairwise relatedness tool in the GenAlEx Excel add-in (Peakall & Smouse 2006, Peakall & Smouse 2012), following Lynch & Ritland (1999) and Queller & Goodnight (1989).

A distance-based redundancy analysis (dbRDA) was used to test for a potential relationship between habitat/feature resistance distance and genetic distance. This followed (Zecherle *et al.* 2020) using the capsscale function in the 'vegan' R package (Legendre, Oksanen & ter Braak 2011). This approach allows distance as a response variable against which different explanatory variables can be regressed (Legendre & Anderson 1999, Buttigieg & Ramette 2014). To utilise this approach, the pairwise relatedness matrices and the habitat/feature resistance matrices were first transformed into one-dimensional explanatory variables using the pcoa function in the 'ape' R package with a Lingoes correction to address negative eigenvalues and preserve variation within the matrices (Paradis & Schliep 2018, Zecherle *et al.* 2020). The number of significant principal coordinates (PCos) to be retained was determined using a broken stick model (MacArthur 1957). This indicated that the first 10 PCos should be retained for the distance control, roads, and water resistance variables; these accounted for >50% of the genetic variance within the samples. Those for the various habitat resistance variables were significantly more due to the low proportion of variability explained. As such the first 10 PCos were used for all resistance variables for consistency (Table 5).

Table 5: Percentage of genetic variance explained by the retained PCos of different improved grassland habitat resistance values

Variable	Variance Explained by Retained PCo (first 10)
Distance	50.62%
Habitats (IG Resistance = 1)	4.17%
Habitats (IG Resistance = 11)	4.24%
Habitats (IG Resistance = 31)	4.30%
Habitats (IG Resistance = 51)	4.32%
Habitats (IG Resistance = 71)	4.34%

Variable	Variance Explained by Retained PCo (first 10)
Habitats (IG Resistance = 91)	4.36%
Habitats (IG Resistance = 99)	4.36%
Roads	50.40%
Water	50.63%
NB: *IG resistance = improved grassland resistance which varies within each variable as identified*	

Twenty models were tested for resistance effects on gene flow with the transformed pairwise relatedness matrix set as the response variable and one of the transformed resistance matrices (geographic distance, habitat with varying resistance for improved grassland, roads, and waterbodies) set as the explanatory variables. Models were also tested that controlled for an effect of geographic distance on habitat/feature resistance. A model was also tested that included all the explanatory variables, with habitat resistance of 31 (low-medium) for improved grassland. This resistance was chosen as it was the closest to being statistically significant of all the habitat resistance layers (Table 10 and Table 11). Models were run twice, the first using the pairwise relatedness matrix generated by the Lynch & Ritland (1999) estimator and the second using the matrix from the Queller & Goodnight (1989) estimator. All models were tested for significance using the anova.cca function with 9999 permutations.

3. Results

3.1. Genetic Analysis

Of 303 samples obtained, 298 samples were successfully genotyped, 98.3% of the samples provided by GBH (Table 6). Our panel of 14 microsatellites had a high level of diversity and information content; mean observed heterozygosity of the microsatellites within the sampled population was 0.656 (range 0.336 - 0.801), mean Polymorphic Information Content (PIC) was 0.614 (range 0.327 – 0.761) and the mean number of alleles per locus was 7.786 (range 3 – 11) (Table 6 and Table 7). There was no significant deviation from the HW equilibrium for any locus (Table 6 and Table 7). This indicates that the microsatellites genotyped meet the HW assumptions and are unlikely to be impacted by issues such as sex-linkage and high

levels of genotyping errors. The estimated frequency of null alleles was under 0.05 for all the microsatellites used with the exception of three, the highest having an estimated frequency of 0.067 (highlighted in red in Table 7). As these estimated null allele rates were low, the potential presence of null alleles is likely to have a negligible effect on population-genetic parameters, so all of the microsatellites were used in further analysis.

A subset of 49 samples was re-genotyped and the two sets compared to determine the error level within the genotyped data. The error rate was zero for 12 of the loci and was very low (<0.04 per allele) for the remaining two loci (Table 8). Furthermore, three individuals where tail and swab samples were taken were successfully genotyped and showed no variation in results, demonstrating that genotypes from non-invasive swabs were consistent with those from tissue samples.

Table 6: Genotyping summary

Displayed are the total number of individuals sampled, total number genotyped, number of microsatellite loci used, mean number of alleles per locus, loci proportion typed, mean expected heterozygosity and mean polymorphic information content (PIC)

Number of individuals sampled	303
Number of individuals genotyped	298
Number of microsatellite loci	14
Mean number of alleles per locus	7.786
Mean proportion of loci typed	0.985
Mean expected heterozygosity	0.656
Mean polymorphic Information Content (PIC)	0.614

Table 7: Genotyping summary

Displayed are the loci used, number of alleles associated with each, number of hedgehogs genotyped using that locus, observed and expected heterozygosity, polymorphic information content (PIC), Hardy-Weinberg variation (HW; NS represent not significant), and estimated frequency of null alleles.

Locus	No of Alleles	No of Hedgehogs Genotyped	Observed Hetero-zygosity	Expected Hetero-zygosity	PIC	HW	Estimated Frequency of Null Alleles
E13	9	298	0.728	0.792	0.761	NS	0.041
EEU1	8	300	0.407	0.403	0.35	NS	-0.012
EEU12H	3	300	0.377	0.42	0.359	NS	0.052
EEU37H	6	295	0.336	0.343	0.327	NS	0.001
EEU4	7	298	0.728	0.783	0.748	NS	0.035

Locus	No of Alleles	No of Hedgehogs Genotyped	Observed Hetero-zygosity	Expected Hetero-zygosity	PIC	HW	Estimated Frequency of Null Alleles
EEU2	9	302	0.636	0.712	0.666	NS	0.056
EEU3	7	298	0.614	0.639	0.581	NS	0.016
EEU5	11	299	0.712	0.767	0.731	NS	0.036
EEU6	6	302	0.586	0.669	0.609	NS	0.067
E36	7	301	0.801	0.781	0.748	NS	-0.016
EEU43H	10	298	0.695	0.72	0.685	NS	0.020
W23	8	285	0.586	0.629	0.573	NS	0.035
W30	10	302	0.689	0.75	0.72	NS	0.044
W8	8	301	0.721	0.77	0.732	NS	0.033

Table 8: Re-genotyping error rate

Locus	Total No of Alleles Re-genotyped	No of Mismatches	Error Rate per Allele
E13	94	0	0
EEU1	92	1	0.011
EEU12H	92	0	0
EEU37H	90	3	0.033
EEU4	94	0	0
EEU2	94	0	0
EEU3	96	0	0
EEU5	94	0	0
EEU6	96	0	0
E36	90	0	0
EEU43H	96	0	0
W23	88	0	0
W30	98	0	0
W8	98	0	0

3.2. Population structure analysis

The Ln Pr(X|K) generated by the Structure analysis across all runs of K indicated a K value of either 3 (2 out 5 runs) or 4 (3 out of 5 runs). The average Ln Pr(X|K) indicated a K value of 4, although there is only a slight difference between this and K = 3 (Figure 2). The Structure Harvester analysis indicated a K value of 3 based the mean LnP(K), just above that for K=4 (Table 9 and Figure 3) while ΔK showed a strong peak at K = 4 (Figure 4). Overall, the analysis indicates that there are most likely 4 genetic sub-populations within the sample population.

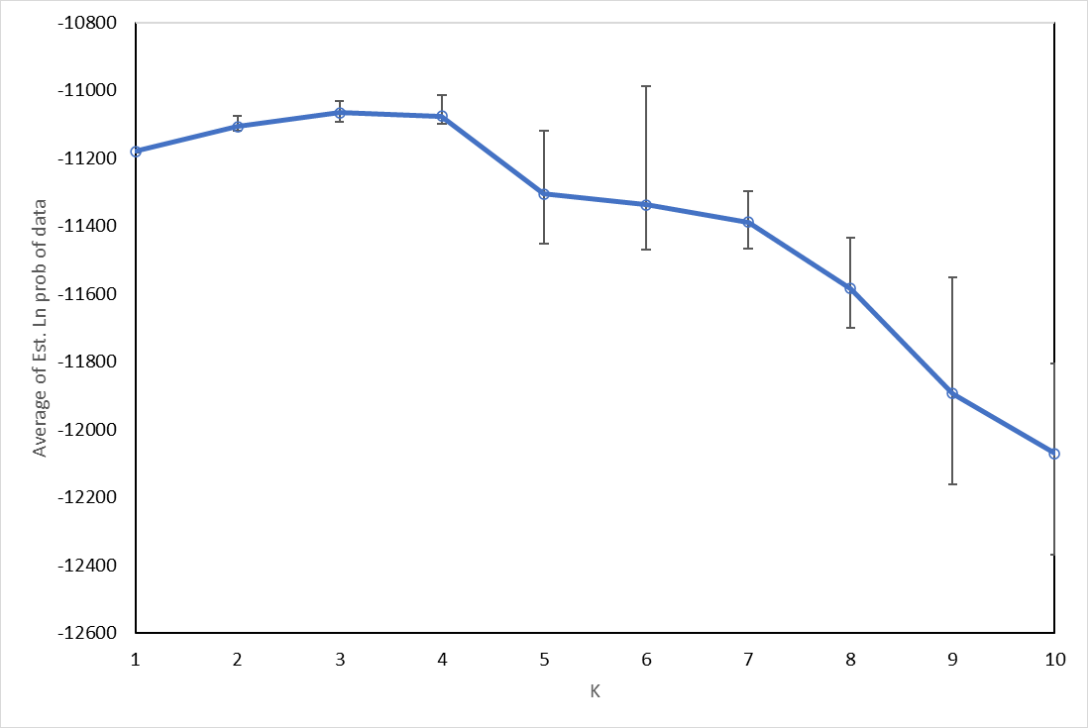

Figure 2: Average Ln Pr(X|K) for K 1-10 across all runs.

Table 9: Evanno output from Structure Harvester

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
2	5	-11106.68	18.480	NA	NA	NA
3	5	-11065.98	23.473	40.70	51.02	2.174
4	5	-11076.30	34.761	-10.32	218.54	6.287
5	5	-11305.16	120.694	-228.86	196.3	1.626
6	5	-11337.72	200.279	-32.56	18.30	0.091
7	5	-11388.58	67.898	-50.86	145.38	2.141
8	5	-11584.82	116.876	-196.24	112.28	0.961
9	5	-11893.34	251.701	-308.52	131.7	0.523
10	5	-12070.16	203.0189	-176.82	NA	NA

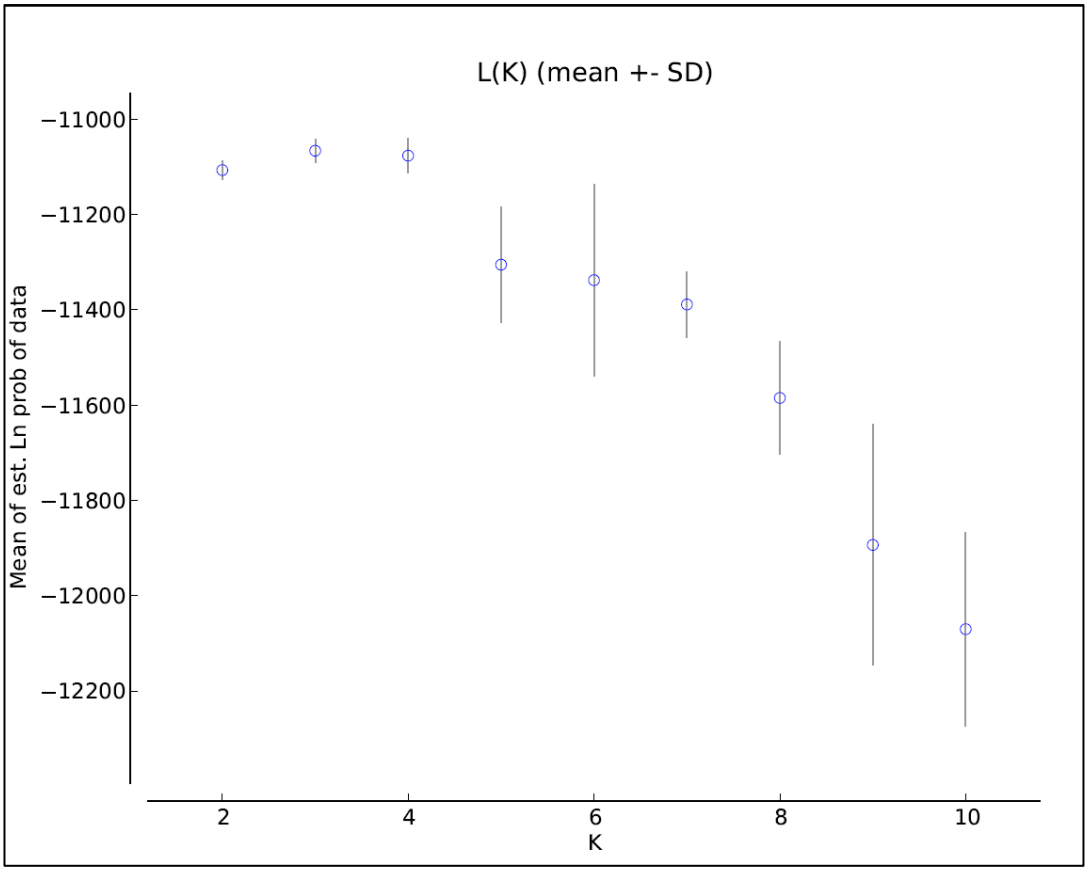

Figure 3: LnP(K) from Structure Harvester

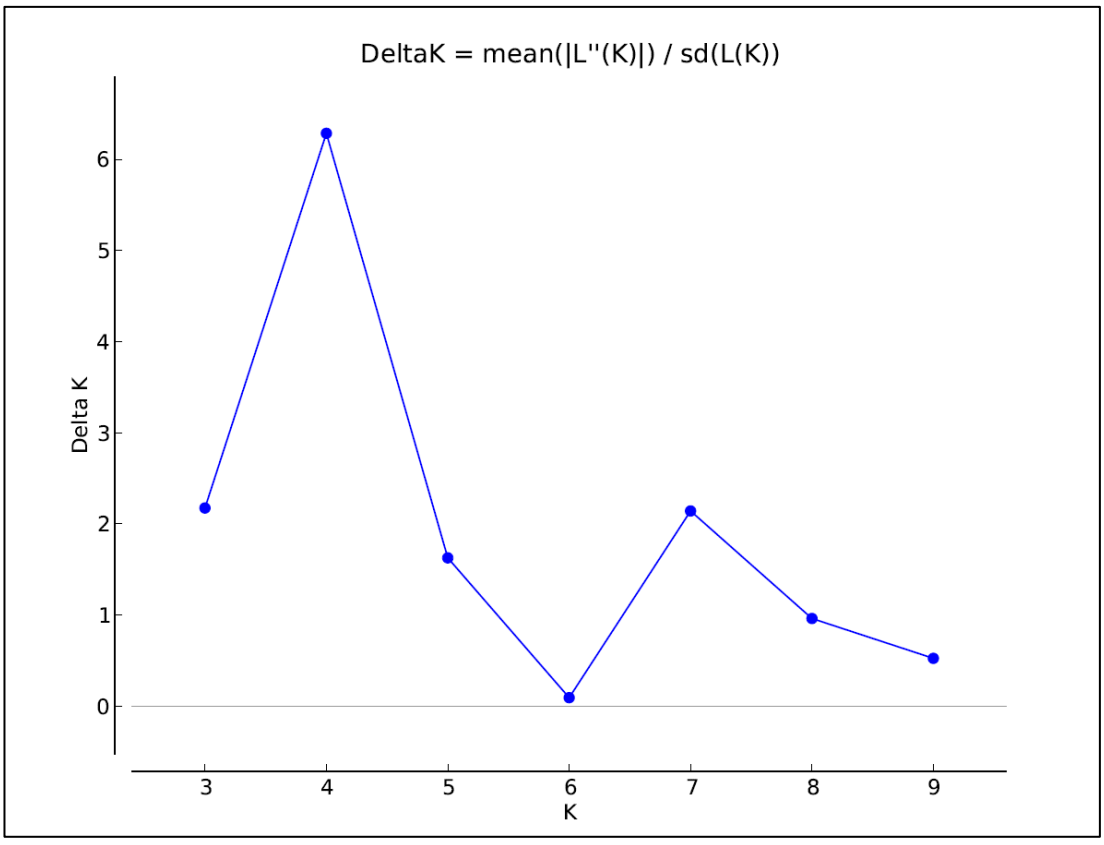

Figure 4: Delta K from Structure Harvester

The Structure ancestry analysis (Figure 5) based on K=4 shows a clear population cluster assigned for 136 (46%) individuals, while 162 (54%) could not be clearly assigned to a single population (q-values <0.7). Mapping the likely populations based on K=4 (Figure 6 and Figure 7) shows the overlap between the population clusters with two populations largely limited to the Gower/Swansea area (red and yellow populations). The green population is largely limited to this area but also has several individuals to the north and east. The blue population also has some geographic limitation to the south east part of the study but this also has some overlap with the other coloured populations. The remaining individuals, shown in white, could not be clearly assigned to a specific population cluster; these are spread throughout the study area.

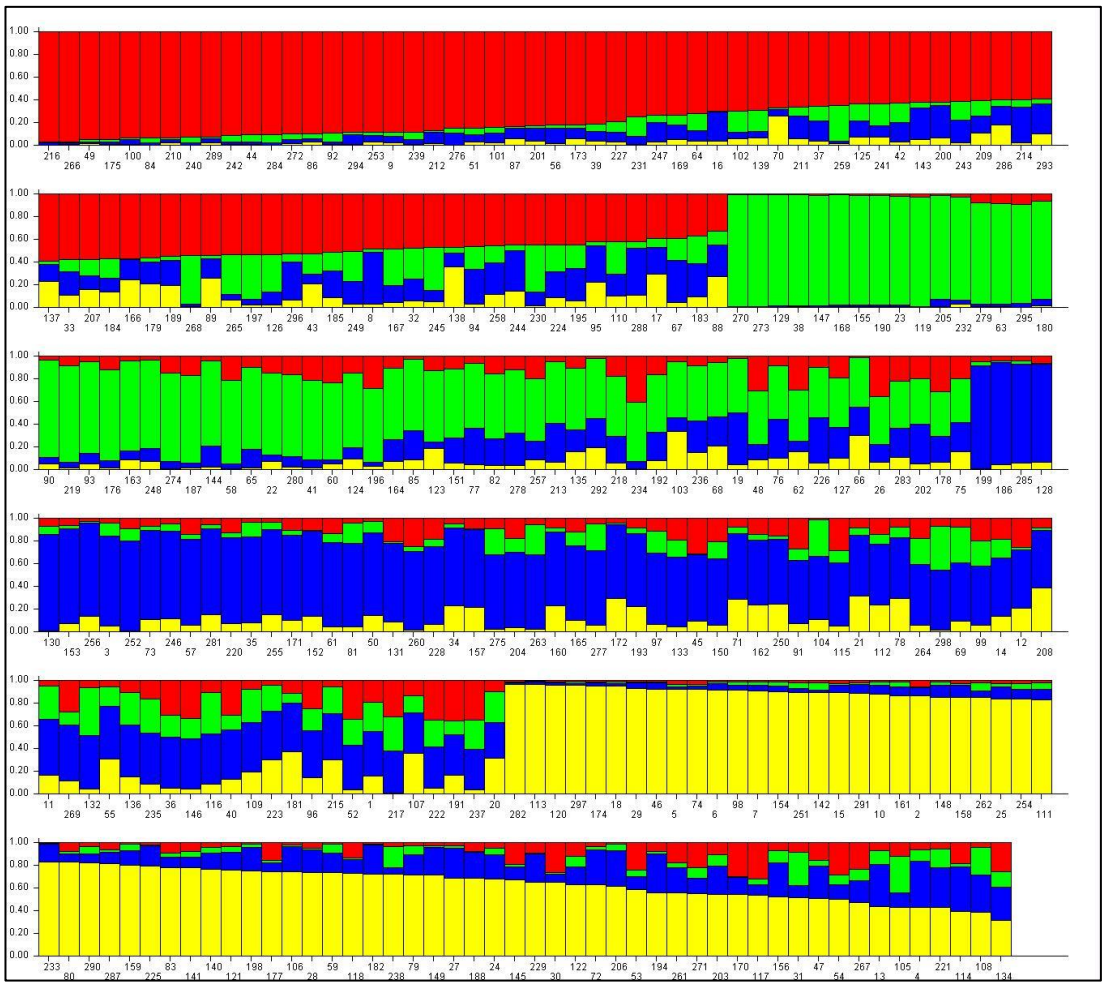

Figure 5: Results of the genetic structure analysis, proportional ancestry for all samples for four genetic clusters (K=4) as estimated by Structure

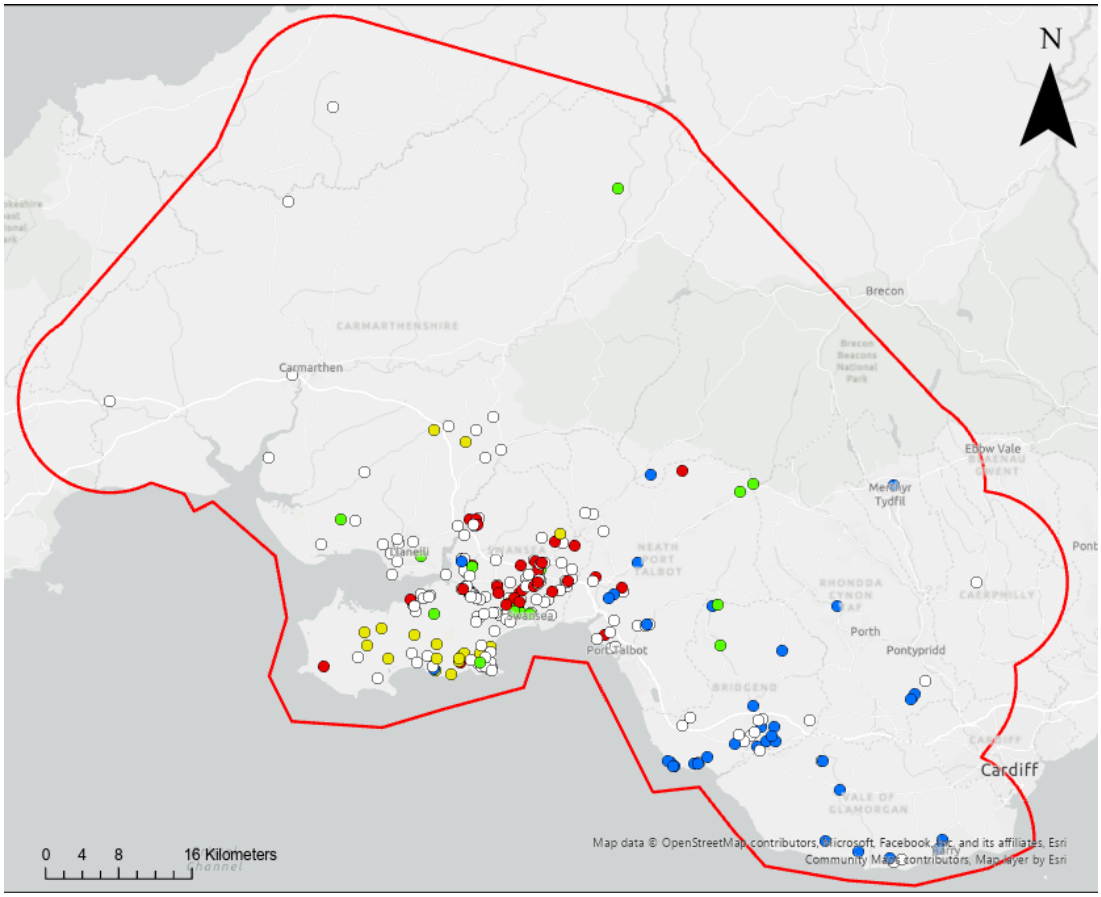

Figure 6: GIS mapping of genetic sub-populations (K=4). The colours shown for individuals with <0.7 assignment to a single cluster match those used in Figure 5. Admixed individuals with <0.7 assignment to a single cluster are shown in white.

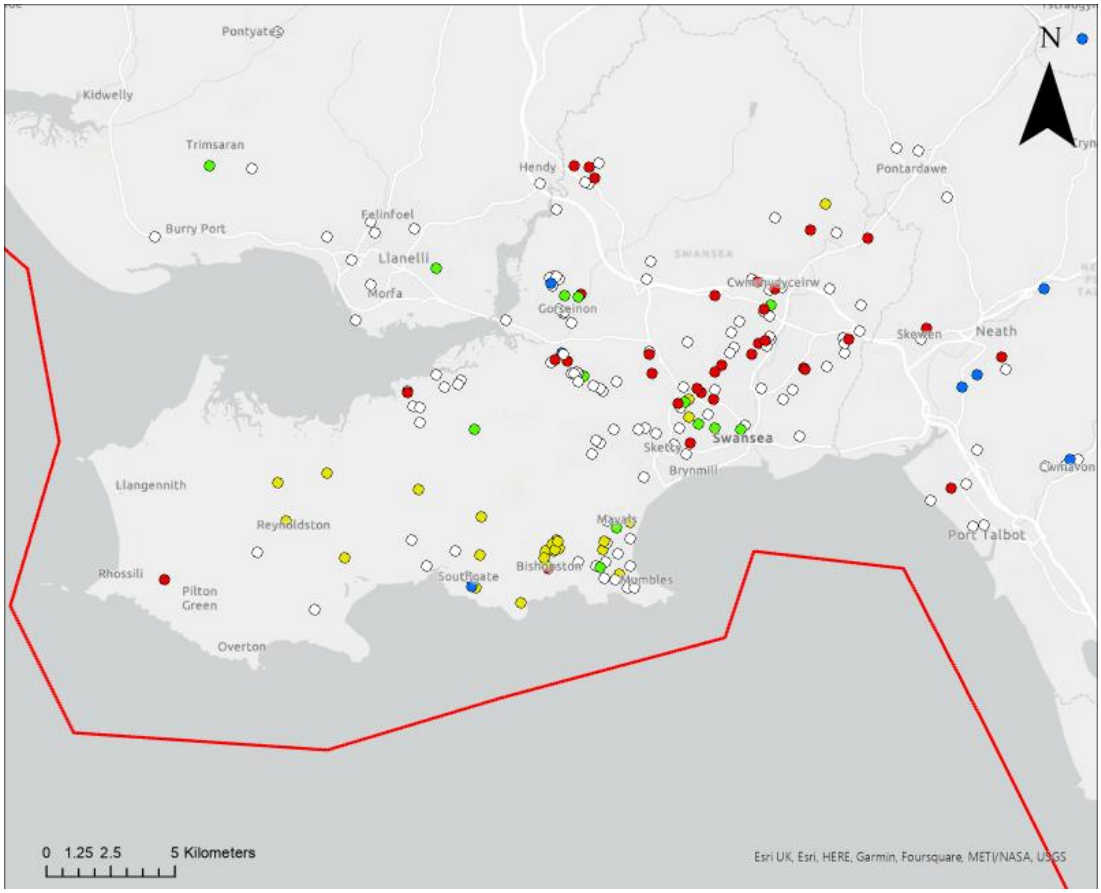

Figure 7: GIS mapping of genetic sub-populations concentrated in the Swansea/Gower area (K=4). The colours shown for individuals with <0.7 assignment to a single cluster match those used in Figure 5. Admixed individuals with <0.7 assignment to a single cluster are shown in white.

3.3. Landscape Genetic Analysis

None of the explanatory variables (geographic distance, habitat resistance, the presence of roads or watercourses) had a significant effect on pairwise genetic relatedness within the sample population, either alone or when controlled for distance (Table 10 and Table 11).

Table 10: Results from the dbRDA (Lynch and Ritland 1999 estimator)

Displayed are the tested models for the Lynch and Ritland 1999 estimator, their total variance (inertia), the % variance explained (R^2) and adjusted % variance explained (adjusted R^2), the degrees of freedom (df), F-statistic (F), and p-value (Pr(>F)) of the permutation tests (9,999). Models controlled for distance are indicated with |.

Variable	Inertia	R^2	Adjusted R^2	Df	F	Pr(>F)
Distance	1.65	3.70%	<1%	10	0.980	0.971
Habitats (IG Resistance = 1)	1.66	3.74%	<1%	10	0.991	0.853

Variable	Inertia	R^2	Adjusted R^2	Df	F	Pr(>F)
Habitats (IG Resistance = 11)	1.68	3.77%	<1%	10	1.000	0.527
Habitats (IG Resistance = 31)	1.68	3.77%	<1%	10	1.000	0.516
Habitats (IG Resistance = 51)	1.66	3.74%	<1%	10	0.992	0.819
Habitats (IG Resistance = 71)	1.66	3.73%	<1%	10	0.987	0.911
Habitats (IG Resistance = 91)	1.64	3.69%	<1%	10	0.977	0.986
Habitats (IG Resistance = 99)	1.64	3.69%	<1%	10	0.977	0.984
Roads	1.65	3.70%	<1%	10	0.980	0.967
Water	1.64	3.70%	<1%	10	0.980	0.971
Roads | Distance	1.64	3.76%	<1%	10	0.995	0.679
Water | Distance	1.64	3.76%	<1%	10	0.995	0.680
Habitats (IG Resistance = 1) | Distance	1.64	3.70%	<1%	10	0.980	0.968
Habitats (IG Resistance = 11) | Distance	1.64	3.72%	<1%	10	0.985	0.901
Habitats (IG Resistance = 31) | Distance	1.64	3.74%	<1%	10	0.989	0.840
Habitats (IG Resistance = 51) | Distance	1.64	3.72%	<1%	10	0.986	0.899
Habitats (IG Resistance = 71) | Distance	1.64	3.72%	<1%	10	0.984	0.922
Habitats (IG Resistance = 91) | Distance	1.64	3.69%	<1%	10	0.976	0.981
Habitats (IG Resistance = 99) | Distance	1.64	3.69%	<1%	10	0.975	0.982
All variables (IG resistance = 31)	44.44	15.03%	<1%	40	0.995	0.844

Table 11: Results from the dbRDA (Queller and Goodnight 1989 estimator)

Displayed are the tested models for the Queller and Goodnight 1989 estimator, their total variance (inertia), the % variance explained (R2) and adjusted % variance explained (adjusted R2), the degrees of freedom (df), F-statistic (F), and p-value (Pr(>F)) of the permutation tests (9,999). Models controlled for distance are indicated with |.

Variable	Inertia	R^2	Adjusted R^2	Df	F	Pr(>F)
Distance	8.82	3.75%	<1%	10	0.995	0.688
Habitats (IG Resistance = 1)	8.76	3.73%	<1%	10	0.987	0.913
Habitats (IG Resistance = 11)	8.83	3.75%	<1%	10	0.995	0.704
Habitats (IG Resistance = 31)	8.84	3.76%	<1%	10	0.996	0.659
Habitats (IG Resistance = 51)	8.81	3.75%	<1%	10	0.993	0.778
Habitats (IG Resistance = 71)	8.80	3.74%	<1%	10	0.992	0.794
Habitats (IG Resistance = 91)	8.80	3.74%	<1%	10	0.992	0.784
Habitats (IG Resistance = 99)	8.80	3.74%	<1%	10	0.992	0.792
Roads	8.83	3.76%	<1%	10	0.996	0.653
Water	8.82	3.75%	<1%	10	0.995	0.693

Variable	Inertia	R^2	Adjusted R^2	Df	F	Pr(>F)
Roads | Distance	8.82	3.78%	<1%	10	1.002	0.452
Water | Distance	8.82	3.77%	<1%	10	1.000	0.525
Habitats (IG Resistance = 1) | Distance	8.82	3.70%	<1%	10	0.979	0.962
Habitats (IG Resistance = 11) | Distance	8.82	3.71%	<1%	10	0.982	0.937
Habitats (IG Resistance = 31) | Distance	8.82	3.71%	<1%	10	0.981	0.937
Habitats (IG Resistance = 51) | Distance	8.82	3.70%	<1%	10	0.978	0.964
Habitats (IG Resistance = 71) | Distance	8.82	3.69%	<1%	10	0.978	0.967
Habitats (IG Resistance = 91) | Distance	8.82	3.70%	<1%	10	0.980	0.958
Habitats (IG Resistance = 99) | Distance	8.82	3.70%	<1%	10	0.980	0.952
All variables (IG resistance = 31)	235.06	15.03%	<1%	40	0.995	0.839

4. Discussion

The analysis showed a weak genetic structure within the hedgehog sample population across the study area, with four genetic clusters; one being primarily found in the south east, another primarily on the Gower peninsula and the remaining two clusters around the Swansea area. However, over half of the individuals sampled were not clearly allocated to a specific cluster, supporting the conclusion of a weak genetic structure. Patterns of genetic relatedness across the study area were seemingly unrelated to geographic distance, habitat resistance, or the presence of large barrier features within the landscape.

4.1. The impact of habitat and feature resistance on gene flow

While previously published research based on individually tracking hedgehogs demonstrates that the species avoids or uses certain habitats less than others (Driezen *et al.* 2007), this doesn't appear to have affected the patterns of relatedness across the study area. This was repeated across all habitat resistance models despite variations in the resistance levels used for improved grassland habitat, which

dominated the study area. This suggests that individual hedgehogs may cross higher resistance habitats with sufficient frequency to allow gene-flow across South Wales. Alternatively, habitat resistance may impact movement, but there may be other drivers to hedgehog movements (not measured in this study) that override the habitat resistance as a factor determining gene-flow, such as availability of resting or nesting habitat, disturbance, food availability and risk of predation (Doncaster 1993, Doncaster, Rondinini & Johnson 2001, Riber 2006, Driezen *et al.* 2007, Berger *et al.* 2020a). However, it should be noted that the drivers not investigated in this study are likely have limited influence on gene-flow on a landscape-scale as we found a weak genetic structure across the whole population.

Contrary to expectations, the presence of roads was found to have no significant impact on genetic relatedness within the study area. Roads present substantial features within the landscape, including motorways and busy A-roads, and based on published research, could be expected to have some form of barrier effect (Orłowski & Nowak 2004, Moore *et al.* 2020). My results suggest that hedgehogs are able to cross the majority of roads, circumvent them, or even to use them as movement corridors. Doncaster (1992) observed individual hedgehogs in London crossing major trunk roads with reports of hedgehogs successfully crossing or circumventing roads also occurring in other studies (Doncaster, Rondinini and Johnson 2001, Dowding *et al.* 2010, Braaker *et al.* 2014, Williams *et al.* 2018 and Barthel *et al.* 2020). Roads have also been reported to aid hedgehog movements in some studies, depending on the suitability of the verge habitats associated with them (Doncaster, Rondinini & Johnson 2001, Rondinini & Doncaster 2002, Hof & Bright 2009, Hof & Bright 2012, Wright *et al.* 2020). When combined with my results, it appears that the road network within the study area has little barrier effect.

While we categorised roads based on their National Highways classification, there are finer scale connectivity features that may be present that are not accounted for within the models, such as crossing structures and traffic calming measures, that are allowing continued safe crossing of roads by hedgehogs in particular locations (Moore *et al.* 2020). Barthel *et al.* (2020) identified the potential for hedgehogs to

use bridges over watercourses for crossing purposes and they may be similarly used to cross larger roads. The presence of underpasses, culverts, and other similar structures could also provide a means for hedgehogs to safely cross roads (Moore *et al.* 2020), reducing the barrier effect of such features.

The presence of several large watercourses was also found to have no significant impact on genetic relatedness within the study area. This is not wholly unexpected given that several published studies that indicate that hedgehogs are able to cross such features (Doncaster 1992). However, given the high number of watercourses present, including several large rivers, some effect on genetic relatedness was expected. As with roads, the lack of significant impact suggests that hedgehogs are able to cross such features either by swimming, circumventing them or using finer scale connectivity features to cross that are not accounted for within the models used, including man-made bridges (Barthel *et al.* 2020).

4.2. Genetic structure

The weak genetic structure identified within the sample population was unexpected given the size of the study area and published research indicating that habitat resistance, roads, and watercourses can and do limit hedgehog movement and therefore gene flow (Driezen *et al.* 2007, Braaker *et al.* 2014, Morris 2018, Wright *et al.* 2020). However, weak population structure is consistent with some other studies operating on a similar scale; Rasmussen *et al.* (2019) found no genetic sub-structuring within hedgehog samples from across Denmark, Barthel *et al.* (2020) identified a lack of genetic structure across hedgehog populations in Berlin, and Bolfíková *et al.* (2013) identified variable genetic structuring, dependent on the genotyping used, from hedgehogs sampled across an area of 500km x 280km within Czech Republic. The significance of geographic distance on the genetic structuring has also shown to be slight, if present at all, by Rasmussen *et al.* (2020), Curto *et al.* (2019), Becher and Griffiths (1998), Braaker *et al.* (2017) and Barthel *et al.* (2020), which indicates that the lack of a significant distance effect within the sample population is relatively common with hedgehogs over a variety of spatial scales. However, some other

studies have shown slight to moderate but significant genetic structure at smaller and larger scales than my study (Braaker *et al.* 2017, Curto *et al.* 2019), which suggests that my sample population has a higher rate of gene flow than these populations, or that the genetic structure of hedgehogs in South Wales is yet to reflect recent changes in anthropogenic habitat resistance and barrier effects in the landscape. It is also possible that landscape features not present in South Wales are the primary determinants of genetic structure. For example, the study area for Curto *et al.* (2019) includes large mountains (the Alps) which likely present a more significant barrier than the features within my study area. Bolfíková *et al.* (2013) showed that New Zealand hedgehog populations had high admixture with several clusters in a similar genetic pattern to my study.

The weak genetic structure seen within hedgehogs in South Wales could be driven by several factors. The presence of unmapped stepping-stone habitats, habitat corridors, and/or habitat networks that allow gene flow between the population clusters is likely given the scale of the mapping used and the limitations within this data (discussed in Section 4.3). The presence of such unaccounted-for features could override the resistance and barrier effects of the habitats, roads, and watercourses that have been included within the models changing the significance of any influence that they do have (Hof & Bright 2012, Moorhouse *et al.* 2014). A similar conclusion was suggested within Barthel *et al.* (2020), albeit on a smaller scale than the current study area, where the results indicated gene flow across the city of Berlin despite the presence of barrier features including large roads and watercourses. There is also the potential for the sample population to be part of a larger metapopulation extending beyond the study area boundaries resulting in genetic inputs from individuals and other subpopulations not sampled as part of this study. This has been observed in other hedgehog species (Abu Baker *et al.* 2017).

A further driver of gene flow could be changing land use patterns, noted when comparing the 2016/2017 habitat mapping and the more recent 2020 aerial imagery as over 20 polygons totalling 31.65km^2 (approximately 0.5% of the overall study area) were changed from semi-natural habitats to urban/sub-urban. Such changes may

result in the migration of animals away from these areas into adjacent habitats (Abu Baker *et al.* 2017, Tarabon *et al.* 2019) particularly during the clearance and construction phases as observed by the author through their professional role and recognised within CIEEM (2018) and European_Commission (2020), resulting in gene flow into existing populations. High levels of widespread migration induced by short term/rapid land-use change might explain the high level of admixture observed, whereby over 50% of the sample population could not be assigned to a single genetic cluster. What is not clear is whether the structure observed represents a stable genetic equilibrium within the study area or whether changes are still in process that may result in more defined population clustering or increased admixture.

The release of animals from rescue and rehabilitation centres may also be contributing to the level of admixture within the sample population (Moore *et al.* 2007, Barthel *et al.* 2020, Ploi 2020), particularly where animals are not released to the same location that they were rescued from. Jensen *et al.* (2017) showed this to have negative implications for the genetic health and success of a population by introducing inbreed individuals or those less genetically suited to an area, although it was not considered to be a significant risk to wild populations. However, Pacioni *et al.* (2017) demonstrated that the release of rehabilitated individuals can benefit wild populations by counteracting genetic drift and boosting genetic diversity. The occurrence of uncoordinated translocations for many species are not quantified or recorded at present (Pyke & Szabo 2018, Barthel *et al.* 2020), but the number of animals rescued by the GBH is indicative of the level of concern the public show for hedgehogs and other studies have shown that hedgehogs are one of the most commonly admitted species to rescues in the UK (Molony *et al.* 2006). While the GBH does release hedgehogs back to their found locations, not all rescues follow this and location data may not always be available (Molony *et al.* 2006).

4.3. Limitations and further research

This study presents a detailed analysis of the landscape genetic structure of hedgehogs across South Wales, based on approaches used in similar analysis for

other mobile mammal species (Zecherle *et al.* 2020). Nevertheless, there are some limitations to the analysis.

Genetic samples were dependent on members of the public taking rescued hedgehogs to the GBH and reporting location data accurately or informing GBH of hedgehogs in need of assistance. This limits the sample population to those areas where hedgehogs and people overlap and interact, such as urban and sub-urban areas and along roads and paths. This could introduce a bias in the sampling to certain individual hedgehogs, such as younger or sick hedgehogs (Bunnell 2001) or areas where hedgehogs move around more or areas where there are more people present, with potential effects on the subsequent analysis and its outcomes by potentially favouring hedgehogs that utilise higher resistance habitats and/or urban/sub-urban habitats. Such a bias could alter the genetic basis of the sample population and the influence of habitat resistance and barrier effects identified (Balkenhol *et al.* 2016).

Future studies could build on the samples gathered to date by collecting samples in the field from within the study area, or targeted locations within the study area, to supplement those received from GBH. This would provide a greater sample population and include animals that may not utilise higher resistance habitats or urban/sub-urban habitats, offsetting any bias that may be present within the study population. A larger dataset could result in clearer sub-population assignment, or greater evidence of admixture across the study area due to the presence of a metapopulation. This additional work may also result in the influence of habitat resistance and/or barrier effects being identified, particularly if field sampling is successful where no samples have yet been obtained. Such work would not only provide a larger data set but allow comparison between the sampling methods to identify if any bias is present.

The genetic samples were collected over a two-year period, within the generation time of hedgehogs (Morris 2018). However, should there be a time lag between changes within the landscape resistance due to land use changes and the genetic

relatedness of individuals, further genetic sampling over an extended time period may be required to reveal the impacts. Such a time lag is most likely in relation to the habitat resistance mapping where changes were noted to have occurred between 2016/2017 and 2020. The effect of these changes on the genetic structure of the sample population may not be visible as yet as insufficient time has lapsed following these changes given that the generation time of hedgehogs is between 2 and 7 years (Morris 2018). It is unlikely that a time lag is responsible for the lack of significant influence of roads as many of the large roads within the study area have been present for several decades, including the M4 which was completed in the 1980s. The watercourses present within the study area have also been present for several decades, and often much longer. Therefore, any barrier effect arising from roads or watercourses is unlikely to be seen as time-lags in their impacts would have passed (Coulon *et al.* 2006, Balkenhol *et al.* 2016, Lecis *et al.* 2022).

The base mapping used to determine the habitat resistance was obtained from freely available sources and was updated based on aerial imagery, therefore is relatively accurate and up to date. However, the scale of mapping doesn't include finer grain features such as tree lines, hedgerows, field margins, road verges etc. Such features are known to be frequently used by hedgehogs (Reeve 1994, Hof & Bright 2010, van de Poel, Dekker & Langevelde 2015, Morris 2018) and the presence of such features may offset the resistance posed by the larger-scale habitat types across the study area (Hof & Bright 2012, Moorhouse *et al.* 2014). The mapping also didn't differentiate gardens from buildings and hard standing within urban and sub-urban areas, which are also known to be utilised by hedgehogs (Hof & Bright 2009, Hubert *et al.* 2011, Braaker *et al.* 2014, Williams, Stafford & Goodenough 2015, Pettett *et al.* 2017, Wilson & Wembridge 2018). This information was not included as it was not freely available and there was not sufficient time to add these through review of aerial mapping or ground truthing, particularly given the extent of the study area. This could be an area of further research to see how the inclusion of such smaller scale features influences the resistance models, either across the study area as a whole or for focused locations within the study area.

The habitat resistances assigned as part of the study was based on research published to date and required the use of proxy habitats and interpretation of conflicting information. This may have introduced inaccuracies in terms of resistance levels analysed, which were unavoidable due to a lack of definitive data. GPS radio-tracking of individual hedgehogs, camera traps, and/or footprint tunnel studies could be undertaken within targeted locations within the study area to augment the analysis and conclusions and test their accuracy in terms of real-world data. Radio-tracking data would provide information on how individual hedgehogs use the areas they are in and when mapped against habitat type would show which habitats are used and which are not. Camera traps and/or footprint tunnels can be placed in different habitat types or features and show whether they are used with some indication of how much based on the number of trigger events recorded or footprints within each tunnel. Collecting and collating both types of data could be used to demonstrate habitat use and therefore confirm or amend the habitat resistance values used in the analysis. GPS data was included as part of the Zecherle *et al.* (2020) study on Asiatic wild ass where it was used to add context to and aid interpretation of the results. Similarly, a review by Müller *et al.* (2023) shows that using GPS/movement data alongside genetic data provides a more robust analytical tool and additional insights that may have been missed otherwise, including identifying the presence of actual and effective barriers in the landscape. The use of GPS data for hedgehog movements may provide similar insights into the conclusions of this study.

4.4. Conservation implications

While this study didn't identify significant correlations between genetic structure and geographic distance, habitat resistance, or the presence of the roads and watercourses, the results do highlight some possible implications for hedgehog conservation efforts. The lack of correlation between genetic relatedness and geographic distance indicates that hedgehogs may travel much further than the 0.7 to 2.5km per night up to 10 to 15km suggested by existing studies (Reeve 1994, Riber 2006, Williams, Stafford & Goodenough 2015, Morris 2018). This suggests that

ecological assessment and conservation efforts need to consider the landscape scale and gene flow between populations across larger distances and areas than may have been considered to date, which are often limited to 1 to 5km depending on the scale of the project. Some species can trigger the implementation of increased distance for impact assessment, which can be up to 30km for some species of bats (Welsh Government 2016a, Welsh Government 2016b), but this is not currently triggered by the presence of hedgehogs. There is a risk that population fragmentation and isolation impacts may be ruled out due to distance from the impact source when such impacts are present so best practice in relation to hedgehogs needs to be reviewed to ensure that appropriate consideration is given to this species.

The lack of correlation with habitat resistance suggests that other factors may be influencing hedgehog movements, such as smaller scale habitats or habitat features which couldn't be incorporated into the study models. It may be that small scale changes and removal of smaller features could have a more significant impact on population fragmentation than larger scale habitat resistance as analysed by this study. Should this be the case replacement, creation, and/or enhancement of smaller scale features could also have greater benefits for reducing population and genetic isolation. This could be achieved by considering small scale measures such as hedgerows, tree-lines, and field margins across a larger landscape scale rather than a site by site basis. This landscape-scale approach has started to be used in conservation, following studies into hedgehogs and other species and the success of agri-environment schemes in supporting wider biodiversity benefits (Donald & Evans 2006, Yarnell & Pettett 2020). It is also being used with increasing frequency within ecological consultancy, for example in relation to bats on the A487 Caernarfon and Bontnewydd Bypass (Welsh Government 2016a, Welsh Government 2016b).

The lack of correlation between genetic structure and the presence of roads and watercourses indicates that these may not pose as much of a barrier to hedgehog movement as earlier studies have suggested (Micol, Doncaster & Mackinlay 1994, Huijser & Bergers 2000, Rondinini & Doncaster 2002, Orłowski & Nowak 2004). While this may be seen as a reason to not implement conservation measures in relation to

these, it shows that any barrier effect they do have may be relatively easy to address with simple approaches rather than providing overly complex crossing features. Such measures could be as simple as providing connecting edge habitats along the sides of such features reconnecting severed habitats (Riber 2006, Hof & Bright 2009, Moorhouse *et al.* 2014), stepping-stone (Fitzgibbon 1997), or similar crossing options within central reservations or across bridges (Clark *et al.* 2001, Moore *et al.* 2020), inclusion of culverts or underpasses beneath roads (Yanes, Velasco & Suárez 1995, Moore *et al.* 2020), use of guidance fencing (Fahrig & Rytwinski 2009, Moore *et al.* 2020), more sensitive management regimes (Coffin 2007, Hof 2009), reduced lighting where possible (Berger *et al.* 2020b), traffic calming measures on smaller roads (Moore *et al.* 2020), and even warning signs and raised awareness for users to be aware of hedgehogs crossing (Hof 2009). These measures would also contribute to reduce the mortality rate associated with roads and hedgehogs, which is a recognised contributor to declining hedgehog numbers in the UK (Wright *et al.* 2020).

5. Conclusion

This study aimed to produce habitat resistance maps and use these to investigate the presence of barriers to hedgehog movements and the implications of these on gene flow. The analysis has shown that:

1. The genetic structure of the sample population had four distinct subpopulations, but this structure was weak, with spatial overlaps and a significant proportion of individuals unassigned to a specific subpopulation.
2. Areas of low habitat suitability (high habitat resistance) did not have a significant influence on the genetic structure of the sample population.
3. Larger landscape features such as roads and watercourses also did not have a barrier effect on hedgehog movement within the sample population.

Several areas for further and future research have been identified to build on the data and results generated by this study. Future research options include building on the current data set with further genetic sampling and including finer scale habitat modelling to include features not included within this study. Real world habitat use

data collected from radio-tracking or similar survey approaches could also be collected and used to confirm or amend the resistance values assigned as part of this study.

6. Appendices

6.1. Appendix B – Habitat Resistance Data

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
A.1.1.1	Broadleaved semi-natural woodland	Positive link to edge habitats	(Huijser & Bergers 2000)	Low	2 to 20	Woodland habitats noted to have low hedgehog densities - boundaries used more
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Used but not as much as gardens and fields	(Rondinini & Doncaster 2002)			
		Extensive use, especially in clearings	(Riber 2006)			
		Second highest observation rate, negative link in greater extents, less so in edge habitats	(Hof & Bright 2012)			
		Positive effect - as linear habitats (edge effect)	(van de Poel, Dekker & Langevelde 2015)			
		Least or second least ranked	(Pettett *et al.* 2017)			
		Negative relationship	(Hof, Allen & Bright 2019)			
		Favourable habitat	(Wright *et al.* 2020)			
		Winter nesting - undergrowth needed	(Reeve 1981)			
		Neither selected for or against but higher than pasture/improved grassland, clearings and edge habitats important	(Hof 2009)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive preference in spring and summer, not so in autumn	(Dowie 1993)			
A.1.1.2	Broadleaved plantation	Positive link to edge habitats	(Huijser & Bergers 2000)	Low-medium	21 to 40	Woodland habitats noted to have low hedgehog densities - boundaries used more
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Used but not as much as gardens and fields	(Rondinini & Doncaster 2002)			
		Extensive use, especially in clearings	(Riber 2006)			
		Second highest observation rate, negative link in greater extents, less so in edge habitats	(Hof & Bright 2012)			
		Positive effect - as linear habitats (edge effect)	(van de Poel, Dekker & Langevelde 2015)			
		Least or second least ranked	(Pettett *et al.* 2017)			
		Negative relationship	(Hof, Allen & Bright 2019)			
		Favourable habitat	(Wright *et al.* 2020)			
		Winter nesting - undergrowth needed	(Reeve 1981)			
		Neither selected for or against but higher than pasture/improved grassland, clearings and edge habitats important	(Hof 2009)			
		Positive preference in spring and summer, not so in autumn	(Dowie 1993)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
A.1.2.1	Coniferous semi-natural woodland	Lowest observation rate, negative link in greater extents, less so in edge habitats	(Hof & Bright 2012)	Medium	41 to 60	Less well used than other woodland habitats, mixed results - edge habitat used more
		Positive effect - as linear habitats (edge effect)	(van de Poel, Dekker & Langevelde 2015)			
		Least or second least ranked	(Pettett *et al.* 2017)			
		High selection	(Hof 2009)			
A.1.2.2	Coniferous plantation	Lowest observation rate, negative link in greater extents, less so in edge habitats	(Hof & Bright 2012)	Medium	41 to 60	Less well used than other woodland habitats, mixed results - edge habitat used more
		Positive effect - as linear habitats (edge effect)	(van de Poel, Dekker & Langevelde 2015)			
		Least or second least ranked	(Pettett *et al.* 2017)			
		High selection	(Hof 2009)			
A.1.3.1	Mixed semi-natural woodland	Highly selected (1 to 5m within habitat)	(Hof & Bright 2010)	Low-medium	21 to 40	Woodland habitats noted to have low hedgehog densities - boundaries used more
		Negative link in greater extents, less so in edge habitats	(Hof & Bright 2012)			
		Positive effect - as linear habitats (edge effect)	(van de Poel, Dekker & Langevelde 2015)			
		Least or second least ranked	(Pettett *et al.* 2017)			
		Neither selected for or against but higher than pasture/improved grassland, clearings and edge habitats important	(Hof 2009)			
A.1.3.2	Mixed plantation	Highly selected (1 to 5m within habitat)	(Hof & Bright 2010)	Low-medium	21 to 40	Woodland habitats noted to have low

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Negative link in greater extents, less so in edge habitats	(Hof & Bright 2012)			hedgehog densities - boundaries used more
		Positive effect - as linear habitats (edge effect)	(van de Poel, Dekker & Langevelde 2015)			
		Least or second least ranked	(Pettett *et al.* 2017)			
		Neither selected for or against but higher than pasture/improved grassland, clearings and edge habitats important	(Hof 2009)			
A.2.1	Dense / continuous scrub	Used for cover	(Rondinini & Doncaster 2002)	Negligible	1	Considered similar to hedgerows? Could depend on extent
		Positive effect - as linear habitats (edge effect)	(van de Poel, Dekker & Langevelde 2015)			
		Confirmed habitat use	(Berger *et al.* 2020a)			
		Positive link	(Yarnell & Pettett 2020)			
		Confirmed use	(Berger *et al.* 2020b)			
		Winter nesting	(Reeve 1981)			
A.2.2	Scattered scrub	Confirmed habitat use	(Berger *et al.* 2020a)	Low	2 to 20	Limited cover but may be used in similar manner to less structured gardens / open habitats, activity focused to edges
A.3.1	Broadleaved parkland / scattered trees	Positive link	(Hof & Bright 2009)	Negligible	1	Hedgehogs reported to use parkland areas
		Confirmed habitat use	(Berger *et al.* 2020a)			
		Confirmed use	(Berger *et al.* 2020b)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
A.3.2	Coniferous parkland / scattered trees	Positive link	(Hof & Bright 2009)	Negligible	1	Hedgehogs reported to use parkland areas
		Confirmed habitat use	(Berger *et al.* 2020a)			
		Confirmed use	(Berger *et al.* 2020b)			
A.3.3	Mixed parkland / scattered trees	Positive link	(Hof & Bright 2009)	Negligible	1	Hedgehogs reported to use parkland areas
		Confirmed habitat use	(Berger *et al.* 2020a)			
		Confirmed use	(Berger *et al.* 2020b)			
A.4.1	Broadleaved recently felled woodland	No references		Low	2 to 20	Open areas but hedgehogs known to use parkland and open spaces, may be limited to edge habitats Proxy = broadleaved woodland and parkland
A.4.2	Coniferous recently felled woodland	No references		Low-medium	21 to 40	Open areas but hedgehogs known to use parkland and open spaces, may be limited to edge habitats Proxy = broadleaved woodland and parkland

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
A.4.3	Mixed recently felled woodland	No references		Low-medium	21 to 40	Open areas but hedgehogs known to use parkland and open spaces, may be limited to edge habitats Proxy = broadleaved woodland and parkland
B.1.1	Unimproved acid grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Margins used but less impact on movement distance	(Moorhouse *et al.* 2014)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			
		Positive correlation	(Hof 2009)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Less preference unless used as pasture	(Dowie 1993)			
B.1.2	Semi-improved acid grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Margins used but less imapct on movement distance	(Moorhouse *et al.* 2014)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			
		Positive correlation	(Hof 2009)			
		Less preference unless used as pasture	(Dowie 1993)			
B.2.1	Unimproved neutral grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Margins used but less imapct on movement distance	(Moorhouse *et al.* 2014)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			
		Neither selected for or against but higher than pasture/improved grassland	(Hof 2009)			
		Positive preference	(Dowie 1993)			
B.2.2	Semi-improved neutral grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Margins used but less imapct on movement distance	(Moorhouse *et al.* 2014)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			
		Neither selected for or against but higher than pasture/improved grassland	(Hof 2009)			
		Positive preference	(Dowie 1993)			
B.3.1	Unimproved calcareous grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Margins used but less imapct on movement distance	(Moorhouse *et al.* 2014)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive correlation, lower nutrients = less inverts = less food = fewer hedgehogs	(Hof 2009)			
		Less preference unless used as pasture	(Dowie 1993)			
B.3.2	Semi-improved calcareous grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Margins used but less imapct on movement distance	(Moorhouse *et al.* 2014)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			
		Positive correlation, lower nutrients = less inverts = less food = fewer hedgehogs	(Hof 2009)			
		Less preference unless used as pasture	(Dowie 1993)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
B.4	Improved grassland (pasture)	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Negative relationship	(Hof, Allen & Bright 2019)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			
		Neither selected for or against	(Hof 2009)			
		Positive preference	(Dowie 1993)			
B.5	Marsh / marshy grassland	Seldom used	(Jackson 2007)	Negligible	1	Pasture and set aside well used
		Highly selected (margins)	(Hof & Bright 2010)			
		Negative effect	(van de Poel, Dekker & Langevelde 2015)			
B.6	Poor semi-improved grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Pasture and set aside well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Scarce in pasture	(Young *et al.* 2006)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Highly selected (margins)	(Hof & Bright 2010)			
		Second highest observation rate	(Hof & Bright 2012)			
		Margins used but less imapct on movement distance	(Moorhouse *et al.* 2014)			
		Middle ranking - changeable	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Less preferred	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		Summer nesting in taller vegetation	(Reeve 1981)			
		Neither selected for or against	(Hof 2009)			
		Less preference unless used as pasture	(Dowie 1993)			
C.1.1	Continuous bracken	Summer nesting in taller vegetation	(Reeve 1981)	Negligible	1	Similar to dense scrub in terms of cover provided
C.1.2	Scattered bracken	No references		Low-medium	21 to 40	Limited cover but may be used in similar manner to less structured gardens / open habitats, activity focused to edges Proxy other habitat

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
C.2	Upland species-rich ledges	No references		High	81 to 98	Unlikely in upland areas, absent above treeline (Williams *et al.* 2018)
C.3.1	Tall ruderal	Highly selected (margins)	(Hof & Bright 2010)	Negligible	1	Similar to dense scrub in terms of cover provided
		Positive link (vegetation cover)	(Yarnell & Pettett 2020)			
C.3.2	Non-ruderal	No references		Negligible	1	Similar to dense scrub in terms of cover provided Proxy dense scrub
D.1.1	Dry dwarf shrub heath - acid	Seldom used	(Jackson 2007)	Low-medium	21 to 40	Research suggests not well used but not necessarily a barrier
		Lowest observation rate	(Hof & Bright 2012)			
D.1.2	Dry dwarf shrub heath - basic	Seldom used	(Jackson 2007)	Low-medium	21 to 40	Research suggests not well used but not necessarily a barrier
		Lowest observation rate	(Hof & Bright 2012)			
D.2	Wet dwarf shrub heath	Seldom used	(Jackson 2007)	Medium-high	61 to 80	Research suggests not well used but not necessarily a barrier, wetter habitats noted to be used less frequently than dry habitats
		Lowest observation rate	(Hof & Bright 2012)			
D.3	Lichen / bryophyte heath	Seldom used	(Jackson 2007)	Medium-high	61 to 80	Research suggests not well used but not necessarily a barrier,
		Lowest observation rate	(Hof & Bright 2012)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
						wetter habitats noted to be used less frequently than dry habitats
D.4	Montane heath / dwarf herb	Seldom used	(Jackson 2007)	Medium	41 to 60	Research suggests not well used but not necessarily a barrier, upland habitats noted to be used in some studies
		Lowest observation rate	(Hof & Bright 2012)			
D.5	Dry heath / acid grassland mosaic	No references		Low-medium	21 to 40	Research suggests not well used but not necessarily a barrier Proxy dry dwarf shrub heath - acid
D.6	Wet heath / acid grassland mosaic	No references		Medium	41 to 60	Research suggests not well used but not necessarily a barrier, wetter habitats noted to be used less frequently than dry habitats Proxy wet dwarf shrub heath and acid grassland

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
E.1.6.1	Blanket bog	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.1.6.2	Raised bog	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.1.7	Wet modified bog	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.1.8	Dry modified bog	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.2.1	Acid / neutral flush / spring	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.2.2	Basic flush / spring	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.2.3	Bryophyte dominated flush / spring	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.3.1	Valley mire fen	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.3.2	Basin mire fen	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.3.3	Flood plain fen	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
E.4	Bare peat	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
F.1	Swamp	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
F.2.1	Marginal vegetation	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
F.2.2	Inundation vegetation	Negative effect	(van de Poel, Dekker & Langevelde 2015)	High	81 to 98	Habitat type unlikely to be used
G.1	Standing water	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Positive but not significant effect on presence	(Hof 2009)			
G.1.1	Eutrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Positive but not significant effect on presence	(Hof 2009)			
G.1.2	Mesotrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Positive but not significant effect on presence	(Hof 2009)			
G.1.3	Oligotrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Positive but not significant effect on presence	(Hof 2009)			
G.1.4	Dystrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Positive but not significant effect on presence	(Hof 2009)			
G.1.5	Marl	Partial barrier	(Hof & Bright 2009)	High	81 to 98	

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive but not significant effect on presence	(Hof 2009)			Hedgehogs known to swim on occasion so not an absolute barrier
G.1.6	Brackish	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Positive but not significant effect on presence	(Hof 2009)			
G.2	Running water	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Negative effect on presence	(Hof 2009)			
G.2.1	Eutrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Negative effect on presence	(Hof 2009)			
G.2.2	Mesotrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Negative effect on presence	(Hof 2009)			
G.2.3	Oligotrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Negative effect on presence	(Hof 2009)			
G.2.4	Dystrophic	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Negative effect on presence	(Hof 2009)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
G.2.5	Marl	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Negative effect on presence	(Hof 2009)			
G.2.6	Brackish	Partial barrier	(Hof & Bright 2009)	High	81 to 98	Hedgehogs known to swim on occasion so not an absolute barrier
		Negative effect on presence	(Hof 2009)			
H.1.1	Intertidal mud / sand	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.1.2	Intertidal shingles / cobbles	No references		High	81 to 98	Habitat type unlikely to be usedProxy sand dune - open
H.1.3	Intertidal boulders / rocks	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.1.(1-2).1	Zostera beds	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
H.1.(1-3).2	Green algal beds	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.1.(1-3).3	Brown algal beds	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.2.3	Saltmarsh / dune interface	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.2.4	Scattered saltmarsh plants	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.2.6	Dense / continuous saltmarsh plants	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
H.3	Shingle above high tide mark	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.4	Boulders / rocks above high tide mark	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.5	Strandline vegetation	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.6.4	Sand dune - dune slack	Foraging	(Jackson 2007)	Medium-high	61 to 80	Low use generally, only 1 study shows use, more due to proximity and presence within home range
H.6.5	Sand dune - dune grassland	Foraging	(Jackson 2007)	Medium-high	61 to 80	Low use generally, only 1 study shows use, more due to proximity and presence within home range

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
H.6.6	Sand dune - dune heath	No references		High	81 to 98	Habitat type unlikely to be used Proxy sand dune - open
H.6.7	Sand dune - dune scrub	Foraging	(Jackson 2007)	Medium-high	61 to 80	Low use generally, only 1 study shows use, more due to proximity and presence within home range
H.6.8	Sand dune - open dune	Not used	(Jackson 2007)	High	81 to 98	Not used
H.8.1	Maritime cliff and slope - hard cliff	No references		High	81 to 98	Habitat type unlikely to be used
H.8.2	Maritime cliff and slope - soft cliff	No references		High	81 to 98	Habitat type unlikely to be used
H.8.3	Maritime cliff and slope - crevice / ledge vegetation	No references		High	81 to 98	Habitat type unlikely to be used

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
H.8.4	Maritime cliff and slope - coastal grassland	No references		Negligible	1	Similar to grassland habitats in terms of potential use
H.8.5	Maritime cliff and slope - coastal heathland	No references		Low-medium	21 to 40	Heathland habitats generally not used
I.1.1.1	Natural inland cliff - acid / neutral	No references		High	81 to 98	Habitat type unlikely to be used
I.1.1.2	Natural inland cliff - basic	No references		High	81 to 98	Habitat type unlikely to be used
I.1.2.1	Natural scree - acid / neutral	No references		High	81 to 98	Habitat type unlikely to be used
I.1.2.2	Natural scree - basic	No references		High	81 to 98	Habitat type unlikely to be used
I.1.3	Natural limestone pavement	No references		Medium-high	61 to 80	Might vary depending on associated habitats
I.1.4.1	Natural other exposure -	No references		High	81 to 98	Habitat type unlikely to be used

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
	acid / neutral					
I.1.4.2	Natural other exposure - basic	No references		High	81 to 98	Habitat type unlikely to be used
I.1.5	Natural cave	No references		High	81 to 98	Habitat type unlikely to be used
I.2.1	Artificial quarry	No references		High	81 to 98	Habitat type unlikely to be used
I.2.2	Artificial spoil	No references		High	81 to 98	Habitat type unlikely to be used
I.2.3	Artificial mine	No references		High	81 to 98	Habitat type unlikely to be used
I.2.4	Artificial refuse tip	No references		High	81 to 98	Habitat type unlikely to be used
J.1.1	Arable	Negative link	(Micol, Doncaster & Mackinlay 1994)	Medium-high	61 to 80	Depends on size of fields but research suggests habitat is not used
		Negative association	(Doncaster, Rondinini & Johnson 2001)			
		Rarely visited	(Riber 2006)			
		Rarely selected	(Hof & Bright 2010)			
		Positive effect	(van de Poel, Dekker & Langevelde 2015)			
		Least or second least ranked	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive relationship	(Hof, Allen & Bright 2019)			
		Positive link (field margins)	(Yarnell & Pettett 2020)			
		Trended to avoid	(Driezen *et al.* 2007)			
		Favourable habitat	(Wright *et al.* 2020)			
		Selected against - use of field margins and hedgerows confuses this (use generally within 5m of edge)	(Hof 2009)			
		Negatively preferred	(Dowie 1993)			
J.1.2	Amenity grassland	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	Amenity grassland noted to be well used
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Preferred	(Rondinini & Doncaster 2002)			
		Lower densities	(Orłowski & Nowak 2004)			
		More abundant	(Young *et al.* 2006)			
		Positive link	(Hof & Bright 2009)			
		Highly selected (females)	(Hof & Bright 2010)			
		Positive effect	(van de Poel, Dekker & Langevelde 2015)			
		Locally high, landscape lower	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			
		Confirmed habitat use	(Berger *et al.* 2020a)			
		Positive link	(Yarnell & Pettett 2020)			
		Strong preference, particularly with structures (bushes, trees etc)	(Braaker *et al.* 2014)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Favourable habitat	(Wright *et al.* 2020)			
		General use	(Reeve 1981)			
		Selected for - edge habitats	(Hof 2009)			
J.1.3	Ephemeral / short perennial	Positive link (vegetation cover)	(Yarnell & Pettett 2020)	Negligible	1	Similar to dense scrub, although less cover provided
		Used	(Hof 2009)			
J.1.4	Introduced shrub (inc INNS)	No references		Negligible	1	Similar to dense scrubProxy dense scrub
J.1.5	Gardens	Preferred habitat	(Micol, Doncaster & Mackinlay 1994)	Low	2 to 20	Boundaries will affect resistance level
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Preferred	(Rondinini & Doncaster 2002)			
		Higher densities	(Orłowski & Nowak 2004)			
		More abundant	(Young *et al.* 2006)			
		High % of shrubs and grass	(Hof & Bright 2009)			
		Strong preference	(Dowding *et al.* 2010)			
		Highly selected (females)	(Hof & Bright 2010)			
		Highest observation rate	(Hof & Bright 2012)			
		Positive effect	(van de Poel, Dekker & Langevelde 2015)			
		Well used - likely under-recorded	(Williams, Stafford & Goodenough 2015)			
		Highest ranked	(Pettett *et al.* 2017)			
		Present	(Williams *et al.* 2018)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive link	(Yarnell & Pettett 2020)			
		Confirmed use	(Berger *et al.* 2020b)			
		Favoured	(Driezen *et al.* 2007)			
		Strong preference, particularly with structures (bushes, trees etc)	(Braaker *et al.* 2014)			
		Favourable habitat	(Wright *et al.* 2020)			
		General use but less than amenity/golf course	(Reeve 1981)			
		Used but limited by connectivity (or lack of)	(Hof 2009)			
		Mixed levels of use	(Dowie 1993)			
		Consistently preferred	(Dowding 2007)			
J.2.1.1	Intact species rich hedge	Positive link	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	
		Positive link to edge habitats	(Huijser & Bergers 2000)			
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Favoured (edge habitats)	(Riber 2006)			
		Highly selected	(Hof & Bright 2010)			
		Positive association	(Hof & Bright 2012)			
		Positive effect	(van de Poel, Dekker & Langevelde 2015)			
		Highly ranked	(Pettett *et al.* 2017)			
		May be attractive	(Hof, Allen & Bright 2019)			
		Positive link	(Yarnell & Pettett 2020)			
		Confirmed use	(Berger *et al.* 2020b)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive correlation	(Hof 2009)			
		Strong preference of use	(Dowie 1993)			
J.2.1.2	Intact species poor hedge	Positive link	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	
		Positive link to edge habitats	(Huijser & Bergers 2000)			
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Favoured (edge habitats)	(Riber 2006)			
		Highly selected	(Hof & Bright 2010)			
		Positive association	(Hof & Bright 2012)			
		Positive effect	(van de Poel, Dekker & Langevelde 2015)			
		Highly ranked	(Pettett *et al.* 2017)			
		May be attractive	(Hof, Allen & Bright 2019)			
		Positive correlation	(Hof 2009)			
		Strong preference of use	(Dowie 1993)			
J.2.2.1	Defunct species rich hedge	No references		Negligible	1	Proxy intact species poor hedge
J.2.2.2	Defunct species poor hedge	No references		Negligible	1	Proxy intact species poor hedge
J.2.3.1	Species rich hedge with trees	Positive link	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	
		Positive link to edge habitats	(Huijser & Bergers 2000)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Favoured (edge habitats)	(Riber 2006)			
		Highly selected	(Hof & Bright 2010)			
		Positive association	(Hof & Bright 2012)			
		Determinant of permeability	(Moorhouse *et al.* 2014)			
		Positive effect	(van de Poel, Dekker & Langevelde 2015)			
		Highly ranked	(Pettett *et al.* 2017)			
		May be attractive	(Hof, Allen & Bright 2019)			
		Positive link	(Yarnell & Pettett 2020)			
		Confirmed use	(Berger *et al.* 2020b)			
		Positive correlation	(Hof 2009)			
		Strong preference of use	(Dowie 1993)			
J.2.3.2	Species poor hedge with trees	Positive link	(Micol, Doncaster & Mackinlay 1994)	Negligible	1	
		Positive link to edge habitats	(Huijser & Bergers 2000)			
		Positive association	(Doncaster, Rondinini & Johnson 2001)			
		Favoured (edge habitats)	(Riber 2006)			
		Highly selected	(Hof & Bright 2010)			
		Positive association	(Hof & Bright 2012)			
		Determinant of permeability	(Moorhouse *et al.* 2014)			
		Positive effect	(van de Poel, Dekker & Langevelde 2015)			
		Highly ranked	(Pettett *et al.* 2017)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		May be attractive	(Hof, Allen & Bright 2019)			
		Positive link	(Yarnell & Pettett 2020)			
		Confirmed use	(Berger *et al.* 2020b)			
		Positive correlation	(Hof 2009)			
		Strong preference of use	(Dowie 1993)			
J.2.4	Fence	No references		Medium	41 to 60	Depends on condition and presence of gaps No proxy
J.2.5	Wall	No references		High	81 to 98	Lower walls could be climbed so pose less of a barrier No proxy
J.2.6	Dry ditch	No references		Negligible	1	Proxy grassland habitats
J.2.7	Boundary removed	No references		Negligible	1	Proxy grassland habitats
J.2.8	Earth bank	No references		Negligible	1	Proxy grassland habitats
J.3.4	Caravan site	No references		Medium-high	61 to 80	Assuming small garden / amenity areas are present amongst caravans, which is common, although often heavily managed and lit

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
						Proxy buildings and track / road
J.3.5	Sea wall	No references		Complete	99	Proxy buildings
J.3.6	Buildings	Highly ranked	(Pettett *et al.* 2017)	Complete	99	Mixed correlations, likely driven by other factors such as gardens, amenity areas, parks etc.
		Positive link - fewer badgers, more food	(Williams *et al.* 2018)			
		Negative relationship	(Hof, Allen & Bright 2019)			
		Favoured	(Driezen *et al.* 2007)			
		Favourable habitat	(Wright *et al.* 2020)			
		Avoided	(Dowie 1993)			
J.3.7	Track / road	Negative link	(Micol, Doncaster & Mackinlay 1994)	Medium	41 to 60	Likely to vary depending on the size of road and traffic levels, adjacent habitats etc.
		Verges used (split into 2)	(Doncaster, Rondinini & Johnson 2001)			
		Less preferred inc verges, reluctance to cross larger roads	(Rondinini & Doncaster 2002)			
		35% lower densities, larger roads bigger barrier effect	(Orłowski & Nowak 2004)			
		Verges used, large roads bigger barrier	(Hof & Bright 2009)			
		Clear aversion but would cross during the night	(Dowding *et al.* 2010)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive for minor roads, negative for major roads	(Hof & Bright 2012)			
		Positive effect - likely linked to lower badger numbers	(van de Poel, Dekker & Langevelde 2015)			
		Positive link - fewer badgers, more food	(Williams *et al.* 2018)			
		Positive link (verges)	(Yarnell & Pettett 2020)			
		Smaller roads no resistence, main streets acted as major barriers	(Braaker *et al.* 2014)			
		Favourable habitat (verges)	(Wright *et al.* 2020)			
		General avoidance	(Reeve 1981)			
		Minor road positively correlated (verge habs), major roads negatively correlated	(Hof 2009)			
		Avoided	(Dowie 1993)			
		Used in nightly ranging, avoided within home ranges, didn't actively avoid crossing	(Dowding 2007)			
J.3.7	Road - including all unpaved and asphalt roads, and railroads.	Negative link	(Micol, Doncaster & Mackinlay 1994)	Low	2 to 20	Likely to vary depending on traffic levels, adjacent habitats etc.
		Verges used (split into 2)	(Doncaster, Rondinini & Johnson 2001)			
		Least preferred	(Rondinini & Doncaster 2002)			
		Lower densities	(Orłowski & Nowak 2004)			
		Verges used	(Hof & Bright 2009)			
		Clear aversion but would cross during the night	(Dowding *et al.* 2010)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive link	(Hof & Bright 2012)			
		Positive effect - likely linked to lower badger numbers	(van de Poel, Dekker & Langevelde 2015)			
		Positive link - fewer badgers, more food	(Williams *et al.* 2018)			
		Positive link (verges)	(Yarnell & Pettett 2020)			
		No resistence	(Braaker *et al.* 2014)			
		Favourable habitat (verges)	(Wright *et al.* 2020)			
		General avoidance	(Reeve 1981)			
		Positively correlated	(Hof 2009)			
		Avoided	(Dowie 1993)			
		Used in nightly ranging, avoided within home ranges, didn't actively avoid crossing	(Dowding 2007)			
J.3.7	Large Road - as above but over 4m wide	Negative link	(Micol, Doncaster & Mackinlay 1994)	High	81 to 98	Likely to vary depending on traffic levels, adjacent habitats etc.
		Verges used (split into 2)	(Doncaster, Rondinini & Johnson 2001)			
		Reluctance to cross	Rondinini & Doncaster 2002			
		Large barrier effect	(Orłowski & Nowak 2004)			
		Large barrier effect	(Hof & Bright 2009)			
		Clear aversion but would cross during the night	(Dowding *et al.* 2010)			
		Negative link	(Hof & Bright 2012)			
		Positive effect - likely linked to lower badger numbers	(van de Poel, Dekker & Langevelde 2015)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		Positive link - fewer badgers, more food	(Williams *et al.* 2018)			
		Positive link (verges)	(Yarnell & Pettett 2020)			
		Barrier effect	(Braaker *et al.* 2014)			
		Favourable habitat (verges)	(Wright *et al.* 2020)			
		General avoidance	(Reeve 1981)			
		Negatively correlated	(Hof 2009)			
		Avoided	(Dowie 1993)			
		Used in nightly ranging, avoided within home ranges, didn't actively avoid crossing	(Dowding 2007)			
J.4	Bare ground	No references		Low-medium	21 to 40	Will be variable depending on what the habitat is and the extent Proxy other habitat
J.5	Other habitat	Waste ground favoured	(Hof 2009)	Low-medium	21 to 40	Will be variable depending on what the habitat is
		Least selected	(Dowding 2007)			
	Other notes	Positive link with uplands, explained by roads and badgers	(Hof & Bright 2012)			
		Absent above tree line	(Williams *et al.* 2018)			
		Avoided high light intensity	(Berger *et al.* 2020a)			
		Food availability and connectivity key drivers to presence, larger	(Yarnell & Pettett 2020)			

Habitat Code	Habitat Type	Summary of References	References	Typical Resistance Level	Typical Resistance Value (0 to 99)	Notes
		field sizes likely to hinder movement				
		Adjusted behaviour in urban areas, able to respond to temporary disturbance	(Berger *et al.* 2020b)			
		Connectivity is less important than quality, although connectivity issues create pinch points	(Braaker *et al.* 2014)			
		Preference for cover over open habs in first year, reverse in second year (due to lack of radio tracking, biased by ability to see animals), clear use of edge habitats even when in open areas	(Reeve 1981)			
		Selected for upland habs (downs, moors, heaths), agri-environment schemes small positive effect	(Hof 2009)			
		Habitat use dictated by need to foraging - need to fill stomachs 2/3 times per night	(Dowding 2007)			

6.2. Appendix B – R Code

```
# Masters analysis August 2023
# data location D:\Research Project\Thesis Work\forR
# set through file menu

# load vegan and ape packages
# load ggplot2

# create explanatory variables from resistance matrices (Circuitscape data) using
weighted PCoA

# import explanatory data as matrices
# note: hab labels contain number ones not lowercase letter L

distance<-read.table("distance.txt", header=TRUE, row.names=1)
habg1<-read.table("habg1.txt", header=TRUE, row.names=1)
habg11<-read.table("habg11.txt", header=TRUE, row.names=1)
habg31<-read.table("habg31rf.txt", header=TRUE, row.names=1)
habg51<-read.table("habg51.txt", header=TRUE, row.names=1)
habg71<-read.table("habg71.txt", header=TRUE, row.names=1)
habg91<-read.table("habg91.txt", header=TRUE, row.names=1)
habg99<-read.table("habg99.txt", header=TRUE, row.names=1)
road<-read.table("roads.txt", header=TRUE, row.names=1)
water<-read.table("water.txt", header=TRUE, row.names=1)

#########################################################################

# import response data as matrices (pairwise genetic info generated through
GenAlEx)
# LRM - Lynch & Ritland (1999) estimator - Mean
# QGM - Queller and Goodnight (1989) estimator - Mean

pwiselrm<-read.table("pairwiselrm.txt", header=TRUE, row.names=1)
pwiseqgm<-read.table("pairwiseqgm.txt", header=TRUE, row.names=1)

# transform into distance matrices

distancea<-as.dist(distance)
habg1a<-as.dist(habg1)
habg11a<-as.dist(habg11)
habg31a<-as.dist(habg31)
habg51a<-as.dist(habg51)
habg71a<-as.dist(habg71)
habg91a<-as.dist(habg91)
habg99a<-as.dist(habg99)
roada<-as.dist(road)
```

```
watera<-as.dist(water)
pwiselrma<-as.dist(pwiselrm)
pwiseqgma<-as.dist(pwiseqgm)

#########################################################################

# PCoAs

# perform PCoA for each explanatory variable (distance, habitats, roads, and water)

pcoadist<-pcoa(distancea, correction="lingoes", rn=NULL)
pcoahabg1<-pcoa(habg1a, correction="lingoes", rn=NULL)
pcoahabg11<-pcoa(habg11a, correction="lingoes", rn=NULL)
pcoahabg31<-pcoa(habg31a, correction="lingoes", rn=NULL)
pcoahabg51<-pcoa(habg51a, correction="lingoes", rn=NULL)
pcoahabg71<-pcoa(habg71a, correction="lingoes", rn=NULL)
pcoahabg91<-pcoa(habg91a, correction="lingoes", rn=NULL)
pcoahabg99<-pcoa(habg99a, correction="lingoes", rn=NULL)
pcoaroad<-pcoa(roada, correction="lingoes", rn=NULL)
pcoawater<-pcoa(watera, correction="lingoes", rn=NULL)

pcoadist$values
pcoahabg1$values
pcoahabg11$values
pcoahabg31$values
pcoahabg51$values
pcoahabg71$values
pcoahabg91$values
pcoahabg99$values
pcoaroad$values
pcoawater$values

#########################################################################

# identify number of PCos to retain - those above red line
# habitats code amended to reflect pcoa columns, bar [,3] and line [,4]

# distance

df.bar<-barplot(pcoadist$values[,2], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoadist$values[,3], col='red')

# habitatsGL1

df.bar<-barplot(pcoahabg1$values[,3], xlab="PCos", ylab="percentage variation
explained")
```

```
lines(x=df.bar, y=pcoahabg1$values[,4], col='red')

# habitatsGL11

df.bar<-barplot(pcoahabg11$values[,3], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoahabg11$values[,4], col='red')

# habitatsGL31

df.bar<-barplot(pcoahabg31$values[,3], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoahabg31$values[,4], col='red')

# habitatsGL51

df.bar<-barplot(pcoahabg51$values[,3], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoahabg51$values[,4], col='red')

# habitatsGL71

df.bar<-barplot(pcoahabg71$values[,3], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoahabg71$values[,4], col='red')

# habitatsGL91

df.bar<-barplot(pcoahabg91$values[,3], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoahabg91$values[,4], col='red')

# habitatsGL99

df.bar<-barplot(pcoahabg99$values[,3], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoahabg99$values[,4], col='red')

# roads

df.bar<-barplot(pcoaroad$values[,2], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoaroad$values[,3], col='red')

# water
```

```
df.bar<-barplot(pcoawater$values[,2], xlab="PCos", ylab="percentage variation
explained")
lines(x=df.bar, y=pcoawater$values[,3], col='red')

#########################################################################

# variation explained by first PCos, relative eigenvalues / relative correlated evs for
habs
# habitats code amended to reflect pcoa columns, [,3]

sum(pcoadist$values[1:1,2])
sum(pcoahabg1$values[1:1,3])
sum(pcoahabg11$values[1:1,3])
sum(pcoahabg31$values[1:1,3])
sum(pcoahabg51$values[1:1,3])
sum(pcoahabg71$values[1:1,3])
sum(pcoahabg91$values[1:1,3])
sum(pcoahabg99$values[1:1,3])
sum(pcoaroad$values[1:1,2])
sum(pcoawater$values[1:1,2])

#########################################################################

# variation explained by first 4 PCos, relative eigenvalues / relative correlated evs
for habs
# habitats code amended to reflect pcoa columns, [,3]

sum(pcoadist$values[1:4,2])
sum(pcoahabg1$values[1:4,3])
sum(pcoahabg11$values[1:4,3])
sum(pcoahabg31$values[1:4,3])
sum(pcoahabg51$values[1:4,3])
sum(pcoahabg71$values[1:4,3])
sum(pcoahabg91$values[1:4,3])
sum(pcoahabg99$values[1:4,3])
sum(pcoaroad$values[1:4,2])
sum(pcoawater$values[1:4,2])

#########################################################################

# variation explained by first 9 PCos, relative eigenvalues / relative correlated evs
for habs
# habitats code amended to reflect pcoa columns, [,3]

sum(pcoadist$values[1:9,2])
sum(pcoahabg1$values[1:9,3])
sum(pcoahabg11$values[1:9,3])
```

```
sum(pcoahabg31$values[1:9,3])
sum(pcoahabg51$values[1:9,3])
sum(pcoahabg71$values[1:9,3])
sum(pcoahabg91$values[1:9,3])
sum(pcoahabg99$values[1:9,3])
sum(pcoaroad$values[1:9,2])
sum(pcoawater$values[1:9,2])

#########################################################################

# variation explained by first 10 PCos, relative eigenvalues / relative correlated evs
for habs
# habitats code amended to reflect pcoa columns, [,3]

sum(pcoadist$values[1:10,2])
sum(pcoahabg1$values[1:10,3])
sum(pcoahabg11$values[1:10,3])
sum(pcoahabg31$values[1:10,3])
sum(pcoahabg51$values[1:10,3])
sum(pcoahabg71$values[1:10,3])
sum(pcoahabg91$values[1:10,3])
sum(pcoahabg99$values[1:10,3])
sum(pcoaroad$values[1:10,2])
sum(pcoawater$values[1:10,2])

# create matrices from first 1/10 (corrected) PCos

dist<-(pcoadist$vectors[,1:10])
habsg1<-(pcoahabg1$vectors[,1:10])
habsg11<-(pcoahabg11$vectors[,1:10])
habsg31<-(pcoahabg31$vectors[,1:10])
habsg51<-(pcoahabg51$vectors[,1:10])
habsg71<-(pcoahabg71$vectors[,1:10])
habsg91<-(pcoahabg91$vectors[,1:10])
habsg99<-(pcoahabg99$vectors[,1:10])
roads<-(pcoaroad$vectors[,1:10])
waters<-(pcoawater$vectors[,1:10])

#########################################################################

# run dbRDA models - one per explanatory variable

# set 1 - LRM pairwise models (ma)
# pairwise matrix as response variable - distance matrices created at start
# distance, habitats, roads, and water as explanatory variables - PCos matrices
created above
```

```
# ma1 - pairwise and distance

ma1<-capscale(pwiselrma~dist, add="lingoes")
print(ma1)
anova.cca(ma1,permutations=how(nperm=9999))

# ma2 - pairwise and habitatGL1

ma2<-capscale(pwiselrma~habsg1, add="lingoes")
print(ma2)
anova.cca(ma2,permutations=how(nperm=9999))

# ma3 - pairwise and habitatGL11

ma3<-capscale(pwiselrma~habsg11, add="lingoes")
print(ma3)
anova.cca(ma3,permutations=how(nperm=9999))

# ma4 - pairwise and habitatGL31

ma4<-capscale(pwiselrma~habsg31, add="lingoes")
print(ma4)
anova.cca(ma4,permutations=how(nperm=9999))

# ma5 - pairwise and habitatGL51

ma5<-capscale(pwiselrma~habsg51, add="lingoes")
print(ma5)
anova.cca(ma5,permutations=how(nperm=9999))

# ma6 - pairwise and habitatGL71

ma6<-capscale(pwiselrma~habsg71, add="lingoes")
print(ma6)
anova.cca(ma6,permutations=how(nperm=9999))

# ma7 - pairwise and habitatGL91

ma7<-capscale(pwiselrma~habsg91, add="lingoes")
print(ma7)
anova.cca(ma7,permutations=how(nperm=9999))

# ma8 - pairwise and habitatGL99

ma8<-capscale(pwiselrma~habsg99, add="lingoes")
print(ma8)
anova.cca(ma8,permutations=how(nperm=9999))
```

```
# ma9 - pairwise and roads

ma9<-capscale(pwiselrma~roads, add="lingoes")
print(ma9)
anova.cca(ma9,permutations=how(nperm=9999))

# ma10 - pairwise and water

ma10<-capscale(pwiselrma~waters, add="lingoes")
print(ma10)
anova.cca(ma10,permutations=how(nperm=9999))

# ma11 - pairwise and roads

ma11<-capscale(pwiselrma~roads+Condition(dist), add="lingoes")
print(ma11)
anova.cca(ma11,permutations=how(nperm=9999))

# ma12 - pairwise and water

ma12<-capscale(pwiselrma~waters+Condition(dist), add="lingoes")
print(ma12)
anova.cca(ma12,permutations=how(nperm=9999))

# ma13 - pairwise and habitatGL1

ma13<-capscale(pwiselrma~habsg1+Condition(dist), add="lingoes")
print(ma13)
anova.cca(ma13,permutations=how(nperm=9999))

# ma14 - pairwise and habitatGL11

ma14<-capscale(pwiselrma~habsg11+Condition(dist), add="lingoes")
print(ma14)
anova.cca(ma14,permutations=how(nperm=9999))

# ma15 - pairwise and habitatGL31

ma15<-capscale(pwiselrma~habsg31+Condition(dist), add="lingoes")
print(ma15)
anova.cca(ma15,permutations=how(nperm=9999))

# ma16 - pairwise and habitatGL51

ma16<-capscale(pwiselrma~habsg51+Condition(dist), add="lingoes")
print(ma16)
```

```
anova.cca(ma16,permutations=how(nperm=9999))

# ma17 - pairwise and habitatGL71

ma17<-capscale(pwiselrma~habsg71+Condition(dist), add="lingoes")
print(ma17)
anova.cca(ma17,permutations=how(nperm=9999))

# ma18 - pairwise and habitatGL91

ma18<-capscale(pwiselrma~habsg91+Condition(dist), add="lingoes")
print(ma18)
anova.cca(ma18,permutations=how(nperm=9999))

# ma19 - pairwise and habitatGL99

ma19<-capscale(pwiselrma~habsg99+Condition(dist), add="lingoes")
print(ma19)
anova.cca(ma19,permutations=how(nperm=9999))

# ma20 - pairwise and all explanatory variables (imp grass = 31)

ma20<-capscale(pwiselrma~habsg31+dist+roads+waters, add="lingoes")
print(ma20)
anova.cca(ma20,permutations=how(nperm=9999))

#########################################################################

# run dbRDA models - one per explanatory variable

# set 2 - QGM pairwise models (mb)
# pairwise matrix as response variable - distance matrices created at start
# distance, habitats, roads, and water as explanatory variables - PCos matrices
created above

# mb1 - pairwise and distance

mb1<-capscale(pwiseqgma~dist, add="lingoes")
print(mb1)
anova.cca(mb1,permutations=how(nperm=9999))

# mb2 - pairwise and habitatGL1

mb2<-capscale(pwiseqgma~habsg1, add="lingoes")
print(mb2)
anova.cca(mb2,permutations=how(nperm=9999))
```

```
# mb3 - pairwise and habitatGL11

mb3<-capscale(pwiseqgma~habsg11, add="lingoes")
print(mb3)
anova.cca(mb3,permutations=how(nperm=9999))

# mb4 - pairwise and habitatGL31

mb4<-capscale(pwiseqgma~habsg31, add="lingoes")
print(mb4)
anova.cca(mb4,permutations=how(nperm=9999))

# mb5 - pairwise and habitatGL51

mb5<-capscale(pwiseqgma~habsg51, add="lingoes")
print(mb5)
anova.cca(mb5,permutations=how(nperm=9999))

# mb6 - pairwise and habitatGL71

mb6<-capscale(pwiseqgma~habsg71, add="lingoes")
print(mb6)
anova.cca(mb6,permutations=how(nperm=9999))

# mb7 - pairwise and habitatGL91

mb7<-capscale(pwiseqgma~habsg91, add="lingoes")
print(mb7)
anova.cca(mb7,permutations=how(nperm=9999))

# mb8 - pairwise and habitatGL99

mb8<-capscale(pwiseqgma~habsg99, add="lingoes")
print(mb8)
anova.cca(mb8,permutations=how(nperm=9999))

# mb9 - pairwise and roads

mb9<-capscale(pwiseqgma~roads, add="lingoes")
print(mb9)
anova.cca(mb9,permutations=how(nperm=9999))

# mb10 - pairwise and water

mb10<-capscale(pwiseqgma~waters, add="lingoes")
print(mb10)
anova.cca(mb10,permutations=how(nperm=9999))
```

```
# mb11 - pairwise and roads

mb11<-capscale(pwiseqgma~roads+Condition(dist), add="lingoes")
print(mb11)
anova.cca(mb11,permutations=how(nperm=9999))

# mb12 - pairwise and water

mb12<-capscale(pwiseqgma~waters+Condition(dist), add="lingoes")
print(mb12)
anova.cca(mb12,permutations=how(nperm=9999))

# mb13 - pairwise and habitatGL1

mb13<-capscale(pwiseqgma~habsg1+Condition(dist), add="lingoes")
print(mb13)
anova.cca(mb13,permutations=how(nperm=9999))

# mb14 - pairwise and habitatGL11

mb14<-capscale(pwiseqgma~habsg11+Condition(dist), add="lingoes")
print(mb14)
anova.cca(mb14,permutations=how(nperm=9999))

# mb15 - pairwise and habitatGL31

mb15<-capscale(pwiseqgma~habsg31+Condition(dist), add="lingoes")
print(mb15)
anova.cca(mb15,permutations=how(nperm=9999))

# mb16 - pairwise and habitatGL51

mb16<-capscale(pwiseqgma~habsg51+Condition(dist), add="lingoes")
print(mb16)
anova.cca(mb16,permutations=how(nperm=9999))

# mb17 - pairwise and habitatGL71

mb17<-capscale(pwiseqgma~habsg71+Condition(dist), add="lingoes")
print(mb17)
anova.cca(mb17,permutations=how(nperm=9999))

# mb18 - pairwise and habitatGL91

mb18<-capscale(pwiseqgma~habsg91+Condition(dist), add="lingoes")
print(mb18)
```

```
anova.cca(mb18,permutations=how(nperm=9999))

# mb19 - pairwise and habitatGL99

mb19<-capscale(pwiseqgma~habsg99+Condition(dist), add="lingoes")
print(mb19)
anova.cca(mb19,permutations=how(nperm=9999))

# mb20 - pairwise and all explanatory variables (imp grass = 31)

mb20<-capscale(pwiseqgma~habsg31+dist+roads+waters, add="lingoes")
print(mb20)
anova.cca(mb20,permutations=how(nperm=9999))

#########################################################################

# extract adjusted R2 for all models

RsquareAdj(ma1)
RsquareAdj(ma2)
RsquareAdj(ma3)
RsquareAdj(ma4)
RsquareAdj(ma5)
RsquareAdj(ma6)
RsquareAdj(ma7)
RsquareAdj(ma8)
RsquareAdj(ma9)
RsquareAdj(ma10)
RsquareAdj(ma11)
RsquareAdj(ma12)
RsquareAdj(ma13)
RsquareAdj(ma14)
RsquareAdj(ma15)
RsquareAdj(ma16)
RsquareAdj(ma17)
RsquareAdj(ma18)
RsquareAdj(ma19)
RsquareAdj(ma20)

RsquareAdj(mb1)
RsquareAdj(mb2)
RsquareAdj(mb3)
RsquareAdj(mb4)
RsquareAdj(mb5)
RsquareAdj(mb6)
RsquareAdj(mb7)
RsquareAdj(mb8)
```

```
RsquareAdj(mb9)
RsquareAdj(mb10)
RsquareAdj(mb11)
RsquareAdj(mb12)
RsquareAdj(mb13)
RsquareAdj(mb14)
RsquareAdj(mb15)
RsquareAdj(mb16)
RsquareAdj(mb17)
RsquareAdj(mb18)
RsquareAdj(mb19)
RsquareAdj(mb20)

##########################################################################

# END
```

6.3. Appendix C –Health and Safety Documentation

Risk Assessment – COVID-19 in the lab environment			
College/ PSU	College of Science	**Assessment Date**	15/09/21
Location (Building / Lab Number)	Wallace	**Assessor**	Dr Hazel Nichols
Activities	See individual risk assessments	**Review Date (if applicable)**	
Associated documents	Individual risk assessments for each activity are stored in the lab		

Part 1: Risk Assessment - COVID-19 controls

Generic COVID-19 controls for labs are detailed below. This document has been amended to reflect the controls required in this lab. Where a number of research groups share a laboratory, co-ordinating your activities will be required. Supervisors / Principle Investigators are responsible for implementing the controls and monitoring work in the lab. You should also review the risk assessments of the activites that you are carrying out, to consider the additional risk of COVID-19. All lab users are to follow the controls identified below.

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
Potential contact with the COVID-19 through contact with an infected person – this person may be symptomatic or asymptomatic.	Staff , Students, visitors, contractors Members of their household	• Worst-case infection can be fatal. • Illness of varying degrees. • Some staff / students may be at higher risk from coronavirus (including older	**Eliminate / reduce** • Staff / students should work from home where possible. See University - Homeworking guidance • Returning staff / students must have be authorised prior to attending campus. Permits will be issued. **Vulnerable groups:** • Staff to complete the HR return to work if you are categorised as at higher risk from coronavirus. Staff who live with someone who is Clinically Extremely Vulnerable or Clinically	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
		people, people with health conditions and pregnant persons). Refer to NHS website.	Vulnerable should contact their line manager / supervisor prior to their return. **Training** • Returning staff / students must complete the Health and Safety Covid Recovery Induction (on Canvas) to reduce the risk of infection. • Staff / students will receive a local induction to familarise them with the additional controls required within their lab to reduce the risk of Covid-19. **Ill-health**: • Covid-19 symptoms include (see NHS website): • • New continuous cough • • High temperature • • Loss of or change to sense of smell or taste. • Staff / students who experience any of these symptoms must not travel to or attend the workplace. They must self-isolate at home and inform their supervisor / manager that they have coronavirus symptoms; then follow NHS Wales advise and Test, Trace and Protect. **Self-isolating:** • If staff / student lives with others *and someone in the household has symptoms of coronavirus or if you have been asked to self-isolate by the NHS (Test, Trace and Protect)*, they must not travel to or attend the workplace. They must self-isolate and not leave the house for 14 days. Individuals should continue to work from	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
			home and inform their line manager. / supervisor.	
Suspected case of Covid-19 in the workplace or suspicion of own infection	Staff - individual	Infection and spreading of virus	• If a person in work becomes symptomatic whilst in work, they should avoid touching anything and return home, where they should follow NHS advise. The line manager / supervisor must be informed. Clean and disinfect any surfaces or equipment the person has come in to contact with. Arrange for safe cleaning of the lab coat. Anyone who may have come in to contact with the person showing symptoms should wash their hands for 20 seconds. NHS Wales Test, Trace and Protect should be used to identify other contacts who may need to self-isolate.	
Contact with the virus whilst travelling to work.			• Staff / students travelling to campus should understand the need to observe social distancing when travelling to and from work. Where possible staff / students should travel alone or with their household group. Refer to H&S information sheet - Travelling on Public Transport and - Travelling in private or other vehicles	
COVID-19, as a result of sharing a lab, bench-space or lab equipment.	Staff /students	Spread of infection through close contact with others, or touching benchspace or equipment that has been contaminated – then touching mouth/ eye/ nose.	• The maximum capacity of the Lab is 4. This maximum capacity must not be exceeded. • All users are to maintain 2 metre social distancing while working in the lab. • Visitors to the lab should be minimized. Use of phone, email, video conferencing.	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
			• Staff / students will be assigned designated bench-spaces. These will be socially distanced to maintain a minimum separation distance of 2 metres. Working side to side, or back to back is preferred. • Where bench-spaces are shared, each will be shared with the minimum number of users. All bench spaces must be cleaned and disinfected before and after use. All staff / students will maintain good levels of personal hygiene, this will include – • Frequent washing of hands, including when you arrive and leave the lab (washing hands with soap and water often for at least 20 seconds using soap and water). See University guidance Hand washing and sanitizing • Avoid of touching eyes, nose and mouth with unwashed hands. • Catching sneezes and coughs in tissues / arm and to wash hands for 20 seconds. • There is a hand washing station in the lab with soap, access to hot and cold water and paper towels – use the Estates helpdesk if soap or paper towels are unavailable. Where a hand wash station is not available hand sanitizer will be provided. We will ensure the maximum number of lab users is not exceeded by:	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
			☒ Pre-booking ☐ Using a student / staff rota ☐ Use fixed shifts, to minimize the number of people you come into contact with. Other - Identify other actions required in this lab to maintain 2 metre social distancing: ☐ *One way system* ☐ *Use of floor tape (2m separation)* ☐ *Tape designating safe bench-spaces* ☐ *Work at alternate fume cupboards* ☐ *Move frequently used equipment to a safe, socially distanced space* ☐ Installing barriers *Other:* There should only be one user within each working bay for each booking slot. Sign up sheets at each bay already exist. Users should also check that no other users are signed up for equipment in that bay for their scheduled time. For further information see Social distancing and shared spaces for further advice The following high touch points have been identified and are to be cleaned regularly: - Door handle	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
			- Bench tops in working bays - Taps at hand wash stations - Refridgerator / freezer / chemical cabinet handles - Fume cupboards controls & railing - Light switch - High use shared equipment within W131A: Nanodrop, Autoclave, Gel Doc, PCR machine lids & Control, Water Purification station, Centrifuges, PCR cabinet controls, BioAnalyzer, QPCR machine & keyboard • • Personal protective equipment must not be shared e.g. lab coats, safety glasses, gloves. Lab coats should be stored separately and must be cleaned regularly (they should not be taken home to clean); contact Hilary Williams for further information. Gloves should be changed frequently and hands washed after use. • If face coverings are used, users should be aware of their correct use and limitations - Face coverings • Identify shared equipment. Minimise the use of shared equipment. All shared equipment is to be cleaned and disinfected before and after use. The disinfectant to be used is: Distel or BioCleanse. 70% Ethanol (used for sanitizing tools before molecular work) should not be used as a regular or primary disinfectant.	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
Risk of exposure and spread of COVID-19 to other personnel when training / demonstating or observing persons working in lab			• Consider providing detailed instructions / video demonstratons. • Minimise contact the time. • Face to face teaching should be avoided, work side by side. • Wear face coverings if 2 metre social distancing cannot be maintained.	
Lone working			• Lone working must not be undertaken where there a reasonably foreseeable risk that the work might result in an adverse event or emergency, which would be sufficiently serious to require a second person to be available to summon help or provide assistance. • A lone working risk assessment is to be completed if lone working cannot be avoided. Refer to the University's lone working policy - https://staff.swansea.ac.uk/healthsafety/policies-and-procedures/general-health-and-safety/ • Lone workers should use SafeZone	No work is planned outside of normal working hours. Students will work under direct supervision, or on their own after suitable training and assessment of their competencies.
Work equipment that has not been maintained / tested	Lab users	Failure of equipment	• Checks are to be made that equipment remains safe to use and that any equipment that requires statutory testing have been tested (e.g. fume cupboards / autoclaves / gas regulators / lifting equipment / portable electric equipment).	
Emergencies – Reduced staffing on Campus / within buildings, first aiders.	All lab users	No first aiders available due to reduced staff presence, delaying emergency first aid treatment.	*First aid* • Lab users should identify the location of their nearest first aider. • In the event of an emergency use 333 from a landline, or use SafeZone https://www.safezoneapp.com/how-it-works.	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
Potential for contact with COVID-19 when giving first aid Fire: Covid directional signs, slowling down the safe evacuation of the building.		Difficulty social distancing when administering first aid Staff not evacuating the building quickly, due to covid directional signs. Staff may congregate at fire assembly points, without social distancing.	• First aiders are to be made aware of new guidance for first aiders • First aiders to follow new Guidance for first aiders This includes new guidance for CPR. • In the event of a fire alarm, staff and students should evacuate the building through *nearest* exit. *Covid directional signs should not be followed in an emergency.* • Once outside and a safe distance away from the building staff / students should not congregate at the fire evacuation point, they should maintain social distancing.	
Adverse Impact on mental health and wellbeing	Staff / Student	Adverse mental health leading to sickness absence or detrimental effect on work and weelbeing	• Regular contact with line manage / supervisor and colleagues • Offer flexible working arrangements where possible • Signpost staff to mental health assistance and professional mental health services should they require them See University Guidance - Health and Wellbeing	

Part 2: Actions arising from risk assessment

Actions	Lead	Target Date	Done Yes/No

Lab users – who require a permit

Name	Staff / student ID
Dr Hazel Nichols	
Samantha Shove	

Swansea University Prifysgol Abertawe | HEALTH & SAFETY IECHYD A DIOGELWCH

RISK ASSESSMENT OF AN ACTIVITY INVOLVING DELIBERATE WORK WITH PATHOGENIC MICROORGANISMS OR SAMPLES WITH POTENTIAL TO HARBOUR PATHOGENIC MICROORGANIMS

This risk assessment form should be used to assist in the assessment of risks from an activity involving deliberate work with an infectious of harmful biological agent. The aim of the assessment is to identify those at risk from infection or other harm and the measures required to eliminate or control the risks to human health and the environment to an acceptable level.

SECTION 1: PROJECT INFORMATION

1.1 Principal Investigator/Academic Supervisor

Name	College
Dr Hazel Nichols	**College of Science**

1.2 Person undertaking this risk assessment (if different from above)

Name	College
Click or tap here to enter text.	Click or tap here to enter text.

1.3 Project title

Understanding sociality in the banded mongoose (Mungos mungo), dwarf mongoose (Helogale parvula), Onager (Equus hemionus), mole-rats (Bathyergidae) and European hedgehogs (Erinaceus europaeus)

1.4 Brief overview of the work *(in layman's terms)*

This project does NOT involve deliberated use of pathogens and will NOT result in an accidental propagation and concentration of pathogenic microorganisms.

Banded mongoose

DNA will be extracted from samples of tissue (e.g., skin) and blood that were collected **from wild banded mongooses in Uganda**. Samples will be stored and maintained in 96% ethanol at 4°C or -20°C in spark proof refrigerators and freezers. Extracted DNA will be analysed using PCR. The resulting data will be combined with behavioural data collected in the field and used to further our understanding of the evolution of social behaviour.

Tissue (skin) and blood samples are i) obtained from a study population that has been observed daily since 1995, and they are not derived from animals known or suspected to be infected with a pathogen which causes a notifiable disease (ii) the samples do not originate from animals in a premises or region or zone of a country that is subject to official restrictions due to a notifiable disease to which the animals are susceptible according to European or other National Animal Health Regulations. Samples are shipped to Swansea for DNA extraction and analyses with a global logistics company. All samples will be shipped in leak proof, impervious, lidded and labelled containers/tubes. On receipt, samples will be inspected for potential damage. Until further processing/analyses, tissue samples will be stored at 4°C or -20°C under Containment Level 2 conditions (Wallace 044). All procedures will follow Good Laboratory Practice and Good Occupational Safety and Hygiene procedures commensurate with HSE guidelines.

Banded mongooses can be infected with two pathogens: leptospirosis and the newly discovered *Mycobacterium mungi,* a member of the *Mycobacterium tuberculosis* complex. However, these two disease-causing agents are not known to be present in our study population (note that this population

has been observed daily for the past 20+ years). In addition, tissue and blood samples **are preserved in 96% ethanol**, **which kills almost all pathogenic and non-pathogenic microbes** (including, *Mycobacterium mungi* and *Leptospira interrogans* the causative agents of tuberculosis and letospirosis) **after 1 minute of exposure**. Samples are never taken from animals known or suspected to be infected with a transmissible disease.

Dwarf mongoose

I will extract DNA from a dwarf mongoose blood sample (stored in herapin) to conduct downstream genetic analyses. The sample is from Chester Zoo in the UK. The individual was observed daily by zoo staff and was not known or suspected to have any transmissible diseases.

Onager

DNA will be extracted from blood and tissue from captive Onagers housed within the EU (Chester and Emmen Zoo). The individuals were observed daily by zoo staff and were not known or suspected to have any transmissible diseases. Samples are stored in 96% ethanol, which kills almost all pathogenic microbes.

Mole-rats

DNA will be extracted from non-invasive skin swabs obtained from captive and wild mole-rats. The mole-rats were located in laboratories at Pretoria University and the Kalahari Research Centre in South Africa. The majority of individuals are captive bred, but some are wild caught under appropriate licences. All were monitored daily by project staff and were not known or suspected to have any transmissible diseases. Samples are stored in 96% ethanol, which kills almost all pathogenic microbes.

Hedgehogs

DNA will be extracted from saliva and tissue samples taken by the Gower Bird Hospital. The buccal swabs are taken from live animals and stored in ethanol or frozen at -20°C, while the tissue samples are taken from the tails or ears of recently deceased hedgehogs and stored in 96% ethanol.

The diseases and parasites that wild hedgehogs carry are generally of little concern to humans. However, there is a risk of exposure to a small number including *Leptospira icterohaemorrhagiae*, the bacterium that causes Weil's disease, and methicillin-resistant Staphylococcus aureus (MRSA). The infection risk from hedgehog-borne MRSA to humans is very low, with no evidence of infection in rescue-centre workers who regularly come into contact with wild hedgehogs[1].

To minimise risk of transfer (1) tissue samples will be stored in 96% ethanol, which destroys almost all pathogenic organisms after 1 minute of exposure (2) all samples will be treated with proteinase-K which breaks down cell walls and hence destroys microorganisms (3) no samples will be collected from any animals suspected of carrying potentially zoonotic diseases and (4) we will apply Containment Level 2 conditions (Wallace 044) to all samples and following the Good Laboratory Practice and Good Occupational Safety and Hygiene procedures commensurate with HSE guidelines.

1. Rasmussen, S.L., Larsen, J., van Wijk, R.E., Jones, O.R., Berg, T.B., Angen, Ø. and Larsen, A.R., 2019. European hedgehogs (Erinaceus europaeus) as a natural reservoir of methicillin-resistant Staphylococcus aureus carrying mecC in Denmark. PloS one, 14(9), p.e0222031.

General

Risk of exposure to microorganisms through working with these samples is very unlikely and it will be further reduced by using disinfectants such as 2% Biocleanse to clean spatulas, glassware, and spills. In addition, the use of the Swansea University Wallace Building Cat 2 facility will provide an extra containment and clear routes for safe disposal if necessary.

SECTION 2: IDENTIFICATION OF BIOLOGICAL HAZARDS			
2.1 List microorganisms deliberately used			
Name of microorganism	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Identified as human pathogen on ACDP list[1]	Choose an item.	Choose an item.	Choose an item.
If yes please state hazard group	Choose an item.	Choose an item.	Choose an item.
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Normal routes of human infection	☐ Inhalation ☐ Oral/ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g. insect) ☐Allergen	☐ Inhalation ☐ Oral/ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g. insect) ☐Allergen	☐ Inhalation ☐ Oral/ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g. insect) ☐Allergen
Multiplicity of infection if known (i.e. number of organisms required to establish an infection)	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Consequence of infection to humans	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Is the microorganism a specified animal pathogen (SAPO[2])	Choose an item.	Choose an item.	Choose an item.
If yes please state SAPO hazard group	Choose an item.	Choose an item.	Choose an item.
Detail of any other harm the microorganism may pose to the environment? e.g. harmful to plants, insects etc.	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Consequence of spread in environment	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Route of transmission for environmental pathogens (including animals)	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Any additional risk to health/environment e.g. hyper	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.

virulence, multiple antibiotic resistance			
Listed on Schedule 5[3]	Choose an item.	Choose an item.	Choose an item.

[1] *ACDP Approved List of (Human) Pathogens*
[2] *SAPO pathogens*
[3] *Schedule 5 Pathogens on the Anti-terrorism & Security Order*

SECTION 3: EXPERIMENTAL PROCEDURES

3.1 Description of experimental procedures:
(Brief details, also indicate any non-standard laboratory operations and any procedures that might require specific control measures e.g. use of sharps, generation of aerosols, in vivo work)

Samples will be maintained for long-term storage through monitoring the condition of the samples and storage containers (predominantly 2 ml plastic screw cap vials) and topping up with 96% ethanol when necessary.

Buccal samples will not be stores long-term, unless in 96% ethanol.

DNA will be extracted from samples using standard DNA extraction kits (e.g. Qiagen blood/tissue kit) following the manufacturer's instructions and protocols and standard Good Laboratory Practice and Good Occupational Safety and Hygiene procedures commensurate with HSE guidelines

3.2 Quantities used and frequency of use:
This information will enable you to determine the likelihood of exposure and therefore the risks from this particular activity. Please indicate maximum culture volumes at any time shown as multiples of flask volumes to give an idea of scale.

Max. volume per culture/sample	100g. Almost all samples are <0.25g.	***Max. volume per experiment:***	0.1g
Frequency of experiments		DNA extractions from approx. 200 samples per year, taking place over a period of approx. 3 weeks per year.	

SECTION 4: MEASURES TO PREVENT OR CONTROL EXPOSURE

Preventing exposure

4.1 Could a less hazardous substance (or form of the substance) be used instead?
(If it can, then it should be used or justification be given here why it is not being used. COSHH requires substitution with less hazardous materials wherever possible, but there may be good reasons for not using them.)

No. Material containing substantial amounts of DNA (blood/tissue) is required for obtaining sufficient quality and quantity of DNA for subsequent analyses.

Controlling exposure

4.2 Containment Level - what containment level is required for this work with regard to COSHH/SAPO?

☐1	☒2	☐3

CL3 only – application for derogation from the following controls (list if relevant and justify)

Click or tap here to enter text.

Premises where this work will be carried out		
Building	*Laboratories*	*Containment level*
Wallace	**044**	**2**

Will the work be segregated from others not involved in the work and if not, how will they be informed of the hazards? :
Samples (tissue/blood) will only be handled in the Category 2 lab. Samples are extremely unlikely to present a risk to those not directly handling them. Dr Nichols has been vaccinated against rabies, tuberculosis, hepatitis A and B, further decreasing the probability of infection.

4.3 Engineering Controls (Containment & Ventilation)	
a) Is a microbial safety cabinet (or isolator for in vivo work) required? These must be used for activities generating potentially infectious aerosols or splashes.	
☐YES ☒ NO	Class: ☐ I ☐ II ☐ III
If required, what processes require its use?	Click or tap here to enter text.
Specify other local ventilation control measures considered appropriate (e.g. downdraft table, isolator):	
Click or tap here to enter text.	
b) Will centrifugation be used?	
☒ YES ☐ NO	
If yes, will buckets and rotors be sealed?	☐ YES ☒ NO
If yes, where will buckets or rotors be opened?	Click or tap here to enter text.
If yes, how will spillages in the centrifuge be dealt with?	Samples will only be centrifuged after (1) treatment/storage in ethanol and (2) digestion with proteinase K. They are therefore extremely unlikely to contain any viable pathogens. Any spillages will be sprayed with 2% Bio-cleanse* disinfectant and removed with absorbent material, and will be disposed of as biohazard material (autoclaved).
c) Will incubators be used?	
☒ YES ☐ NO	
If yes, what type (e.g. shaking)?	Standard (non-shaking) incubators may be used for digestion of the sample with proteinase K.
If yes, how will spillages in the incubator be dealt with?	Any spillages will be removed with absorbent material, disposed of as biohazard material and affected surfaces treated with disinfectant (Bio-cleanse* 2%).
d) Will sharps be used:	
☒ YES ☐ NO	
If yes, list and justify their use:	Needed to cut tissue samples to the appropriate size.
Control measures	Sharps will be disposed of as biohazard material in the appropriate sharp bin available in Wallace 044.
e) Will animals be deliberately infected with these biological agents?	

☐ YES ☒ NO	
If yes, describe the procedure, control measures and whether shedding of infectious agents by animals is expected?	Click or tap here to enter text.

Practical controls:	
Will any further treatment of the sample be undertaken prior to or during use?	
☒YES	☐ NO
If yes, please provide further information:	
Tissue samples are stored in 96% ethanol, which renders almost all pathogens inviable. Further treatment with proteinase K during DNA extraction will provide an additional step to remove viable pathogens.	

4.4 Personal Protective Equipment (PPE):			
Lab coat	*Gloves*	*Eye or face (specify if yes)*	*Other (specify)*
☒YES	☒ YES ☐ NO	☒ YES ☐ NO	Click or tap here to enter text.
Details:	*Details*:	*Details:*	
Suitable for Category 2 material	Nitrile gloves	Safety spectacles	

4.5 Transportation	
a) How will viable material be transported within the laboratory ?	
No viable pathogens are expected to be present in the samples. Samples will be stored and transported in sealed vials within closed boxes.	
b) How will viable material be transported locally outside the laboratory ?	
No viable pathogens are expected to be present in the samples. It is not anticipated that samples will be transported outside of the laboratory, however if this is required, samples will be transported in sealed vials inside closed boxes.	
c) Will viable material be shipped anywhere (off campus)?	☐ YES ☒ NO
If yes, what will be shipped?	**No viable pathogens are expected in tissue/blood samples. It is not anticipated that any samples will be transported off-site.** If required, however, tissue and blood samples, DNA and PCR products will be shipped.
If yes, how will this be shipped (e.g. Category A, Category B, Exempt, Non-hazardous)?	No viable pathogens are expected to be present in the samples (tissue and blood). **It is not anticipated that any samples will be transported off-site**. If transportation of tissue and blood is required, samples will be shipped as Category B material and packaged appropriately according to HSE guidelines.

	DNA and PCR products may be shipped off site. However, all biological agents (i.e., microorganisms and potential pathogens) will have been removed through DNA extraction processes so these are non-hazardous materials.

4.6 Waste disposal procedures:
(Disinfectants, concentrations, exposure times, autoclaving procedures, incinerator procedures, include any animal related wastes.)

Waste	Decontamination method (include details on efficacy)	Disposal route e.g. drain/incineration/landfill
Liquid waste	No liquid disposal is anticipated with the exception of the ethanol and the DNA extraction reagents. These will be disposed according to Swansea University chemical waste procedure if applicable.	Swansea University chemical disposal.
Solid waste	1) No tissue or blood will be discarded a priori, as it will be archived for further experiments. However, if necessary any waste will be disposed in appropriate containers (labelled solid red-lidded yellow containers) and incinerated through Natural UK. 2) Tips, spent buccal swabs and other plastics that have been in direct contact with the samples will be disposed in autoclave bags. 3) Tips and plastics used for DNA extraction (no direct contact with samples) will be disposed in the chemical contaminated waste boxes.	1) Incineration by Natural UK. 2) Autoclaving. 3) Incineration by Swansea University.
Sharp waste	Swansea University disposal procedures for sharps will be followed.	Incineration or alternative method will be followed according to Swansea University.

4.7 Emergency procedures
(spillages – if not covered by local rules/standard operating procedure) Remember to take into account route of exposure

Inside primary containment (if relevant e.g. MSC, isolator)
NA
Outside primary containment but within the laboratory (secondary containment)
Any spillages will be removed with absorbent material, disposed of as biohazard material and affected surfaces treated with disinfectant (2% Bio-cleanse*).
Outside secondary containment (if relevant):
Any spillages will be removed with absorbent material, disposed of as biohazard material and affected surfaces treated with disinfectant (2% Bio-cleanse*).
Other procedures (e.g. following any kind of accidental exposure, needlestick etc.):
If cuts occur, clean wound thoroughly with soap and water, seek medical attention from GP or emergency department if necessary.

*** Bio-cleanse (Teknon®) has powerful bactericidal and virucidal properties. Bio-cleanse concentrate is free from sodium hypochlorite, phosphates and enzymes and effectively removes blood, fat, proteins, grease and other organic and non-organic contaminants whilst simultaneously disinfecting the treated surface. It is safe to use on ferrous and non-ferrous metals, ceramics, glassware and plastics. Bio-cleanse kills *E. coli*, *Salmonella*, *Listeria*, *Candida* and *Penicillium* at 1% dilution and MRSA at 2% dilution. Efficacy against *C. difficile*, Avian Flu, HIV and HBV is proven at 5% dilution. In addition, under COSHH Regulations, the product does not require a maximum exposure limit and when diluted to normal use concentrations, is not irritating to skin.**

SECTION 5: PERSONNEL AND HEALTH ISSUES

5.1 Vaccination

For ACDP 2 or above human pathogens - to be completed by Occupational Health
Is an effective vaccination available for any of the pathogens associated with this work?

NA

5.2 Is health surveillance/health clearance required?

Staff and postgraduate research students	☐ YES ☐ NO
Taught students (undergrad and MSc)	☐ YES ☐ NO *(initial Health clearance only)*

5.3 Identify any particular groups of workers who may be at increased risk from this material: *(for example pregnant workers, young persons under 18, disabled workers, those with pre-existing disease that increases susceptibility.)*

Click or tap here to enter text.

Anyone who might have compromised resistance to disease for any reason should seek advice from the University Occupational Health Service regarding the need for additional precautions.

5.4 Information, instruction and training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity.

044 Laboratory training and inductions will be provided Dr Christopher Coates and/or Dr Almudena Ortiz-Urquiza. If concerns are raised regarding pre-existing conditions, advice will be sought from University Occupational Health Service.

SECTION 6: DECLARATIONS AND APPROVAL

Principal Investigator:

I the undersigned:

- Confirm that all information contained in this assessment is correct and up to date
- Will ensure that suitable and sufficient instruction, information and supervision is provided to all individuals working on the activity
- Will ensure that no work will be carried out until this assessment has been completed and approved and that all necessary control measures are in place
- Will ensure that all information contained in this assessment will remain correct and up to date and re-submit for approval if any significant changes occur
- Work will only be undertaken in appropriate facilities

Name	Signature	Date
Dr Hazel Nichols		**12/02/2019**

Approval on behalf of the College (Head of College approval required for ACDP HG3/4, SAPO 2-4 and organisms listed on Schedule 5 process) – College BSO and University BSA)
(The person supporting this proposal must not be involved in the project being proposed.)

Name	Signature	Date
Click or tap here to enter text.	Click or tap here to enter text.	Click or tap to enter a date.

Approval on behalf of the University (for ACDP HG2-4, SAPO2-4 and organisms listed on Schedule 5 process) – College BSO and University BSA		
Name	Signature	Date
Click or tap here to enter text.	Click or tap here to enter text.	Click or tap to enter a date.

SECTION 7: LIST OF WORKERS UNDER THIS PROJECT						
Full Name (	Worker type					Signature and date
	Staff	Postgrad - Research	Postgrad - taught	UG	Other	
Dr Hazel Nichols	☒	☐	☐	☐	☐ Details	**12/02/2019**
Samantha Shove	☐	☒	☐	☐	☐ Details	**Samatha Shove 1/01/21**
Click or tap here to enter text.	☐	☐	☐	☐	☐ Details	Click or tap here to enter text.
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Click or tap here to enter text.	☐	☐	☐	☐	☐ Details	Click or tap here to enter text.

(this can also be completed manually and kept as a hard copy in the laboratory – copies must be available for review by BSO/BSA – a blank copy of this table can be found here.) ‘

Additional information:

Laboratory 044 has restricted access at the discretion of Dr Christopher Coates and Dr Almudena Ortiz. This lab is equipped with a Salto electronic lock.

A designated autoclave is located in the Wallace Building room 001. This autoclave is regularly validated using a 12-point thermocouple technique. Staff technicians keep records of the validation. Once autoclaved, the waste will be place in tiger bags and disposed into the autoclave skip situated in the car park between Margam and ILS1.

There is a designated lidded bin to store and transport double-bagged waste from lab 044 to the autoclave. Autoclave facilities are in the same building.

Risk Assessment for Teaching, Administration and Research Activities

Swansea University; College of Science

Name Samantha Shove **Signature** **date**…11/12/2021

Supervisor* Hazel Nichols ..... **Signature**.....**date**.....

Activity title DNA extractions from tissue samples ..... **Base location (room no.)** .....

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOC)

University Activity Serial # (enter Employee No. or STUREC No.
Start date of activity (cannot predate signature dates) .....
End date of activity (or 'on going') .....

Level of worker (delete as applicable) .....

UG,PG, research assistant, technician, administration, academic staff, other (state)

Approval obtained for Gene Manipulation Safety Assessment by SU ? not applicable
Licence(s) obtained under "Animals (Scientific Procedures) Act (1986)" ? not applicable
Approval obtained for use of radioisotopes by COS ? not applicable

Record of specialist training undertaken

Course	date

Summary of protocols used; protocol sheets to be appended plus COSHH details for chemicals of category A or B with high or medium exposure

Protocol Details						Protocol Details					
#	Assessment					#	Assessment				
	1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential		1st date	Frequency of re- assessment	Hazard category	Secondary containment level	Exposure potential
1	19/05/21	annually	C	OB	4	11					
2						12					
3						13					
4						14					
5						15					
6						16					
7						17					

8						**18**					
9						**19**					
10						**20**					

See notes in handbook for help in filling in form (Continue on another sheet if necessary)

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #	**Title: DNA extraction and purification from tissue**
Associated Protocols #.....	**Description: Extracting DNA from tissue samples of banded mongoose (Mungo mungo), dwarf mongoose (Helogale parvula), Onager (Equus hemionus) and mole-rats (Bathyergidae).**

Location:

circle which Bioscience and Geography Local Rules apply –

Laboratory

Identify here risks and control measures for work in this environment, <u>additional</u> to Local Rules

Chemicals	**Quantity**	**Hazards**	**Category (A,B,C,D)***	**Exp. Score**
Isopropanol	32ml	Flammable Liquid (Category 2) Serious eye damage/eye irritation (Category 2A) Target Organ Systematic Toxicant – Single Exposure (Category 3) H225: Highly flammable liquid and vapour H336: Vapours may cause drowsiness and dizziness. H319: Causes serious eye irritation.	C	4
HBC Buffer	500 µl per sample vial	Acute toxicity (oral) (Category 4) Skin corrosion/irritation (Category 2) Serious eye damage/eye irritation (Category 2A) Specific target organ toxicity-single exposure (Category 3).	C	2

		H302 – Harmful if swallowed. H315 – Causes skin irritation. H319 – Causes serious skin irritation. H332 – Harmful if inhaled.		
TL Buffer	200 µl per sample vial	Not a hazardous substance/mixture	D	2
BL Buffer	220 µl per sample vial	Acute toxicity (oral) (Category 4) Skin corrosion/irritation (Category 2) Serious eye damage/eye irritation (Category 2A) Specific target organ toxicity – single exposure (Category 3) H302 – Harmful if swallowed. H315 – Causes skin irritation. H319 – Causes serious skin irritation. H332 – Harmful if inhaled.	C	6
Proteinase K Solution	25 µl per sample vial	Skin irritation (Category 3) Respiratory sensitisation (Category 1)	C	1
Ethanol	220 µl per sample vial + 100 ml added to DNA Wash Buffer	Flammable liquids (Category 2) Serious eye damage/eye irritation (Category 2) Specific target organ toxicity (single exposure) (Category 1) Target organs – central nervous system (CNS), optic nerve. Respiratory system. Specific target organ toxicity – (repeated exposure) (Category 1). Target organs – kidney, liver, spleen, blood. Highly flammable liquid and vapor. Causes serious eye irritation. Causes damage to organs.	C	6

DNA wash buffer	700 µl per sample vial	Causes damage to organs through prolonged or repeated exposure. Not a hazardous substance or mixture.	D	2
Elution Buffer	100-200 µl per sample vial	Not a hazardous substance or mixture.	D	2

Hazard Category (known or potential)

A (e.g. carcinogen/teratogen/mutagen)

B (e.g. v.toxic/toxic/explosive/pyrophoric)

C (e.g. harmful/irritant/corrosive/high flammable/oxidising)

D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low **Medium** **High**

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :-

BL Buffer

Keep containers tightly closed in a dry, cool and well-ventilated place. Protect from moisture.

TL buffer

Keep container tightly closed in a dry, well-ventilated place.

HBC buffer

Keep containers tightly closed in a dry, cool and well-ventilated place. Protect from moisture.

Proteinase K Solution

Keep container tightly closed in a dry and well-ventilated place. Recommended storage temperature: 2-8°C

Ethanol

Store locked up in a well-ventilated place. Keep cool.

Isopropanol

Store in a cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Handle open containers with care.

DNA wash buffer

Keep container tightly closed in a dry, well-ventilated place.

Elution buffer

Keep container tightly closed in a dry, well-ventilated place.

Secondary containment (of protocol) open bench/fume hood/special (state) :- open bench

Disposal SU chemical waste

Isopropanol

Dispose of contents and container to appropriate waste site of reclaimer in accordance with local and national regulations.

Ethanol

Dispose of contents/container to an approved waste disposal plant.

BL Buffer

Disposal should be in accordance with applicable regional, national and local laws and regulations.

S29 – Do not empty into drains.

S57 – Use appropriate container to avoid environmental contamination.

TL Buffer

Disposal should be in accordance with applicable regional, national and local laws and regulations.

HBC Buffer

Disposal should be in accordance with applicable regional, national and local laws and regulations.

S29 – Do not empty into drains.

S57 – Use appropriate container to avoid environmental contamination.

DNA Wash Buffer

Disposal should be in accordance with applicable regional, national and local laws and regulations.

Proteinase K Solution

Disposal should be in accordance with applicable regional, national and local laws and regulations.

Identify other control measures (circle or delete) – nitrile gloves; lab coat, safety glasses

Justification and controls for any work outside normal hours NA

Emergency procedures (e.g. spillage clearance; communication methods)

Isopropanol

Ingestion: Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

Eyes: Immediately flush eyes with large amounts of water for at east 15 minutes while holding eyelids open. Transport to the nearest medical facility for additional treatment.

Skin: Remove contaminated clothing, wash off with plenty o water and soap. Consult a physician if any symptoms arise.

Inhaled: Remove to fresh air. If rapid recovery does not occur, transport to nearest medical facility for additional treatment.

First aid facilities: Eye wash fountains and safety showers should be available for emergency use.

Most important symptoms acute and delayed:

EXPOSURE TO HIGH CONCENTRATIONS: Coughing, dry/sore throat, central nervous system depression. Dizziness. Headache Narcopsis.

AFTER ABSORPTION PF HIGH QUANTITIES: Central nervous system depression, headache, dilation of the blood vessels, low arterial pressure, nausea, vomiting, abdominal pain, disturbed motor response, disturbances of consciousness.

FOLLOWING SYMTOMS MAY APPEAR LATER: Body temperature fail, slowing respiration.

ON CONTINUOUS/REPEATED EXPOSURE/CONTACT: Re skin, dry skin. Itching, cracking of the skin, skin rash/inflammation, impaired memory.

HBC Buffer

General advice: Exit to a safe area. Consult a physician. Seek medical attention.

If inhaled: move to fresh air. If not breathing, give artificial respiration. Seek medical attention.

In case of skin contact: exit to a safe area. Wash thoroughly with soap and water. Seek medical attention.

In case of eye contact: flush eyes wit clean water for a minimum of 15 minutes, keeping eye open. Seek medical attention.

If swallowed: rinse mouth with water. Never give anything by mouth to an unconscious person. Seek medical attention/.

Most important symptoms and effect acute and delayed: irritating to eyes, respirator system and skin. Narcotic effect.

Recommendations for immediate medical attention and special treatment: treat symptomatically. Symptoms may be delayed.

TL Buffer

General advice: Wear protective gloves/protective clothing/eye protection/face protection.

In case of skin contact: Wash with soap and water.

I case of eye contact: remove contact lenses, if present and easy to do. Continue rinsing.

If swallowed: N/A

Most important symptoms ad effects acute and delayed: N/A

Recommendations for immediate medical attention and special treatment: Treat symptomatically. Symptoms may be delayed.

BL Buffer

General advice: Exit to safe area. Consult a physician. Seek medical attention.

If inhaled: Move to fresh air. If not breathing, give artificial respiration. Seek medical attention.

In case of skin contact: Exit to safe area. Wash thoroughly with soap and water. Seek medical attention.

If swallowed: rinse mouth with water. Never give anything by mouth to an unconscious person. Seek medical attention.

Most important symptoms and effects accuse and delayed: Irritating to eyes, respiratory system and skin. Narcotic effect.

Recommendations for immediate medical attention and special treatment: treat symptomatically. Symptoms may be delayed.

Proteinase K Solution

General advice: move out of dangerous area. Consult a physician. |Show this safety data sheet to the doctor in attendance.

If inhaled: if we breathe in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact: wash off with soap and plenty of water. Consult a physician.

In case of eye contact: rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed: never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

Recommendations for immediate medical attention and special treatment: treat symptomatically. Symptoms may be delayed.

Ethanol

Response IF exposed: Call a POISON CENTER or doctor/physician.

Skin IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

Eyes IF IN EYES: Rinse cautiously with wate for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists get medical advice/attention.

Fire: in case of fire use CO2, dry chemical or foam for extinguishing.

DNA Wash Buffer

General advice: wear protective gloves/protective clothing/ eye protection/face protection.

If inhaled: If not breathing, give artificial respiration.

In case of skin contact: wash with soap and water.

In case of eye contact: remove contact lenses, if present and easy to do. Continue rinsing.

If swallowed: N/A

Most important symptoms and effects acute and delayed: N/A

Recommendations for immediate medical attention and special treatment: treat symptomatically. Symptoms may be delayed.

Elution Buffer

General advice: wear protective gloves/protective clothing/eye protection/face protection.

If inhaled: if not breathing, give artificial respiration.

In case of skin contact: wash with soap and water.

In case of eye contact: remove contact lenses, if present and easy to do. Continue rinsing.

If swallowed: N/A.

Most important symptoms and effects acute and delayed: N/A

Recommendations for immediate medical attention and special treatment: treat symptomatically. Symptoms may be delayed.

Supervision/training for worker (circle)

None required Already trained Training required Supervised always

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker Samantha Shove

Name & counter-signature of supervisor..... *Date*.....

Date of first reassessment Frequency of reassessments

Risk Assessment for Teaching, Administration and Research Activities

Swansea University; College of Science

Name Samantha Shove **Signature** **date** 11/12/2021

Supervisor* Hazel Nichols ..... **Signature**.....**date**.....

Activity title preparing, running and visualising agarose gels..... **Base location (room no.)**
.....

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOC)

University Activity Serial # (enter Employee No. or STUREC No.
Start date of activity (cannot predate signature dates) .....
End date of activity (or 'on going') on going .....

Level of worker (delete as applicable) .....

UG,PG, research assistant, technician, administration, academic staff, other (state)

Approval obtained for Gene Manipulation Safety Assessment by SU ? not applicable
Licence(s) obtained under "Animals (Scientific Procedures) Act (1986)" ? not applicable
Approval obtained for use of radioisotopes by COS ? not applicable

Record of specialist training undertaken

Course	date

Summary of protocols used; protocol sheets to be appended plus COSHH details for chemicals of category A or B with high or medium exposure

Protocol Details						Protocol Details					
#	Assessment					#	Assessment				
	1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential		1st date	Frequency of re- assessment	Hazard category	Secondary containment level	Exposure potential
1	19/05/21	annually	C	OB	4	11					
2						12					
3						13					
4						14					
5						15					
6						16					
7						17					
8						18					
9						19					
10						20					

See notes in handbook for help in filling in form **(Continue on another sheet if necessary)**

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol # 3	**Title:** Agarose gels
Associated Protocols #.....	**Description:** Making up, loading and running agarose gels for separation of DNA fragments

Location:

circle which Bioscience and Geography Local Rules apply –

Boat Field Genetic-Manipulation Laboratory Office/Facility Radioisotope

Identify here risks and control measures for work in this environment, additional to Local Rules

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp. Score
Agarose powder	1g	Not classified	D	2
TAE buffer	50ml	Skin Corrosion/Irritation (Cat 2) Serious eye damage (Cat2B) Specific Target Organ Toxicity, Single exposure (Cat 3) H315: Causes skin irritation H320: Causes eye irritation H335: May cause respiratory irritation	C	4
SYBR Safe DNA gel stain	5µl	Flammable liquids (Cat 4) H227: Combustible liquids	C	2
DNA ladder	5µl	Not hazardous	D	1
Gel loading dye	1µl pe well	Not hazardous	D	1

Hazard Category (known or potential)

A (e.g. carcinogen/teratogen/mutagen)

B (e.g. v.toxic/toxic/explosive/pyrophoric)

C (e.g. harmful/irritant/corrosive/high flammable/oxidising)

D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low **Medium** **High**

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :-

Agarose powder, TAE buffer, SYBR Safe DNA: Store in a closed container in a cool, dry, well ventilated area. DNA ladder : Store frozen at -20C Gel loading dye: Store refrigerated at 4C
Secondary containment (of protocol) open bench/fume hood/special (state) :- open bench
Disposal SU chemical disposal
Identify other control measures (circle or delete) – Nitrile gloves, lab coat, safety glasses
Justification and controls for any work outside normal hours NA
Emergency procedures (e.g. spillage clearance; communication methods)
Supervision/training for worker (circle) None required Already trained Training required Supervised always
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker* Samantha Shove *Name & counter-signature of supervisor*..... *Date*.....
Date of first reassessment Frequency of reassessments

Guidance for Completion of Bioscience and Geography Protocol Risk Assessment Form

Note – you are strongly advised to complete electronic versions of this form, enabling you to readily expand and contract sections as required to ensure clarity and adequate documentation. Do **not** delete any sections! Instead, mark inappropriate sections with NA (not applicable) and contract the section to save space on the final printed form.

Protocol - any self-contained procedure. This could be any activities undertaken, be they lab-work, use of equipment, fieldwork or office work. Your complete research/teaching/administration **activity** (e.g. undergraduate project, PhD study, research grant, other) is therefore made up from separate **protocols**. If the protocol is mainly of low hazard, but with one or more hazardous components, consider making the manipulation of the latter a separate protocol and tie them together by completing the "*Associated Protocol*" box. This is because the entire protocol must be conducted under conditions required for the handling of the most hazardous component.

Title/Description - give sufficient detail to make it obvious what the protocol involves.

Location – identify which local rules apply. More than one rule may apply. Then add any additional risks and control measures peculiar to this protocol (e.g. site-specific fieldwork information; use of autoclaves, sonicators; mechanical, electrical hazards). You may also wish to stress any particularly important risks and controls even if indicated in local rules.

Chemicals etc. - give name, maximum quantity used, list hazards, hazard category (see Table 1) and calculate the **Exposure Score** (see Table 2) for **every** chemical used. Expand the area in the table as required.

Exposure Potential (see Table 3) - complete this section for the chemical which has the **highest** exposure score in your chemical list as this defines the highest risk factor.

Primary containment/Storage - detail how and where, and for how long, the resultant product from the protocol will be stored. The product must be labelled with the date of synthesis, and disposed of (see below) before the maximum duration time has elapsed.

Secondary containment - detail where the protocol will be performed (refer to Table 4).

Disposal - detail how you will dispose of surplus reagents and the product of the protocol. Final disposal must be undertaken within the period noted in the 'maximum duration' under 'Storage' (above).

Identify other control measures – typically these refer to special protective clothing etc.

Justification and controls for any work outside normal hours – out of hours working is only allowed under special conditions (e.g. 24h sampling, sampling related to tides etc.); convenience is not an acceptable reason.

Emergency procedures - detail how spillages etc. would be handled, including clearance of the laboratory etc. as required. For field work indicate emergency communication and first-aid coverage.

Supervision/training - detail here what special supervision and training is required by the worker named at the bottom of the form. Note that all undergraduates are always considered as research incompetent. First-year PhD students and MSc students are not to be used to supervise the activities of others.

Declaration - both the worker and the supervisor must sign this on the date entered here.

Reassessment - the first reassessment must be undertaken as soon as possible after the first time the protocol has been undertaken in order to identify any unforeseen hazards. After this first reassessment, the protocol should be reassessed every 6-12m, depending on the nature of the chemicals, to take account of changing knowledge concerning the hazardous nature of chemicals. The protocol must be reassessed immediately if new knowledge on the chemical hazards becomes available.

NOTE - standard protocols can be produced for each environment BUT each worker must have their own personalised version, signed by them and their supervisor, and dated. These completed personalised protocols must then be appended to the SU risk assessment form for the Teaching/Research activity belonging to the individual.

COSHH Assessment - modified from "COSHH in Laboratories" published by the Royal Society of Chemistry, July 1989

Hazards, Risks and Containment - Definition of terms

Hazard potential for doing harm, *e.g*. toxic, flammable, carcinogenic *etc*

Exposure potential the risk to the user depends very much on the exposure, which depends on the physical properties of the material, the quantity used and for how long.

Risk = "Hazard" x "Exposure Potential"

The risk is decreased to a safe level by:

a) Containment
b) Personal Protection
c) Good Laboratory Practice (GLP)

Levels of containment

The containment required for a given activity is of two basic kinds: the primary (or intrinsic) containment provided by the apparatus or equipment in which the substances are handled and the additional (or secondary) containment needed to ensure appropriate control of exposure.

HAZARD CATEGORY

TABLE 1- General Guidelines for determining hazard categories

A	EXTREME HAZARD	Substances of known or suspected exceptional toxicity (e.g. carcinogen, teratogen, potential mutagen)
B	HIGH HAZARD	All substances whose toxicity exceeds that of the medium hazard category, except for those known or believed to be so highly toxic as to merit special precautions (i.e. those in the “extreme” category)
C	MEDIUM HAZARD	Substances meeting criteria for CPL* classification as “Harmful” or ‘Irritant'
D	LOW HAZARD	Substances not matching criteria for CPL* classification as “Harmful” or “Irritant”

CPL = the Classification, Packaging and Labelling Regulations 1984.

NOTE:

1. The toxicity considered should be that of the substance or mixture handled, including any impurities.
2. Substances may have other properties (*e.g*. flammability) which may call for additional precautions.
3. The above general guidance may need to be supplemented by developing additional criteria with the help of expert toxicological advice. (Additional criteria may be developed using, for example, data given in HSE Guidance Notes such as EH40).
4. Time factors, such as frequency and duration of activity should also be considered. Short duration tasks, involving a few seconds exposure at infrequent intervals, should not affect the initial estimate, whereas continuous operations on a daily basis would probably raise the estimate to the next highest category.

EXPOSURE SCORE

TABLE 2 - exposure score to be calculated for all chemicals used in a protocol

EXPOSURE SCORE				
Calculation Value		**1**	**2**	**3**
(i)	Quantity	<1g	1-100g	>100g
(ii)	Properties	Dense solid Non- volatile liquid No skin absorption	Dusty solids Lyophilised solids Volatile liquids (b.p.>80ºC)	Gases, Aerosols Highly volatile liquids (b.p.<80º C) Solutions promoting skin absorption
(iii)	Pressure	Normal	Low/Vacuum	>1 atmosphere
(iv)	Temperature	Room temperature	25ºC - 100ºC	>100ºC

Exposure Score calculation = (i) x (ii) x (iii) x (iv)

The Exposure Potential

TABLE 3 - Rough calculation of exposure potential

	EXPOSURE SCORE (FROM TABLE 2)		
Total score	**<10**	**10-54**	**>54**
Exposure Potential	**L** (low)	**M** (medium**)**	**H** (high)

Secondary containment level calculation

Table 4 - use to determine secondary containment

SECONDARY CONTAINMENT LEVEL					
Hazard Category		**A**	**B**	**C**	**D**
Exposure potential (from table 3)	**H**	SA	SA	FH	FH
	M	SA	FH	FH	OB
	L	FH	FH	OB	OB

OB = **O**pen **B**ench; **FH** = **F**ume **H**ood; **SA** = **S**pecial **A**ttention (see supervisor)

Risk Assessment for Teaching, Administration and Research Activities

Swansea University; College of Science

Name Samantha Shove **Signature** **date** 11/12/2021

Supervisor* Hazel Nichols **Signature** ....**date**.....

Activity title PCR setup ..... **Base location (room no.)** .....

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOC)

University Activity Serial # (enter Employee No. or STUREC No.
Start date of activity (cannot predate signature dates) .....
End date of activity (or 'on going') on going .....

Level of worker (delete as applicable) .....

UG,PG, research assistant, technician, administration, academic staff, other (state)

Approval obtained for Gene Manipulation Safety Assessment by SU ? not applicable
Licence(s) obtained under "Animals (Scientific Procedures) Act (1986)" ? not applicable
Approval obtained for use of radioisotopes by COS ? not applicable

Record of specialist training undertaken

Course	date

Summary of protocols used; protocol sheets to be appended plus COSHH details for chemicals of category A or B with high or medium exposure

Protocol Details						Protocol Details					
#	Assessment					#	Assessment				
	1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential		1st date	Frequency of re- assessment	Hazard category	Secondary containment level	Exposure potential
1	19/05/21	annually	D	OB	1	11					
2						12					
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4						14					
5						15					
6						16					
7						17					
8						18					
9						19					
10						20					

See notes in handbook for help in filling in form **(Continue on another sheet if necessary)**

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol # 2	**Title: PCR**
Associated Protocols #.....	**Description: PCR to amplify DNA using microsatellites**

Location:

circle which Bioscience and Geography Local Rules apply –

Laboratory

Identify here risks and control measures for work in this environment, additional to Local Rules

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp. Score
Primer DNA	1.2µl per reaction	Not Hazardous	D	1
Target DNA	2-3µl per reaction	Not Hazardous	D	1
RNAse free water	1.8µl per reaction	Not Hazardous	D	1
Mineral oil	10µl per reaction	Not Hazardous	D	1
Qiagen master mix	6µl per reaction	Not Hazardous (contains very small quantities of glycerol, which may irritate eyes and skin and can be toxic if large amounts are swallowed (LD50= 12,000mg/kg in rats), but the over-all mix is not considered hazardous)	D	1

Hazard Category (known or potential)

A (e.g. carcinogen/teratogen/mutagen)
B (e.g. v.toxic/toxic/explosive/pyrophoric)
C (e.g. harmful/irritant/corrosive/high flammable/oxidising)
D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low **Medium** **High**

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :- All components should be stared frozen. No maximum storage duration

Secondary containment (of protocol) open bench/fume hood/special (state) :- open bench

Disposal chemical waste

Identify other control measures (circle or delete) – nitrile gloves; lab coat, safety glasses
Justification and controls for any work outside normal hours NA
Emergency procedures (e.g. spillage clearance; communication methods) Eye contact: Remove contact lenses. Protect unharmed eye. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician If swallowed : If accidentally swallowed obtain immediate medical attention. Rinse mouth with water. Never give anything by mouth to an unconscious person. General advice : Show this safety data sheet to the doctor in attendance. If inhaled : Move to fresh air. If symptoms persist, call a physician. In case of skin contact : Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. If symptoms persist, call a physician.
Supervision/training for worker (circle) None required Already trained Training required Supervised always
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker* Samantha Shove *Name & counter-signature of supervisor*..... *Date*.....
Date of first reassessment Frequency of reassessments

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Location – identify which local rules apply. More than one rule may apply. Then add any additional risks and control measures peculiar to this protocol (e.g. site-specific fieldwork information; use of autoclaves, sonicators; mechanical, electrical hazards). You may also wish to stress any particularly important risks and controls even if indicated in local rules.

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Hazard potential for doing harm, *e.g*. toxic, flammable, carcinogenic *etc*

Exposure potential the risk to the user depends very much on the exposure, which depends on the physical properties of the material, the quantity used and for how long.

Risk = "Hazard" x "Exposure Potential"

The risk is decreased to a safe level by:

a) Containment
b) Personal Protection
c) Good Laboratory Practice (GLP)

Levels of containment

The containment required for a given activity is of two basic kinds: the primary (or intrinsic) containment provided by the apparatus or equipment in which the substances are handled and the additional (or secondary) containment needed to ensure appropriate control of exposure.

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NOTE:

1. The toxicity considered should be that of the substance or mixture handled, including any impurities.
2. Substances may have other properties (*e.g*. flammability) which may call for additional precautions.
3. The above general guidance may need to be supplemented by developing additional criteria with the help of expert toxicological advice. (Additional criteria may be developed using, for example, data given in HSE Guidance Notes such as EH40).
4. Time factors, such as frequency and duration of activity should also be considered. Short duration tasks, involving a few seconds exposure at infrequent intervals, should not affect the initial estimate, whereas continuous operations on a daily basis would probably raise the estimate to the next highest category.

EXPOSURE SCORE

TABLE 2 - exposure score to be calculated for all chemicals used in a protocol

EXPOSURE SCORE				
Calculation Value		**1**	**2**	**3**
(i)	Quantity	<1g	1-100g	>100g
(ii)	Properties	Dense solid Non- volatile liquid No skin absorption	Dusty solids Lyophilised solids Volatile liquids (b.p.>80ºC)	Gases, Aerosols Highly volatile liquids (b.p.<80º C) Solutions promoting skin absorption
(iii)	Pressure	Normal	Low/Vacuum	>1 atmosphere
(iv)	Temperature	Room temperature	25ºC - 100ºC	>100ºC

Exposure Score calculation = (i) x (ii) x (iii) x (iv)

The Exposure Potential

TABLE 3 - Rough calculation of exposure potential

	EXPOSURE SCORE (FROM TABLE 2)		
Total score	**<10**	**10-54**	**>54**
Exposure Potential	**L** (low)	**M** (medium**)**	**H** (high)

Secondary containment level calculation

Table 4 - use to determine secondary containment

SECONDARY CONTAINMENT LEVEL					
Hazard Category		**A**	**B**	**C**	**D**
Exposure potential (from table 3)	**H**	SA	SA	FH	FH
	M	SA	FH	FH	OB
	L	FH	FH	OB	OB

OB = **O**pen **B**ench; **FH** = **F**ume **H**ood; **SA** = **S**pecial **A**ttention (see supervisor)

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