UNIVERSITY OF GLOUCESTERSHIRE

The Thermal Properties of Artificial Reptile Refugia

A THESIS SUBMITTED TO THE UNIVERSITY OF GLOUCESTERSHIRE IN ACCORDANCE WITH THE REQUIREMENTS OF THE DEGREE OF MASTER OF RESEARCH IN THE SCHOOL OF NATURAL AND SOCIAL SCIENCES

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Abstract

The global decline of reptile species makes their conservation increasingly important. To aid conservation efforts we must have detailed information about reptile populations and any changes or trends that emerge amongst them. For this reason, rigorous population surveys must be carried out and it is in the best interest of these efforts that the survey tools used are as effective as possible.

Artificial refugia surveying is a commonly used technique to survey reptiles in temperate climates. In this thesis we examine the thermal properties of traditional and novel refugia materials (aluminium, brass and polystyrene), and their performance in the field, in an attempt to prove that the technique can still be improved upon. The thermal properties of the materials were examined in a laboratory experiment and then used in a survey of Grass Snakes (*Natrix natrix*) across three different sites to determine which material performed the best.

Overall, the new materials did not perform as well as the traditional however new alterations to traditional materials did result in an increase in performance. Iron outperformed bitumen when it was painted black and attracted to highest number of reptiles in the field survey. This shows that tried and tested methods can still always be improved upon and bitumen, the most popular refugia material according to a survey conducted as part of this thesis, may not necessarily be the best.

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text. No part of the thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.
Any views expressed in the thesis are those of the author and in no way represent those of the University.

Signed
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Chapter 1: Introduction

It has been widely accepted by the scientific community that species are in decline across the globe (Diaz *et al*., 2019). According to Barnosky *et al*., (2011) the amount of species lost over the last few centuries are higher than would be expected based on evidence from the fossil record, which indicates the possibility that a sixth mass extinction event is underway. This loss of species began with terrestrial environments but has recently spread to the ocean as well (McCauley *et al*., 2015). The five vertebrate groups of Fish, Amphibia, Reptilia, Aves and Mammalia are all showing a worldwide loss in population and biodiversity (Harshbarger et al., 2000; Light and Marchetti, 2007; Wake, 1991; Alford et al, 2001; O'hanlon *et al*., 2018; Gibbons et al., 2000; Winne et al., 2007; Mullin and Seigel, 2009; King et al., 2008; McLoughlin et al., 2003).

The common cause that links these events is human activity, considered to be one of, if not the major cause of species loss since the late Pleistocene (Diaz *et al*., 2019; Boivin *et al*., 2016; Faurby and Svenning, 2015; Barnosky *et al*., 2011; Burney and Flannery, 2005). In recent years, human-induced climate change in particular has been cited as the cause for many species decline and extinction (Waller *et al*., 2017; Urban, 2015; Bestion *et al*., 2015). Perhaps more so than other taxonomic groups, Reptiles are especially sensitive to climate change (Böhm *et al*., 2016; Meng *et al*., 2016; Hatten *et al*., 2016) because they are ectotherms. This means that the ambient temperature of their environment effects every aspect of their biology, from digestion to locomotion and even breeding.

1.1 Reptile Thermo-ecology

Reptiles are ectothermic, meaning they rely on external sources of heat to regulate their body temperature (Cowles and Bogert, 1944; Christian and Weavers, 1996; Spellerberg, 1972; Shine and Madsen, 1996; Sunday *et al*., 2014). If they need to warm up, they must find a warm patch and absorb heat from their environment, and if they need to cool down, they must find a cool spot and let the excess heat dissipate. They lack any homeostatic methods for maintaining optimum body temperature.

This reliance is a key factor influencing reptile distribution. Spellerberg, (1972) concluded that the environmental temperatures most limiting to the distribution of reptiles were cold temperatures referred to as the “critical minimum”, the lower limits wherein the animal can still right itself. While determining the temperature limits of multiple reptile species Spellerberg, (1972) also recorded instances of acclimation to these critical temperatures, showing that while these temperatures limit reptile abundance, they do not always block it. Garter snakes (*Thamnophis sirtalis*) have adapted to colder, temperate habitats where they often face a shortage of suitable hibernation spots by utilising communal hibernation locations called a hibernaculum (Costanzo, 1986).

Reptiles are typically more diverse and abundant in the Tropics, as seen in figure 1a below, mainly because ambient temperatures are high and warm microhabitats are readily available (Shine and Madsen, 1996). In the higher temperatures of the tropics many reptiles can maintain a high body temperature without engaging in as much thermoregulatory behaviour as reptiles in temperate regions (Luiselli and Akani, 2002; Shine and Madsen, 1996). Not only are there more reptile species in the tropics but the largest terrestrial reptiles are also concentrated there. The saltwater crocodile (*Crocodylus porosus*) is considered to be the largest species of terrestrial reptile by mass (Britton, 2003) and is distributed from the east coast of India (Meganathan *et al*., 2010) to the Philippines (Britton *et al*., 2012) and to North Australia (Fukuda *et al*., 2011). The largest snake, the Green Anaconda (Feldman *et al*., 2016), is distributed through the Amazon rainforest, the world’s largest tropical rainforest (Rivas, 2001). Many have suggested that the higher temperatures at the tropics are responcible for the increase in diversity, not just for reptiles but for all life (Brown, 2014). Higher temperatures result in higher rates of metabolism which dictates the pace of life (Allen *et al*., 2002). Essentially, life happens faster in the tropics. Rohde (1992) claimed that the species richness in the tropics is the result of shorter generation times, faster mutation rates, and faster selection at greater temperatures. Temperature dictates the distribution of all life, and while that may be true for all species it is especially so for Ectotherms.

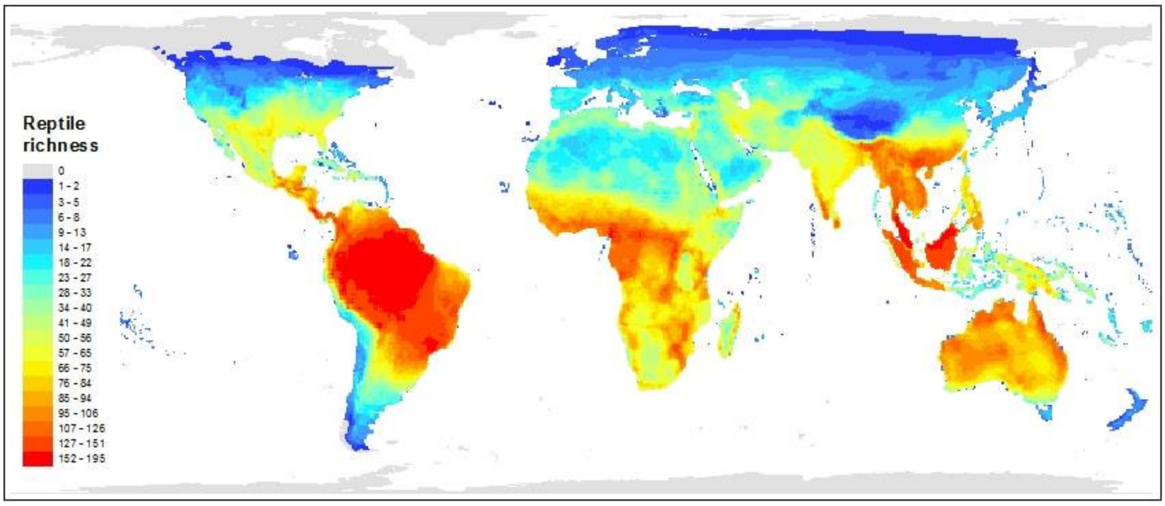

Figure 1a) global reptile richness (Roll et al., 2017)

1.1.1 Behavioural Adaptations for Thermoregulation

Most ectotherms do not have physiological adaptations to adjust their body temperatures, and so rely on behavioural adaptations (Sunday *et al*., 2014). Behavioural adaptations to vary body temperature are termed behavioural thermoregulation. Such adaptations can be as simple as choosing the optimal time of the day for activity (Porter *et al*., 1973).

A lacertid *Podarcis hispanica atrata* displays all the behavioral adaptations typical of reptiles; activity times, use of microhabitats, sun and shade patches, basking, and shuttling. Because of these adaptations it has been observed to maintain a consistent body temperature despite significant variability in available operative temperatures (Bauwens *et al*., 1996).

The Canyon lizard (*Sceloporus merriami*) demonstrates the importance of combining activity times with the use of thermal microclimates (Grant and Dunham, 1988). A microclimate is defined as the climate of a small or restricted area, especially when this differs from the climate of the surrounding area (Suggitt *et al*., 2011). Reptiles often use microclimates that provide more favorable conditions than the wider environment (Guillon *et al*., 2013). It was believed that the thermal environment would have a greater effect of the Canyon lizards because they have a lower body temperature on average than any other North American desert iguanid (32.2°C). Canyon lizard feeding strikes on prey and social displays were limited to a 2-hour period beginning around local sunrise and at its maximum within an hour after sunrise. During these active periods, the lizards makes little and random use of thermal microclimates. It is when the lizards were less active that they made deliberate use of microclimates to maintain their temperature (Grant and Dunham, 1988). The marbled gecko

(*Christinus marmoratus*) is nocturnal, which presents it with the challenge of maintaining its temperature during the much colder nights. During the daytime the lizards shelter beneath rocks until they reach the optimal temperature. At night they have a multitude of refuge options and the geckos can select the refuge closest to their optimal temperature (Kearney and Predavec, 2000).

The most commonly exhibited behaviour of reptiles to regulate their temperature is basking, whereby they expose themselves to a direct source of heat. When in danger of overheating a reptile will seek shade to cool down. For reptiles in temperate zones basking can be essential for maintaining metabolism in cooler environments. For the northern map turtle (*Graptemys geographica*), an aquatic reptile native to north America, basking was shown to be essential for maintaining optimum body temperature for metabolism and could increase their metabolic rate by 17.2 to 30.1% (Bulté and Blouin-Demers, 2010). Man-made structures can also provide basking sites for reptiles near human settlement. Despite the dangers of roadkill many reptiles use man-made roads as basking sites (Forman and Alexander, 1998). The unfortunate side effect is that roads have become areas of high mortality for reptiles whether they were there for basking or simply trying to cross them (Enge and Wood, 2002; Ashley and Robinson, 1996; Langen *et al*., 2009).

Thermoregulation is a high priority for reptiles. When presented with an opportunity to reach their optimal temperature the common lizard (*Zootoca vivipara)* would, without fail, engage in appropriate thermoregulatory behaviour (Herczeg *et al*., 2006). However, thermoregulation is not always the highest priority. Herczeg *et al*., (2006) also found instances of thermoconformity, where the reptiles do not attempt to increase their body temperature because the optimal temperature could not be reached. This indicates an awareness of whether attempting thermoregulation would be worthwhile. Other instances of reptiles sacrificing thermoregulation occur when reptiles seek to avoid the associated risks. Downes and Shine, (1998) studied reptile habitat choice in relation to thermoregulation and predator avoidance and found that reptiles will sacrifice warmer basking sites to avoid predation risk. It has been shown by Chelazzi and Calzolai, (1986) that familiarity with the local environment, including basking sites and microclimates, is of great benefit to reptiles. In a study conducted on Hermann's tortoises (*Testudo hermanni)* it was found that the resident tortoises were more efficient at raising their temperature through basking than individuals who had recently been introduced to the area.

Reptiles can also feature physiological adaptations to thermoregulate. Information on this can be found in appendix 1.

1.2 Threats Facing Reptiles

The ectothermic nature of reptiles makes them especially susceptible to climate change, which is one of the six significant threats facing them. The other five include: habitat loss, invasive species, pollution, disease, and unsustainable use (Gibbon *et al*., 2000; Araújo *et al*., 2006; Schlaepfer *et al*., 2005; Johnson, 2019). Climate change and habitat loss are threats that affect their ability to thermoregulate by changing the local weather and destroying microclimates and basking spots. Disease and pollution threaten reptiles but not in a way that affects thermoregulation. Details of these threats can be found in appendix 1.

1.2.1 Climate Change

Reptiles and their habitats will be affected by the changes in climate predicted over the next few decades (Bickford *et al*., 2010; Araújo *et al*., 2006; Hamann *et al*., 2007; Booth, 2006; Hawkes *et al*., 2007). The distribution of reptile populations mirrors climatic factors, predominantly temperature; reptile abundance is higher in warmer, tropical regions (Shine and Madsen, 1996). Any instance of heating or cooling would affect reptiles in some way, adjusting the climate envelopes in which they could in principle live. Cooling especially would be harmful because it limits their ability to warm up and function (Araújo *et al*., 2006) as well as move to more favourable locations. On the other hand, warming could increase the range of certain reptile species if they were able to move into new areas, as was seen in southeastern Australia after clearing forests for power lines (Shine *et al*., 2002). As climate changes so do local temperatures; reptiles unable to adapt to these changes may be forced to migrate to more suitable areas. In some instances, migration may be impossible and if adaptation does not come quickly enough it will lead to local extinction events, especially for reptiles in isolated areas such as islands (Bickford *et al*., 2010).

For egg laying reptiles the temperature of the nest influences hatching success and sex ratio (Booth, 2006; Gutzke and Crews, 1988), and so climate change can damage both the population of the next generation but also the breeding capacity as the sex ratio shift reduces the number of breeding pairs (Hamann *et al*., 2007). A detailed example of this effect of loggerhead sea turtles (Caretta caretta) can be found in a paper by Hawkes *et al*., (2007) who noticed a high female bias in more southern parts of the United States and predicted that trend to increase with as little as 1°C of warming.

Temperature can affect the physiology of reptiles in other ways, such as their resistance to disease and synthesis of antibodies (Evans and Cowles, 1959; Agha *et al*., 2017). As climate change alters temperatures it can change the distribution of wildlife as more suitable temperatures can now be found elsewhere (Perry *et al*., 2005; Kelly and Goulden, 2008; Levinsky *et al*., 2007). A big concern is that this also applies to diseases and wildlife that acts as vectors for diseases (Medlock and Leach, 2015; Ogden and Lindsay, 2016; Medone *et al*., 2015). This can introduce new species to diseases they have no resistance to which can decimate local populations (Maynard *et al*., 2015). If a reduction in temperature can reduce antibody production, then climate change could also result in reptile populations suffering an increased sensitivity to disease. For more information on disease as a treat to reptiles see appendix 1.

1.2.2 Habitat Loss and Urbanisation

Gibbon *et al*., (2000) claimed that the degradation and loss of habitats are the direst threats to reptile species. According to Brooks *et al*., (2002) one-third of all terrestrial vertebrates, including reptiles, are endemic to 25 “hotspots” of biodiversity and none of these hotspots have more than one-third of their pristine habitat remaining.

One of the leading causes of habitat loss is urbanisation, a process that is rapidly expanding worldwide and has the effect of depleting or fragmenting habitats (Roe, Rees and Georges, 2011; Driscoll, 2004; McKinney, 2008; Hamer and McDonnell, 2010). Urbanisation is a recent phenomenon and its effect on reptiles is relatively poorly studied (McKinney 2002). Many studies have been conducted on reptile habitat choice in the wild (Stumpel, 2012; Semlitsch and Bodie, 2003; Kanowski *et al*., 2006), but less consider habitat choice in urban areas, and how well reptiles can adapt to this new habitat.

Luck *et al*., (2004) found that human population density in Australia has a strong positive correlation with species richness for a variety of animal taxa, but not reptiles, implying that reptiles are less likely to survive urbanisation. Within

Melbourne, Australia, a study was carried out on the probability of frog and reptile populations surviving urbanization. The results found that 81% of frog species and 56% of reptile species had ≥95% probability of being extant in 2006 after urbanization (Hamer and McDonnell, 2010). This suggests that reptiles have a greater sensitivity to urbanization than amphibians and supports the idea that reptiles have a greater overall vulnerability to urbanization that other animal groups. It was suggested by Hamer and McDonnell (2010) that conservation of herpetofauna in urban areas requires structural complexity in remnant habitat patches and implementing management actions to protect habitat corridors. Janiawati *et al*., (2016) found that maintaining a level of vegetation cover as well as water sources can provide sanctuary for reptiles in urban areas. Relatively small changes to urbanisation plans can be implemented to protect local reptiles. However, even when such measures are taken reptiles can prove to be susceptible to urbanization. Vegetation corridors were found to do little to preserve the reptile population in central New South Wales, Australia, with five species showing significantly lower populations and two species being locally extinct (Driscoll, 2004). Species richness of reptiles if often higher at sites further away from development and urban areas (Hunt *et al*., 2013).

Despite the frequently documented damage urbanization can do to reptile populations there some rare cases of reptiles enduring or even benefiting. When Garden *et al*., (2007) studied reptile habitat choice in an area fragmented by urbanisation, they found reptile populations surviving near human settlements focused around areas with prey. This suggests the possibility that urbanisation is not itself a direct cause of reptile decline, but instead causes populations of prey items to decline. Reptiles can even make use of the man-made structures and materials as refuges, as seen by the Gila monster (*Heloderma suspectum)* by Kwiatkowski *et al*., (2008).

In some instances, urban areas can act as sanctuaries to reptile populations, with man-made alterations improving the habitat. A study by Barrett and Guyer (2008) found significant increases in reptile species richness in urban watersheds. Roe *et al*., (2011) studied eastern long-necked turtle (*Chelodina longicollis*) in both an Australian suburban environment and an adjacent nature reserve during drought. Turtles in the suburbs were found to be nearly 3 times more abundant, grew 5 times faster, and had larger adults than nature reserve populations. There was also a net movement of turtles from the nature reserves into the suburbs. This suggests that turtles can survive better in suburban water bodies than in nature reserves, at least during a drought. It opens the possibility of using the suburbs as a refuge for turtles either in the long term or simply transferring them whenever droughts strike.

An important question regarding habitat loss is whether species can reclaim the habitats if they are restored. A landscape-level survey of reptile populations was carried out by McAlpine *et al*., (2015) in the Brigalow Belt of eastern Australia, a region highly modified by recent agricultural expansion. They found that the total abundance of reptiles increased with remnant forest extent meaning the reptiles were not able to re-establish populations in the regrown habitat, at least not to the level in remnant forests. Therefore, efforts should be made to minimise habitat loss in the first place instead of attempting to return habitats to their original state later.

1.3 Importance of Reptile Conservation

With so many threats facing reptiles it will take considerable time and resources to properly preserve them. Because of this we must ask if the conservation of reptiles is worth the effort and resources. It is important therefore, to look at the benefits reptiles provide. Reptiles play numerous important roles in their ecosystems, and so protecting them will help protect their environment. They also have a variety of uses to us that make them worthy of preservation.

1.3.1 Importance to Humans

Reptiles are important bio-indicator species (Crain and Guillette, 1998; Thompson and Thompson, 2005) meaning their presence, abundance and health can indicate the habitat health. This is crucial information for ecologists who are looking to find which areas are most in decline and so require greater conservation effort.

Reptile bioindicators can be used to assess the effects that certain human activities can have on an ecosystem. Marsili *et al*., (2009) developed a methodology using the terrestrial lizard *Podarcis sicula* to assess the ecotoxicological effects associated with onshore oil extraction. Similarly, Read, (1998) investigated the response of gecko communities to sulphur dioxide and salt spray from a mine and industrial site, finding an increase in capture rates followed a reduction in peak sulphur dioxide emissions, indicating that geckos are sensitive to air pollution. Predatory reptiles can be used to examine pollution levels in an environment due to the process of biomagnification. The predatory reptiles will accumulate the pollutants from the prey animals eaten and so will have a higher concentration that other animals. Frossard *et al*., (2019) used this to study cadmium pollution by examining the snake *Bothrops jararaca* and assess the levels of cadmium poisoning in the local environment.

A variety of environmental and chemical factors can affect the development of a reptile embryo into male or female animals, which opens the possibility of the sex ratio of a local population being an indicator for pollution. Crain and Guillette, (1998) found that exposure to certain man-made chemicals such as PCBs (polychlorinated biphenyls, used as coolants and lubricants in transformers, capacitors, and other electrical equipment) and common herbicides can affect the development of alligator embryos and therefore the sex ratio of a local population. Consequently, the degree of contamination in a habitat could potentially be determined by analysing the sex ratio of local alligator populations, although such an approach is confounded by other factors including temperature that may be difficult to disentangle.

Certain animals have a commercial use that makes them valuable to humans. This often motivates people to protect species that they can derive value from and helps support local conservation. This concept is called conservation through commerce. The most notable example for reptiles would be the farming of crocodiles for their leather (Revol, 1995). Many of these farms also release individuals back into the wild to maintain the local populations (Blake and Loveridge, 1975). A regulated industry of crocodile and alligator farming has been found to damage black market profits and reduce incentive for poaching (Moyle, 2013). Some believe that commercial farming could be the only hope for certain crocodilian species. The Chinese alligator, *Alligator sinensis,* is on the verge of becoming extinct in the wild due to the destruction of its habitat (Thorbjarnarson *et al*., 2002). The total population of wild Chinese alligators is estimated to be <130 individuals left. Watanabe, (1983) suggested that commercial farming was the only hope left for the Chinese alligator.

Certain reptiles have a pharmaceutical potential. A peptide was extracted from the Gila monsters' (*Heloderma suspectum)* venom that could mimic the activity of an "Incretin" hormone designated glucagon-like peptide 1 (GLP-1) (Furman, 2012).

GLP-1 increases insulin secretion, β-cell proliferation, and survival, suppresses glucagon secretion, delays gastric emptying, and suppresses appetite, contributing to a potential anti-diabetic effect (Baggio and Drucker, 2007). Unfortunately, GLP-1 breaks down rapidly but the peptide extracted from the Gila monster, designated exendin-4, has much greater stability which led to its introduction to the market in 2005.

Hemotoxic venoms from certain snake species can be used to assay fibrinogen dysfunction, a condition where fibrinogen, a protein involved in blood clotting, is produced defective and cannot perform its function (Marsh and Williams, 2005). Some snake venoms also contain proteases functionally and structurally related to thrombin, an enzyme that is responcible for blood clotting (Castro *et al*., 2004). Snake venoms that affect a prey's blood could potentially have a variety of used in the field of thrombosis and haemostasis (Matsui *et al*., 2000).

1.3.2 Importance to Local Ecology

Food webs are exceptionally complex and easily disrupted, and the removal of any species from its habitat can have knock-on effects for the entire local ecosystem (Dunne and Williams, 2009). For example, a change to vegetation resulted in a simplification of trophic interactions with most of the energy flowing through less trophic pathways than before which caused significant loss of species abundance and diversity (Zeng *et al*., 2014). Lister and Garcia (2018) found that arthropods in the Luquillo rainforest have been declining for the past two decades due to increased temperature, and as the arthropods have declined so too have the local insectivorous lizards, frogs, and birds.

The invasive cane toad (*Bufo marinus)* has caused massive disruptions to the local ecology (Shine, 2010; Burnett, 1997; Smith and Phillips, 2006). Three species of local predatory monitor lizards (*Varanus panoptes*, *V. mertensi*, and *V. mitchelli)* are rapidly declining due to cane toads and their decline caused a marked increase in the numbers of a mesopredator, the common tree snake (*Dendrelaphis punctulatus*) (Doody *et al*., 2013). This is called "mesopredator release" where the loss of an apex predator leads to an uncontrolled rise in mesopredators which often in turn leads to declining prey populations, (Prugh *et al*., 2009). Many reptile species are apex predators in their habitats and the removal of apex predators from a habitat has been shown to have disastrous results for the entire local ecosystem (Reading *et al*., 2010; Myers *et al*., 2007;

Ritchie and Johnson, 2009). The conservation of reptile predators is therefore vital to the conservation of their entire habitat. This is a concept called “top-down” conservation. Sergio *et al*., (2005) found that biodiversity was higher in sites with an apex predator indicating that the presence of an apex predator is beneficial for the ecosystem.

The charisma of high-profile vertebrate predators often makes them candidates for the title of “flagship species”. Flagship species are often used to draw in public support for conservation (White *et al*., 1997), a practice that has had success but also been criticized for limiting the focus of conservation efforts (Andelman and Fagan, 2000). An example of reptile species used as a flagship for conservation are sea turtles which have been used to raise awareness for pollution in the ocean. The use of sea turtles in the ecotourism industry and in social marketing could lead to changes in environmental policy (Eagle *et al*., 2016).

1.4 Importance of Surveying to Conservation

It is essential to the conservation of animals that we have as much information on them as possible. To this end, population surveys have become an essential part of ecology and with good reason. Information gleaned from a thorough and comprehensive survey informs all our conservation efforts. Van der Meij *et al*,. (2015) claimed that: “Robust information on trends in bat populations at a range of geographic scales is essential to the long-term conservation of bats.” In their study, data on hibernating bat populations was aggregated across many European countries revealing a trend that 9 out of 16 bat species had increased at their hibernation sites in Europe between 1993 and 2011, and only one had decreased. This suggests that after a period of population decline in the 20th century some populations of European bat species are stabilising or recovering. This discovery on population trends will be of great value to conservation efforts and was only made possible by examining data from many population surveys.

<u>1.4.1 Informing Conservation Efforts</u>

By monitoring species with periodic surveys, we become aware of changes and trends in their population size and can assess their population status. A prime example of this would be the British Trust for Ornithology (BTO), which designed an alert system to warn them when avian populations were dropping at a rate that warranted investigation or even preventative measures (Baillie & Rehfisch, 2006; Thaxter *et al.,* 2010). The system identifies rapid (>50%) and moderate (>25%)

declines over several timescales, focusing on the longer time periods and using short-term changes to identify continuing, accelerating, ceasing or reversing changes. The alerts advise bodies such as the Birds of Conservation Concern (BOCC) with their listings e.g. the red and amber lists (Eaton *et al.* 2009).

When populations and their changes are properly monitored it allows the appropriate organisations to decide which species are of greatest concern (Laycock *et al*., 2009). The UK Biodiversity Action Plan (BAP) was set out in January 1994 and identifies which species are the highest conservation priority in the UK. Their action plans commit the government to achieving agreed targets (Holloway *et al*., 2003). The document sets out both the rationale for conserving the biodiversity in the U.K. and the resources we devote to sustaining it. It goes on to consider the need to improve the collection of scientific data and species monitoring (Sharp, 1995). The BAP has triggered a wave of research into how we can achieve the goals it sets out. Humphrey *et al*., (2002) discussed the potential contribution of conifer plantations to the conservation of woodland biodiversity to fulfil commitments to biodiversity enhancement outlined in the UK BAP. They found that planted stands had similar or richer fungal communities including many rare and threatened species normally associated with native pine wood, concluding that conifer plantations can make a positive contribution to biodiversity conservation.

1.4.2 Managing Invasive Species

The National Invasive Species Management Plan (NISMP) of the U.S. Department of Agriculture defines the term invasive species as “a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health.” (Beck *et al*., 2008).

For invasive species, measures of population size can help in understanding the extent of the threat the species may pose to other taxa (Sakai *et al*., 2001; Nagy and Korpelainen, 2015; Blossey, 1999). By improving our methods of detecting invasive species we increase the chances of finding them at a smaller population level, lessening the damage, making it easier and cheaper to control (Mehta *et al*., 2007). One of the issues with detecting an invasive species is their low population densities which are typical of species that are just establishing in a new habitat (Mehta *et al*., 2007).

Himalayan Balsam (*Impatiens glandulifera*) is a well-known invasive species in Britain, known for damaging native plant species populations and has been the subject of frequent studies and surveys as a result (Prowse, 2001; Ammer *et al*., 2011; Nagy and Korpelainen, 2015).

1.5 Reptile Survey Methods

The methods that currently exist to survey for reptiles include:

1. Line/Belt Transect (Sewell *et al*., 2012)
2. Refugia
3. Pitfall Trapping (Thompson and Thompson, 2005)
4. Mark Recapture
5. Radio Tracking
6. Camera Trapping

A transect is a path through the habitat being studied along which the surveyor counts occurrences of the species being surveyed (Buckland *et al*., 2001). In some instances, distance from the transect is also measured to estimate density. Transects typically require no equipment as the surveyor is merely walking along a path and recording what they see. Specialised equipment can be used to increase efficiency for example when surveying birds it can help to bring recording equipment to record vocalisations that occur along the transect (Stowell *et al*., 2016). Transects can easily be implemented with other survey methods as the surveyor can employ one when they are walking to collect data from another survey method. For example, a surveyor has set out several traps evenly distributed across the study area. A transect survey can be carried out while the surveyor is walking to each trap. An individual carrying out a transect survey does not require any special training to do so beyond the ability to identify species, which can be assisted with identification guides or other such material. However, many species are skittish around humans and may flee from an individual carrying out a transect survey (Parent and Weatherhead, 2000). Even if the species does not flee from the surveyor the likelihood of encountering any animals can depend on pure luck or the location of the transect. Species are not always homogenously distributed throughout a habitat and so depending on the location an individual transect could not give results that accurately represent the habitat. Multiple transects distributed across the habitat are needed to remove location bias.

Belt transects are used to estimate the distribution of organisms along a belt of habitat. Belt transects record the number and abundance of species found between two lines. The distance between the lines varies and can be as thin as a metre (Smith *et al*., 2005) or as thick as five meters (Velander and Mocogni, 1999). The surveyor walks along the transect and records all species they see (Doan, 2003). On some survey's quadrats are places at certain intervals along the belt; these intervals should be an equidistance apart (Bell and Donnelly, 2006). Animals within the quadrat are counted and sometimes collected to assess the population size and species abundance (Thomas *et al*., 2002). Quadrats are sampled at each marked point on the line or even randomly. Some reptile surveys even incorporate the refugia as markers in the habitat to mark where the quadrat goes. While surveying any disturbance or destruction must be kept at a minimum to avoid scaring the animals off. Animal movement during the transect, sometimes caused by the surveyor themselves, can cause animals to be missed or recorded more than once (Glennie *et al*., 2015). Refugia surveying on the other hand, has very little chance of disturbing the animals. They are placed in the habitat before the reptiles become active, to give them time to warm up, and so the surveyor is often finished before the reptiles are active. Transects involve no method for luring the reptiles in, and simply hope that they will wonder past or stay in place, whereas refugia lure reptiles in by offering them a warmer microclimate. Consequently, it can take a great deal of time and many transects to get sufficient data.

Refugia surveying is a commonly used method for surveying or preserving reptile species in temperate climates, both in the UK and internationally (Hampton, 2007; Grillet *et al*., 2010). It consists of laying out artificial refuges for reptiles to take shelter under or use for basking. Refugia are typically made from metal sheets, either tin or galvanised iron, or bitumen roofing tile (Froglife, 1999; Reading, 1997). These materials are chosen for their ability to conduct heat fast as well as convenience and relatively low cost. The purpose of a refugia is to provide a warm shelter for reptiles (Grillet et al., 2010; Lelièvre et al., 2010) and so it by necessity it needs to be warmer than its surroundings. They create a local microclimate underneath them that is warmer than the surrounding environment and attractive to reptiles looking to raise their temperature, shelter from predators and possibly find food. Once they are set up throughout the reptile's habitat, they are checked periodically for reptiles taking shelter. The reptiles are counted and identified, giving us data to estimate population size and species richness. Refugia should also be properly labelled to prevent members of the public from removing them by mistake. Figure 1b below shows refugia placed in the field.

Figure 1b) artificial refugia placed in the field, image taken from fieldwork taken from this thesis

Pitfall traps are a form of passive collection that involved a pit dug into the ground, often baited, that traps terrestrial animals that fall into them. The pitfalls are designed so that the animals that fall into them are not able to escape. Two main forms of pitfall traps exist, dry and wet (Enge, 2001). Dry pitfall traps consist of a container with a rim at surface level which traps mobile animals, whereas wet pitfall traps contain a chemical solution to kill and preserve the trapped animals. Dry pitfall traps are not designed to kill the animals they trap and so are usually covered by a sloped lid to reduce the amount of rain, sunlight or predators that can enter the trap (Palmer *et al*., 2013; Hobbs and James, 1999). These measures are essential in the case of reptiles. Reptiles in pitfall traps may overheat during midday or die of cold if left overnight. They require daily checks to collect data and to prevent the animals for also starving to death. Wet pitfall traps are fatal to any animal that falls into them, which can be an issue from an animal welfare standpoint. The fluids used include formalin (10% formaldehyde), methylated spirits, alcohol, ethylene glycol, trisodium phosphate, picric acid or even plain water. In all cases this method is lethal to the animals that fall in (Pearce *et al*., 2005). The technique is therefore impossible in any habitat where endangered or protected species might fall into them. Dry pitfall traps are not fatal but require more labour-intensive surveys as they need to be checked daily. However, in the case of many reptiles, pitfall traps can be easily climbed out of making them less effective (Thompson and Thompson, 2007). The technique does not require any specialist training, only access to the required chemicals if employing wet traps and the ability or materials to identify the species caught.

The mark-recapture method is used to estimate a population size when counting each individual would be impractical or even impossible (Pradel, 1996). Firstly, the surveyor captures a number of individuals from the population. Those individuals are then marked in some way to identify them later. Later, a second survey will be carried out capturing more members of the population and the number of marked individuals is counted. This technique assumes that the number of marked individuals within the second sample will be proportional to the number of marked individuals in the whole population (Marten, 1970). Therefore, by dividing the total number of marked individuals by the proportion of marked individuals in the second sample a rough estimation of population size can be achieved. The mark-recapture method relies on having some way to capture animals and so needs to be used alongside another survey technique that lures animals in. the surveyor must also be careful that the method of marking an animal does not cause harm or make it less likely to survive. Capture can be a stressful occurrence for an animal and so care must be taken not to cause any unnecessary fatalities (Gallagher *et al*., 2014; DeNicola and Swihart, 1997; Moore *et al*., 2000). Additionally, many animals require permission to handle and so the surveyor must acquire that permission before undertaking any action. Depending on the status of the species, whether it is protected or endangered, this could be hard or even impossible. What is more, certain species can be too dangerous to handle making this technique dangerous as well as impractical. Additional training might be required to handle a species safely.

Radio tracking involves attaching a transmitter to an animal to receive information in real time about their whereabouts and movements (Cochran and Lord, 1963). The transmitter may be attached to the exterior of the animals or surgically implanted to last longer (Martinelli *et al*., 1998; Weatherhead and Anderka, 1984). The transmitter will send the signal to either a radio receiver or a satellite. The radio receiver will have a directional antenna which will receive the signal most strongly when pointed in the direction of the transmitter. They typically include a means of measuring the strength of the received signal, allowing the surveyor to home in on the correct direction. To keep track of the signal the surveyor must follow the animal manually using the receiver. Satellite tracking has the advantage of not requiring the surveyor to follow the animal in the field. The satellite picks up electronic signals from the transmitter to determine the precise location and track the animal as it moves (Gavashelishvili and McGrady, 2007). Additionally, the satellite transmitters can also provide physiological such as temperature and habitat use (Gavashelishvili *et al*., 2012). This technique could be combined with mark-recapture techniques to great success, as placing a transmitter on the animals requires them to be captures in the first place providing the surveyor with the opportunity to utilise both methods. The transmitters themselves can be used as the mark that denotes an animal as having been captured. The disadvantage of radio tracking, however, is the cost and availability of the required equipment. Transmitters and receivers, especially satellite receivers, are expensive, not readily available and require specialist training to use. Only the most well-funded and professional surveys can use this technique. Potential welfare issues also exist with the transmitters being attached to the animals. There is a possibility that the attached transmitter can impact the health of the animal for example with its weight or from complications with surgical implants (Martinelli *et al*., 1998; Rudolph *et al*., 1998).

A camera trap is a camera that is triggered by movement and placed in an animal's habitat, designed to capture footage of the animals (Kucera and Barrett, 2011). It is a method used for surveying wild animals when the surveyor is not present. Camera traps allow for surveys that involve as little human interference as possible and do not disturb the animals. The exact camera used in the survey must be chosen while considering many factors, such as weather, climate, species present, sturdiness and battery life (Swann *et al*., 2011). Choosing the right camera for the survey will require research into the different models available and the challenges it will face when placed in the study area. The lack of human disturbance is one of the key advantages of camera trapping. When compared to many other techniques, like mark-recapture or radio tracking, there are no welfare issues with camera trapping as it is a non-invasive, discrete survey method that does not impact the animals in any way. The other main advantage is that it is another form of passive data collection with little labour required from the surveyor. However, much like mark-recapture it requires expensive equipment that the surveyor must be trained to use and so would only be available to well-funded surveys carried out by professionals.

Overall, there are four main factors to consider when choosing a survey method: advantages, disadvantages, level of training required and possible animal welfare issues. These factors are summarised for all six techniques in table 1a below.

Table 1a) a summary of the pros, cons, training, and welfare issues of the six main survey techniques

Method	Pros	Cons	Training	Welfare
Refugia	Cheap and effective, animals are drawn to the refugia by their basic need to thermoregulate.	Animals are free to leave; the surveyor may miss the animals that use the refugia.	No real training required beyond being able to identify different species.	Presence of refugia may be beneficial to the animals as they assist thermoregulation.
Pitfall Trapping	Species cannot escape trap, so all individuals can be accounted for, data collected passively.	Covers a small area, requires specific chemicals which are fatal to animals	No real training required beyond being able to identify different species.	Technique is lethal to the animals, cannot be employed on protected or endangered species.
Line Transect	Requires no equipment and can easily be used with other techniques.	Many reptiles are likely to take steps to hide from or avoid the surveyor.	No real training required beyond being able to identify different species.	May force animals back into shelters while trying to thermoregulate.
Mark Recapture	The surveyor knows whether an individual has been caught before and gets a greater idea of population size.	Depending on the species can carry risk for the surveyor or be impractical.	The Surveyor must be legally allowed to capture and handle the animals.	Capture can be distressing for animals; could impact survivability.

Radio Tracking	Provides large amount of data on animal movements and individuals already caught are easily identified.	Expensive equipment not always available to a survey or available in large quantities.	Surveyor must be able to operate the radio tracker.	Equipment weight could affect animal locomotion and impact survivability.
Camera Tracking	Surveyor does not have to be physically present to collect data; cameras will capture most if not all individuals that pass through the study area.	Expensive equipment not always available to a survey or available in large quantities.	Surveyor must be able to operate the camera traps	No real detriment to the animal.

This study will use and focus on the artificial refugia technique, on its strengths and weaknesses and whether it can be improved upon.

1.5.1 Comparison with other techniques

Probably the biggest advantage refugia surveying has is its low impact on the animals themselves. The reptiles are neither harmed nor disturbed by the refugia, and in fact refugia help with thermoregulation and conceal reptiles from predators (Grillet *et al*., 2010; Lelièvre *et al*., 2010). The technique also involves a lot less maintenance when compared to drift fence or pitfall traps (Grant *et al*., 1992). This is especially useful compared to some of the alternatives such as pitfall or funnel trapping, which has been known to carry mortality risks for the animals (Enge, 2001).

Refugia take advantage of the reptile's need to thermoregulate to lure them in whereas transects have no way of luring the animals in; they rely on luck. Refugia do also have a degree of luck because the reptiles are free to come and go and so it is possible that the surveyor can arrive too late or early and miss the animal. However, it remains true that refugia cater to a biological need to thermoregulate which rewards the reptiles for using them. While reptiles in the habitat can be relied upon to visit refugia there is no guarantee that any reptiles will cross the surveyor's path during a transect. Reptile distribution across their habitat is never homogenous but based on a variety of factors (Watling, 2005; Sato *et al*., 2014) and poor luck can mean a transect goes through an area with low density. This also means that with good luck a transect can pass through an area of high density but in either case the transect will provide misleading data. Multiple transects must be carried out across a habitat multiple times to provide enough data, making them much more labour intensive than refugia.

The usefulness of a transect is based on how easy it is to detect the survey animal. Particularly large animals that can be easily seen with the naked eye are ideal (Waltert *et al*., 2008), as are animals that can be identified by their cries which the surveyor could record for later analysis. Smaller, more discrete animals are hard to detect during a transect as they are hard to see with the naked eye and are likely to flee from passing people. While large species of reptiles do exist, most are smaller and tend to seek shelter when disturbed making them hard to detect (Mazerolle *et al*., 2007). A surveyor can often disturb the animals while moving along the transect, making them flee or seek shelter and be less likely to be recorded Refugia provide the animals with shelter. A reptile will likely not flee a refugia until it has been lifted which gives the surveyor time to record the species. It has been recorded in surveys that different reptile species are better recorded by different methods (Reading, 1997).

As such, refugia are more likely to produce a result provided the surveyor visits in the optimal time window. However, the inverse is also true, outside the optimal time window of refugia the animals will have successfully competed thermoregulation and so will be active, making them more likely to be found on a transect.

Refugia and pitfall traps both have the advantage of being able to lure the target animals in, refugia by providing them with thermoregulation opportunities and pitfalls by way of bait. At first glance pitfalls have the additional advantage of trapping the animals whereas refugia allow them to come and go. A dry pitfall trap designed for reptiles needs to be larger than the average one (Morton *et al*., 1988) and have specific modifications to prevent climbing. These pitfall traps require still more modification as there must be a roof to prevent overheating in the sun

(Hobbs and James, 1999) and must also be visited regularly, as reptiles left in overnight are likely to die of cold (Enge, 2001). The purpose of using a dry pitfall trap over a wet one is to avoid fatalities but in the case of reptiles this requires a lot of additional modification and labour. Refugia by comparison has no risk to the reptiles. Refugia can be visited daily, and it is better to do so, but it is not necessary to prevent deaths among the target species as it is with pitfall trapping. More so than any other animals, reptile activity is heavily dependent on weather and therefore so is the performance of pitfall traps (Read and Moseby, 2001).

Only wet pitfall traps require no additional modifications or labour when used against reptiles. They can also reliably prevent escape, but this is because the animals are killed by the solution. This makes pitfall trapping costly to the species and the habitat overall. This technique can therefore only be used when the reptile population is robust enough to withstand the casualties inflicted upon it by the traps. Many species of reptile are protected and so techniques that are fatal are not an option when surveying them. Pitfall traps can also lure in animals outside the target species and therefore cause unintentional fatalities among other local species.

Comparing mark-recapture and refugia is difficult as there are fundamental differences between the two survey methods and what they are trying to accomplish. Refugia surveying is a method to lure in and detect reptiles. Mark-recapture involves marking caught reptiles and carrying out an additional survey that looks at the proportion of marked animals caught. The reptiles have already been detected and caught. A surveyor cannot carry out a mark-recapture survey without using an additional method to lure in the reptiles; it depends on another survey method to function. In that respect, refugia has a distinct advantage over mark-recapture as it can be used independently without relying on other techniques. Additionally, mark-recapture surveys are quite limiting as they exist for one specific function: to estimate population size. It performs this function well (Moore *et al*., 2010), better than refugia surveying alone, but cannot be used outside this narrow focus. Refugia surveying on the other hand can be used in a variety of academic and environmental studies, making it more flexible. Mark-recapture may be better at that one and only purpose but for any other survey purpose refugia would be a better choice.

Rather than simply compare them against each other, it can be helpful to look at how they work together. Mark-recapture relies on another technique to draw in the reptiles, and refugia is a relatively easy technique to do so that does not require expensive or specialist equipment or training (Boughton *et al*., 2000). However, it has a drawback in that it does not actually trap the reptiles. The reptiles are free to come and go beneath the refugia and once it is lifted the surveyor must act quickly to capture it before it escapes. Other survey methods exist which do not allow the reptile to leave, such as pitfall and funnel traps, which might be better partner techniques than refugia. Instead of directly comparing mark-recapture to other techniques it might be better to look at it as a potential "add on" to those techniques. An extra step that increases their power and usefulness.

Comparing refugia to radio tracking is in many ways like comparing refugia to mark-recapture. Again, refugia is a method for detecting reptiles whereas radio tracking requires reptiles to have already been caught. Radio tracking has a single specific purpose: tracking the movements of the reptiles in real time (Koenig *et al*., 2001; Lee *et al*., 2009; McMahon *et al*., 2007). Refugia has a more general purpose and can be used in a wider variety of studies. The main advantage of refugia when compared to radio tracking is the lack of specialist equipment and training required to perform a refugia survey. A refugia survey is incredibly easy when compared to radio tracking and additionally is a great deal cheaper. However, it cannot provide the kind of information or data that radio tracking can. Refugia has no way of tracking an individual reptile's movement, all the surveyor knows is that the reptile moved from an unknown starting point to the refugia. Radio tracking can provide detailed data on reptile activity and habitat use. The two survey techniques accomplish different things for the surveyor.

Refugia and camera trapping are both methods to detect reptiles in the field. At first glance, refugia has the advantage of being able to lure the reptiles in whereas camera traps only capture images of the animals if they happen to wander past, relying heavily on luck. This, however, can be mitigated by strategic placement of the traps where the reptiles are most likely to pass (Cusack *et al*., 2015). The disadvantage of this is that it requires a good deal of research into the habitat before hand and possibly some scouting or prior survey to locate the best spots. Additionally, deliberate placement of camera traps in areas expected to produce results can result in a bias withing the data (Kolowski and Forrester, 2017). A lot of groundwork needs to have been completed to ensure that the cameras are able to maximise their potential. This is also true for refugia to an extent, as they also need to be placed strategically, but to a lesser extent. Refugia facilitate thermoregulation, a basic need of reptiles, meaning that the reptiles will seek them out if they are there whereas the camera traps offer the reptiles nothing. This problem can be overcome by using bait to lure the animals to the trap. Much like radio tracking, camera traps are expensive and require training to use, whereas refugia are cheaper and require no training beyond an ability to identify different reptile species. This makes a refugia survey more accessible as it requires a lower budget, level of training and refugia gone missing are easier to replace. On the other hand, camera traps can be used for more than just reptiles whereas refugia is reptile exclusive. This means that if the surveyor habitually surveys more than just reptiles then camera traps become more cost efficient as they provided a survey technique for many different surveys. This point was brought up by Welbourne *et al*., (2015) in their study which directly compared camera trapping with traditional methods. They found that camera traps did give more results but not significantly more. Camera traps have typically struggled to capture images of smaller animals but Hobbs and Brehme, (2017) developed modifications to the technique to mitigate this which would be of particular use surveying smaller reptiles.

Both techniques have their weaknesses, but a lot of those can be overcome by using them together. Camera traps need to be placed in a location the reptiles are going to frequent; refugia can provide that location as the reptiles will visit them often to thermoregulate. Reptiles visiting a refugia are free to come and go, meaning the surveyor can miss them. A camera trap placed near a refugia can capture images of every reptiles that visited, countering that weakness provided the refugia is placed in a location that does not have too much cover so that the reptiles can be seen approaching it.

1.5.2 Criticism of Refugia

A major disadvantage of refugia surveying is its seasonal nature. Reptile activity is known to vary with the seasons (Brown and Shine, 2002). In many parts of the world reptiles hibernate, meaning that refugia surveying can only take place for as little as half of the year (Nussear *et al*., 2007; Carver *et al*., 2000). In the spring and autumn of temperate regions temperatures are milder and so reptiles are more likely to need the refugia for basking. During the summer, the higher ambient temperatures make reaching the optimal body temperature easier, reducing the need to bask and making the refugia less attractive to reptiles. This means that more data are likely to be collected from surveys completed in the spring or autumn (Thompson and Thompson, 2005). Many surveys therefore take place in either spring or autumn (Gamble, 2003) or in the dry season (Labanowski and Lowin, 2011). In tropical regions where reptiles do not hibernate it is possible to survey year-round but higher ambient temperatures make refugia a less effective surveying method overall. Visual searches and quadrats are known to be better than refugia surveying in tropical rainforests for instance (Doan, 2003), where high temperatures and canopy covers render refugia ineffective.

As well as being limited to certain times of year, refugia surveying is also limited to certain times of day. Reptile activity typically peaks in the morning to bask in the rising sun, drops off at midday to avoid overheating and peaks again in the afternoon (Hailey and Coulson, 1996). It is during these two peaks that reptile surveying can take place, as the reptiles are looking to bask and are more likely to make use of refugia. Reptiles in other parts of the world will be active at different times of the day due to climate differences and even difference in sexes (Kerr and Bull, 2006). No-one has yet to devise a method for surveying reptiles outside of these daily peak times when they are sheltering. The result is that refugia are a very time sensitive method and are in fact useless more than they are useful. Only being useable at certain times of the day and at certain times of the year are clearly very limiting restrictions.

The refugia technique has little standardisation. There are three materials commonly used (galvanised iron, tin and bitumen) but there exists little to no literature about which material produces the best results. Some organisations and studies use other materials or mix of different materials. Some studies have reported greater success with one material (Hodges and Seabrook, 2016), but to date, there are no studies that focus on finding the best material. In this instance, we define the “best” material by the following criteria:

1. Attracts the most reptiles and produces more data in field studies.
2. Stays at optimum temperature for the longest, giving surveyors a larger time window
3. Convenient to acquire and not too expensive to be used in large numbers

No experimentation exists for finding new materials that may yield better results. The technique has been standardised for many years with no research into improvements or alterations.

1.5.3 Advances made in Scientific study using Refugia Surveying

Since refugia surveying is so effective it is used in a great number of studies, and the method has contributed to many advances and discoveries made in reptile ecology and conservation. For example, refugia surveying can be used to measure different aspects of reptile populations, including the species richness, the relative abundance of each species and population sizes.

Sewell *et al*., (2012) used refugia in a comprehensive survey of all reptile species in the UK, with the aim to design a survey method that could be used to detect declines in species. To do this they needed a method that was highly efficient in detecting the species present and their abundance so that, through regular surveys, a detailed record could be compiled over time and trends could be detected. For the four widespread reptile species in the UK, grass snake (*Natrix natrix)*, adder (*Vipera berus)*, common lizard (*Zootoca vivipara)* and slow worm *(Anguis fragilis)*, three to four survey visits using a combination of transects and refugia resulted in 95% chance of detecting species presence. They found that refugia significantly increased detection rates and, with the chance to see the species up close, reduced the chance of misidentification.

Refugia were used to investigate the role of the introduced house gecko (*Hemidactylus frenatus*) in causing the decline of the endemic night gecko (*Nactus coindemirensis*) of the Mascarene Islands in the south of the Indian Ocean (Cole *et al*., 2005). Competition for space was tested using refugia, the abundance of each species measured in positions close to and on the refugia. This was a comparative study, measuring the number of each species that made use of the refugia and using those figures to determine which was more successful at competing for space. The house gecko was observed to be displacing the night gecko from the refugia, increasing the risk of predation and reducing the efficiency of thermoregulation. It was therefore concluded that the house gecko was contributing to the decline of the night gecko.

1.6 The Aims of this Thesis

The purpose of this thesis is to improve the technique of artificial refugia. The primary aim is to prove empirically which out of the commonly used refugia material performs the best. For the purpose of this thesis, the “best” performing refugia is considered to be the refugia which:

a) Reaches the optimum body temperature for reptiles most reliably

b) Does not regularly reach temperatures hazardously high for reptiles

c) Heats up swiftly and cools down slowly, providing the reptiles with more time to bask and the surveyor with a larger time window to find them

d) Attracts the greatest number of reptiles and the widest variety of species providing more data for surveys

Of the four factors that make up the ideal refugia the fourth is considered to be the most important as attracting reptiles is the singular purpose of a refugia. The secondary aim is to investigate possible new materials for refugia in an attempt to further improve the technique and possibly inspire further investigation by proving the technique can still be advanced.

The thesis will be divided into three chapters beyond this one, one for each of the methods used. The second chapter will cover the laboratory work and the investigation into the thermal properties of classic refugia materials and the new ones we have introduced. From this data we will for our first hypotheses about the materials that will perform better in the field. The third chapter will cover a global online survey conducted to investigate trends among the academic community as to how they use refugia. From this we will learn which materials are favoured by which organisations and what other factors are considered important for surveyors. The fourth chapter covers the fieldwork where the refugia are tested in real reptile surveys. The hypotheses formed in the second chapter and built upon in the third chapter will be tested in the field and the results of these test will form out final conclusions. After those there will be a fifth chapter that will outline those conclusions, how we arrived at them and the significance of them.

Chapter 2: A Global Survey of Refugia use

2.1 Introduction

When carrying out surveys it can sometimes be an unintended consequence that the surveyor can discover something new about the methods they are using. In the study by Čeirāns and Nikolajeva (2017) on diet preference of the smooth snake (*Coronella austriaca*) the duration artificial refuges need to be kept on site to be most effective was found during the survey itself, as well as the number of visits required to determine a species' absence. This is an example of people learning more about how a method works by using it. Sometimes ecologists launch entire research projects simply to test new methods. Michael *et al*., (2018) launched a study to investigate the viability of a methodology they had invented for surveying arboreal reptiles, something they had found difficult with traditional refugia methods. Their new method, artificial bark refuges consisting of closed-cell foam attached to eucalypt trees, found nearly 132 times more individuals of the arboreal southern marbled gecko (*Christinus marmoratus*) than the traditional refugia. These studies show that the scientists actively carrying out refugia surveys are the ones innovating it and learning more about how best to use it and its limitations. Therefore, to learn about refugia they are the best sources of information.

The purpose of this survey is to investigate trends among the academic community for which refugia material or mix thereof has given the best results for them, and why they believe that is. The hope is to discover a noticeable trend among the scientists, a specific material that they claim has the best results. Refugia surveying is a common practice among herpetologists, but they do not all use the technique in the same way. With an online survey we can find out the prevalent trends among the scientific community. For example, we can find out which materials are most popular among ecologists. It is in the best interest of professional ecologists to use the best possible technique for surveying. Therefore, the techniques used by the most herpetologists are likely to be the most tried and tested and so are of great interest to this research. It was also worth finding out what they aren't using. Part of the purpose of this research in to find potential new materials for refugia surveying that may be better performing that the standard ones. Materials that have been identified as possible replacements in this research but are not mentioned by the responders will be of particular interest.

2.2 Methodology

The World Wide Web (WWW) is a frequent platform for survey research, offering two types of electronic or online surveys: the email and Web based survey (Van Selm and Jankowski, 2006). A Web-based survey is the collection of data through a self-administered questionnaire online. The email-based survey involves emailing members of your study group directly. The weakness of a web-based survey is that any member of the public can stumble across it and influence the results. Schaefer and Dillman, (1998) pointed out that online surveys are limited to people that possess internet connection and thus cannot always be representative of the general public. It is a limitation of online surveys that there are certain demographics and parts of the world where they cannot be effectively employed. Among certain population groups however, the internet is a staple of life and thus they can be surveyed online with ease. The target demographic for this research are academics in Britain and beyond that have undertaken reptile surveys. Since the academic community is online then there will be no problem reaching out to them with an online survey.

Stanton, (1998) made a comparison between online and paper surveys. They found that the online surveys had fewer missing values than the paper survey, suggesting that online surveys have a higher completion rate than paper ones. Two psychiatric interviews about child mental health were administered to parents by Heiervang and Goodman, (2011). In 2003 the parents were interviewed face-to-face, whereas in 2006 they completed the interview online. Both interviews were preceded by paper questionnaires. The number of online participants was comparable to the response rate for face-to-face interviews. However, the number of participants who completed all sections of the interview was much lower for web-based interviews, a result that contradicts the findings of Stanton, (1998). Although less participants completed the online survey, the time and cost were only a quarter of that for face-to-face interviews. While the online surveys have questionable completion rates, and cannot necessarily reach all groups of people, their ease of use combined with low costs and quick completions times are why it was decided to employ them in this study. An online survey spread through social media will be used to collect data and opinions from professional herpetologists about their use of refugia. The expert opinions of the herpetologists will be compared to the findings from the quantitative methods, which will be lab and field work.

The survey was written of a website called SurveyMonkey, a website for hosting free online surveys. Once written it was circulated on herpetology communities on social media sites, including Facebook, Twitter, and Reddit. Herpetology pages or individual herpetologists with a large following were messaged directly asking to spread the survey throughout their follower base. Other methods for spreading the survey included direct emails to organisations and universities. Every university in the UK with an ecology or related degree was directly messaged, asking to circulate the survey throughout staff and students. Every branch of the wildlife trust was also messaged as well as various ecological consultancies.

Five questions were asked, each with their own purpose in the survey.

Question 1: Where are you based? This question allows us to see if the trends in opinions are localised to specific regions or countries. If so, then it is likely that factors unique to those countries, like climate, are affecting the prevalent trends in surveying.

Question 2: What kind of organisation do you survey for? With this question we can see which organisation carries out more surveys. If more responses come from one organisation than any other, then we can assume that organisation is more common and carries out more surveys. The material they use will be of greater interest.

Question 3: What are your main materials? This is likely the most important question of the survey. With this we can find out directly what materials are used, and which are the most popular. We can then link the results of this question to the previous one and find out which organisations favour which materials.

Question 4: On average, how many do you use when surveying any one site? This question lets us gauge how important cost is to determine which material is best. If surveys typically use dozens, if not hundreds of materials then it is important for the best material to be relatively cheap as well as well performing.

Question 5: How big are your refugia? Like the previous question, this is about cost. Larger refugia cost more and so if refugia are typically quite large then it is important for them to remain quite cheap. Additionally, it could be that size affects performance. Larger refugia may take longer to heat up and smaller ones may take less time. This could alter how suitable they are for surveying.

Once we have collected the data from our survey, it was important to test if there were any correlations or relationships between the answers. We were looking to see if responders from specific organisations favoured specific materials. In order to test this a chi squared association test was performed on the results of questions 2 and 3. This test would find out if there was a statistically significant association between the organisations and their favoured materials, which we would use to determine which material was preferred by the professionals who carry out more surveys.

2.3 Results

Question 1: Where are you based?

The purpose of this questions was to see if the materials used by herpetologists varies between regions. Of the 117 responses 17 were outside the UK. None of the responses outside the UK used the most popular materials within the UK, iron and bitumen. This implies that those materials are a most effective locally and might not produce results as strong outside the UK climate. In light of this we must consider that any results gained from this study will only be relevant within the UK and that the local climate has a strong effect on the effectiveness of materials for reptile refugia surveying.

Question 2: What kind of Organisation do you survey for?

By asking this question, we can identify trends in material use between different professional bodies that carry out reptile surveys. These organisations carry out regular reptile surveys and so it would be safe to assume a high level of competency and awareness of the most effective methods and materials. Therefore, the materials used by them will be of great interest. Additionally, the number of responses for each category gives us an idea of which organisation carries out more reptile surveys and thus is likely to have greater knowledge and experience. Environmental consultancies were the most common responders with 35%, closely followed by Environmental NGO’s at 34%. If these organisations made up the highest number of responses, then it is safe to assume they carry out more surveys than the other organisations. The materials used by

Environmental consultancies will therefore be of higher interest than other organisations with NGO's being a close second.

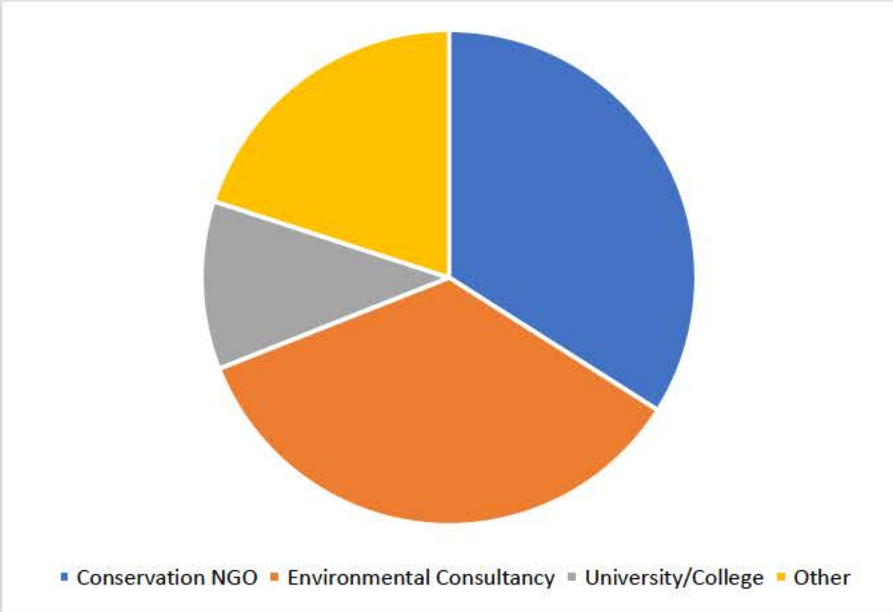

Figure 2a) responses for each option in question 2

Question 3: What are your main materials?

This question links into the focus of this research: to quantify the best material for refugia surveying. With this question we find out what materials are most favoured by the professional herpetologists. As stated above it is assumed that professionals will be using materials that provide strong results and so whichever material is most popular among them is likely to be the best performing material overall, at least as far as the responders are aware. Bitumen was the most popular by a large degree at 50%. The next most common material was the "other" category. However, among the "other" category were a number of responders who used an alternate term for bitumen, including onduline or roofing tiles. This means that the real number of responders who favour bitumen is even larger than 50%. The next most popular single material was tin. However, when gathering materials for study tin was found to be especially hard to come by in the dimensions needed (50cm x 50cm, common size for refugia). Additionally, tin is a highly expensive metal, far more so than iron which was not as popular a metal. The popularity of tin was surprising as is does not seem a readily available or practical material. It is possible that there has been a misunderstanding. An alternate name for refugia surveying is "Tinning" which implies the use of tin but does not necessarily mean tin is used. Additionally, tin is very rarely used as a pure metal but alloyed with others. So called tin cans are actually made from tinplate, which is steel sheet metal coated with a thin layer of tin. It is therefore assumed that responders did not refer to pure tin, but iron or steel coated with tin instead.

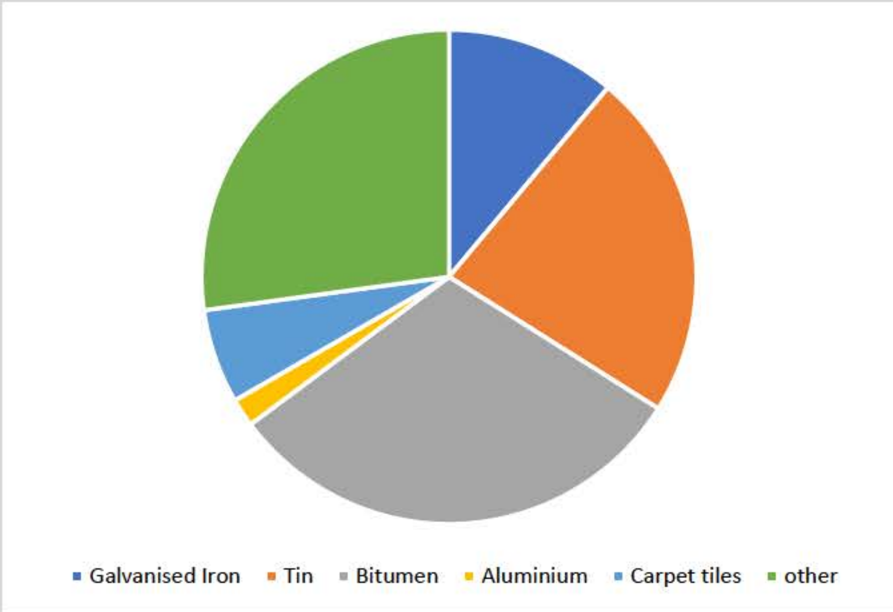

Figure 2b) responses for each option in question 3

Question 4: On average, how many do you use when surveying any one site?

Depending on the material there can be a significant cost to refugia surveying. Larger sites will require more refugia and if they are made of expensive material then the cost of the survey could rise impractically high. Therefore, cost can be an important factor to consider when carrying out a refugia survey depending on how many refugia are needed. Cheaper materials may be more practical for surveys. The purpose of this question is to try and gauge the average amount of refugia used on any given survey. The answers received were of a huge variety; the most refugia used was 300 whereas the least was 2. The only consistent answer given was that the amount of refugia used depended massively on the size of the site being surveyed. Bearing this in mind the ideal material will not simply be the one that performs the best but is also cheap enough to allow thorough surveying of a larger site.

Question 5: How big are your refugia (mm x mm)?

This question follows the same principle as the previous one; the larger the refugia the more an individual one will cost and the more a survey will cost. There is also the possibility that a larger or smaller refugia will perform better in the field. A larger refugia will take longer to heat up but conversely will not overheat as fast.

A smaller refugia will have the opposite, heating up faster but getting too hot faster. The most common answer was 500mm x 500mm. These are the dimensions sold by many ecology survey suppliers and therefore the most commonly used. It is safe to assume that this size is the most effective or strikes the best balance between effectiveness and cost.

A Chi squared association test was used to determine whether there was a statistically significant relationship between the materials used and the organisations using them. Table 2a below summarises the data, showing how many of each organisation said each material. Many organisations responded that they used more than one material. It is important to note that for this test every response of "other" that listed alternative names for bitumen (such as onduline or roofing tiles) were considered as bitumen responses instead, increasing the number of bitumen responses in this test compared to the original survey. The test revealed that there was a significant association between materials used and organisations using them (chi squared test: chi square = 26.2, df = 15, P = 0.036).

Table 2a) the number of responses for each material from each organisation

	Conservation NGO	Environmental Consultancy	University/College	Other
Iron	9	7	1	2
Tin	15	16	1	7
Bitumen	19	31	3	5
Aluminium	1	2	0	1
Carpet	4	5	1	1
Other	8	10	8	12

2.4 Discussion

The final conclusion we can draw from the survey is that 500mm by 500mm bitumen squares are the most popular refugia material with herpetologists in Britain. This is consistent with the literature we have ready where surveyors utilised bitumen refugia exclusively (McInerny, 2017; Hubble and Hurst, 2006; McInerny, 2019). The amount used will vary based on the size of the site but generally herpetologists prefer to use as many as reasonably possible. At least 30 is the number recommended by Sewell et al (2012). On question 4 34% of responders stated they used more than 30, although most simply said the amount used varied based on site. The reason for this is most likely due to its proven effectiveness in the surveys carried out by the organisations but there may be an element of cost involved as organisations must also strive to minimise the expenses of their surveys. Cost is such a significant factor that studies have been launched to find methods that minimise cost. Welbourne *et al*., (2015) tested the costs and effectiveness of camera traps vs traditional methods and found that they were both more effective at detected mammals and squamates and cheaper in the longer run.

Environmental consultancies made up the majority of the responses and answered bitumen for preferred material more than any other material and more than any other organisation did. Environmental NGOs were the next highest by a margin of only 1%. They also responded with bitumen as their most used material but not as much as consultancies. There was also less of a gap between bitumen and their next most preferred material, which was tin. As stated above, it is assumed that professional organisations have a high degree of competency in reptile surveying and the organisations that made up the largest number of responses carry out a larger number of surveys. Therefore, with the two organisations that carry out the most surveys choosing bitumen as their preferred materials the next step is to find out if this relationship is statistically significant. Of course, it is always possible that these assumptions, and therefore these conclusions, are incorrect.

The Chi squared test showed that there was a relationship between the organisations and the materials they used. The organisations that carry out the most surveys all used bitumen as their most commonly used material. With this, the online survey suggests that bitumen is the most optimal material known for reptile refugia. It is favoured by professional bodies who carry out frequent reptile surveys and so likely strikes the best-known balance between results and cost effectiveness. This is valuable information for anyone carrying out a reptile survey looking for a reliable technique that produces strong results. It’s popularity over other materials implies that it has been found to out-perform them by the organizations that carry out the surveys and choose to use it. The next step will be to test these materials in the field to try and prove quantitatively that bitumen is the superior material. Alternatively, it may be revealed that there is another material that outperforms bitumen and that the popularity of the material is based on a misconception among the scientific community. Going forward, the hypothesis was that bitumen is the optimal material for artificial refugia surveying of reptiles in Britain.

Chapter 3: Lab work

3.1 Introduction

Refugia in the field provide a twofold purpose, providing the reptiles with a microclimate that is warmer than the surrounding environment and facilitates basking and providing them shelter from predators. Snakes, mainly smaller ones, will sometimes sacrifice efficient thermoregulation for safety from predation (Webb and Whiting, 2005) and so refugia also fill the purpose of shelters as well as thermal microclimates. Thermoregulation, however, is the primary reason reptiles visit refugia and so this chapter is concerned with how the refugia act as microclimates and how they behave thermally.

Reptile are known to actively seek out microclimates in their environment for thermoregulatory purposes (Jones, 2015; Grant and Dunham, 1988; Guillon *et al*., 2013) and do not discriminate between natural and man-made refuges (Zappalorti and Reinert, 1994). Different materials will have different thermal properties and so the suitability of any one material will differ from any other. Studies have sometimes been launched to assess the suitability of specific materials as refugia (Glorioso and Waddle, 2014). Different materials perform differently in the field; some attract more individuals overall or more of one sex than the other (Hodges and Seabrook, 2016). It is necessary then, to conduct a rigorous thermal study of different refugia materials.

The purpose of the lab work was to study the thermal properties of the different materials that could be used in a reptile survey. With the results of these tests predictions would be made as to which material will perform the best in later field tests. These results would also be compared to the results of a global survey of herpetologists on how they use refugia, to see if the best material according to this research was the material most commonly used. The lab tests will measure three factors likely to be important to the usefulness of a refugia:

1. How quickly does the material heat up?
2. How high does the temperature get?
3. How quickly does the material cool down?

The ideal material would be one that heats up quickly but does not reach a temperature too high and stays at that temperature for an extended period of time.

The hypothesis was that whichever material best fits this model will be the material that is most popular according to the online survey and will perform best in the field.

3.2 Methodology

3.2.1 Material Selection

The first step in the study was to select materials. Iron and bitumen are the most commonly used materials according to the various guides to reptile surveying (Reading, 1997; Fish, 2016; Glorioso and Waddle, 2014) and according to our online survey (Chapter 3). Aluminium and brass were used because they were supposedly better conductors of heat than iron and were readily available from the same suppliers, therefore are just as easy to acquire. Carpet tiles were also mentioned in the online survey and are both cheap and readily available. Therefore, the materials that were to be tested were: iron, bitumen, carpet, aluminium, and brass. One of each material was purchased to be used in this experiment. The standard refugia shape is either corrugated or sinusoidal, however we were unable to acquire carpet, brass, or aluminium in that shape. Therefore, for the sake of managing independent variables, all the materials were kept flat. A possible advantage bitumen had over the other materials is its colouration. Black is known to absorb more heat than any other colour. Shiny materials, like metals, reflect light and so do not heat up as quickly. It was decided to include two versions of every metal, one standard and one painted black. By painting them black we give the materials every chance to perform as well as possible. This also ensures fairness as the bitumen and carpet were also very dark colours and darker colours absorb more heat.

3.2.2 Setup

This examination involved using strong heating lamps to heat the materials for set periods of time; they were then left to cool for an equal period. The specific lamp used was a 25cm Deluxe Porcelain Brooder lamp, with a 100w lamp emitting infra-red, as opposed to visible light. To ensure the lamp would trigger a noticeable change in temperature and thus create usable data it was hung close to the material, approximately 30cm. When used in the field a refugia is heated by the sun until it becomes a warm refuge for reptiles. The purpose of this lab method is to mimic that heating. It is a weakness of the method however, that the lamps will only ever approximate the real field conditions. Since they may not accurately mimic the effects of the sun the results from this test will not be as reliable as we would like. The alternative would be to leave the refugia outdoors to be warmed by the real sun. This, however, suffers from the inconsistency of natural weather where we will be left to wait for a day with favourable conditions. We would also be unable to take measurements during the Autumn and Winter months as reptiles are known to hibernate (Carver *et al*., 2000). The advantage of this lab study is, while it may not be fully accurate of real conditions, it is accurate enough to inform our later fieldwork and can be done all year round without waiting for the right weather. The materials were tested on turf, which brought the experiment closer to real world conditions thus increasing its reliability. To ensure that any anomalous increases or lack thereof of temperature were not the cause of abnormally high or low temperatures in the lab, ambient temperature was taken from the room each day.

3.2.3 Process

A heat probe was placed underneath the material, directly under the lamp. Specifically, a LogIT explorer data logger was used. Thirty minutes was deemed a manageable length of time but still long enough for the material to heat up noticeably and so every material was subject to thirty minutes of heating and then thirty minutes of cooling. How fast the material cooled and how long it retained its heat are important factors in determining how suitable a refugia it would make. The longer it held heat the longer a reptile population could use it. Starting temperature and peak temperature were used to measure the total temperature increase after thirty minutes of heating. Measuring temperature at two-minute intervals gave enough data points to track precisely the increases and decreases in temperature. This resulted in thirty data points per replicate. Five replicates were conducted per material. It is important to note that since the experiment was conducted during the months of May and June, ambient temperatures were higher that they would have been at other times of year. It is possible that the ambient temperatures of the room had an effect on the temperatures of the materials.

To precisely measure exactly how long the materials can hold heat after the source of heating has been removed an additional experiment was run where the materials were heated until their temperature increased by any measurable degree, then left to cool until they returned to the starting temperature. The purpose of this experiment was to determine how much faster the materials gained heat compared to how fast they lost it. For example: material X increased by 1 degree in ten minutes. When allowed to cool it took twenty minutes to lose 1 degree. By simply dividing time spent cooling by time spent heating (2 ÷ 1 = 2) we can assess how much faster the material gained heat compared to losing it. In this case, material X heats up twice as fast as it cools down. For the purpose of this experiment the amount of heat gained is not relevant, only the time taken to gain and lose it. 0.1 degree is the smallest unit the probe could detect changes in. by only heating by this increment the experiment could be run faster.

After the data has been collected it will be tested for statistically significant differences. Because there are more than two samples, we used a one-way ANOVA to find out if there is a statistically significant difference and Tukey post hoc to find which means are statistically different from.

The lab work results were compared to the fieldwork results to help determine the kind of refugia that gain the best results. In this instance, the best results were defined as the most reptiles found underneath the refugia. For example, do the refugia that hold their heat for longer attract more reptiles, or do the materials that attain a higher temperature? Our aim was to try and standardize the practice of refugia surveying. Currently, different organisations favour different materials or a mixture of them. We wanted to find if a mixture of different materials is the most effective, or if there is a single material that outperforms the others and if so, why?

3.3 Results

3.3.1 First Experiment

In terms of swift heating and temperature increase Bitumen outperformed all other materials. Starting at an average temperature of 22.98°C in half an hour it reached an average of 26.82°C and peaked two minutes later at 26.84°C. This amounts to an overall temperature increase of 3.86°C, the highest of all materials. Figure 3a below shows the temperature over time of Bitumen during the lab experiment. We see an initial, rapid increase of temperature within the first ten minutes. After that temperature increase slowly plateaus until the heating bulb is turned off when we see a swift decline. After thirty minutes without heating the material had cooled to an average of 24.68°C, which is 2.16°C lower than peak temperatures and 1.7°C higher than starting temperatures. While this is a considerable drop in temperature the material did not return to its starting temperature; 44% of temperature gained within half an hour of heating remained after half an hour of cooling.

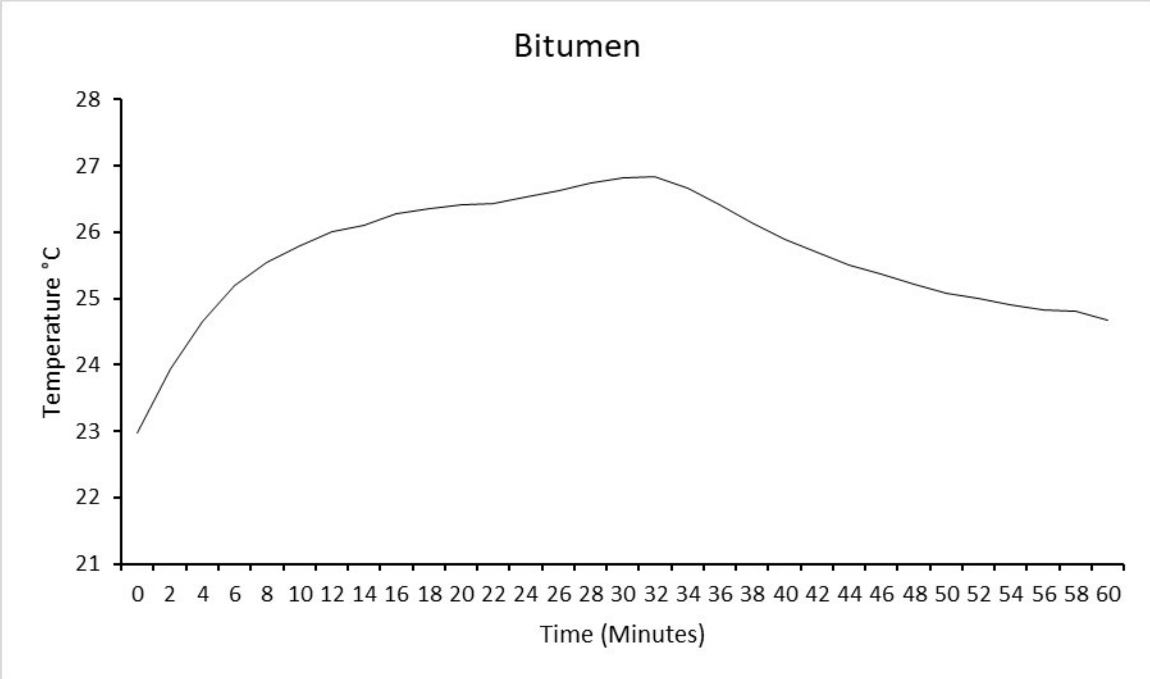

Figure 3a) temperature profile of bitumen

The second most common material, iron, displayed significantly lower results. Starting at an average temperature of 23.02°C it peaked at an average of 23.3°C, this means a total average increase of 0.28°C. At 3.86°C, bitumen increased by a factor roughly thirteen times greater. After the heating bulb had been turned off for thirty minutes the iron sheet was at an average temperature of 23°C, slightly less that its average starting temperature. Figure 3b shows the curve of temperature over time and how, compared to Bitumen, temperature change was minor.

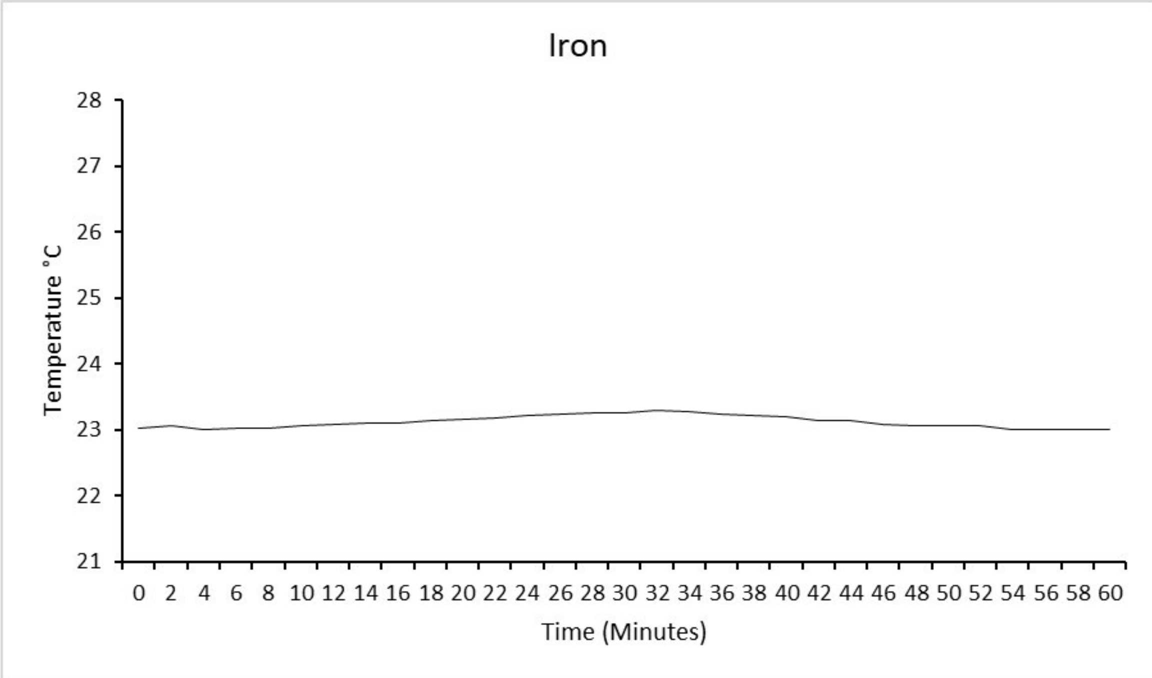

Figure 3b) temperature profile of iron

Another commonly used material was carpet. This material had a much more pronounced curve, shown in figure 3c below. Carpet had the lowest starting temperature of all materials, averaging at 21.16°C, however this is not one of the factors we deemed important. Peaking at an average of 22.36°C the average total temperature increase was 1.2°C. The end temperature was 21.58°C, 0.42°C higher than its starting temperature. This means that 35% of temperature gained within thirty minutes was kept after thirty minutes.

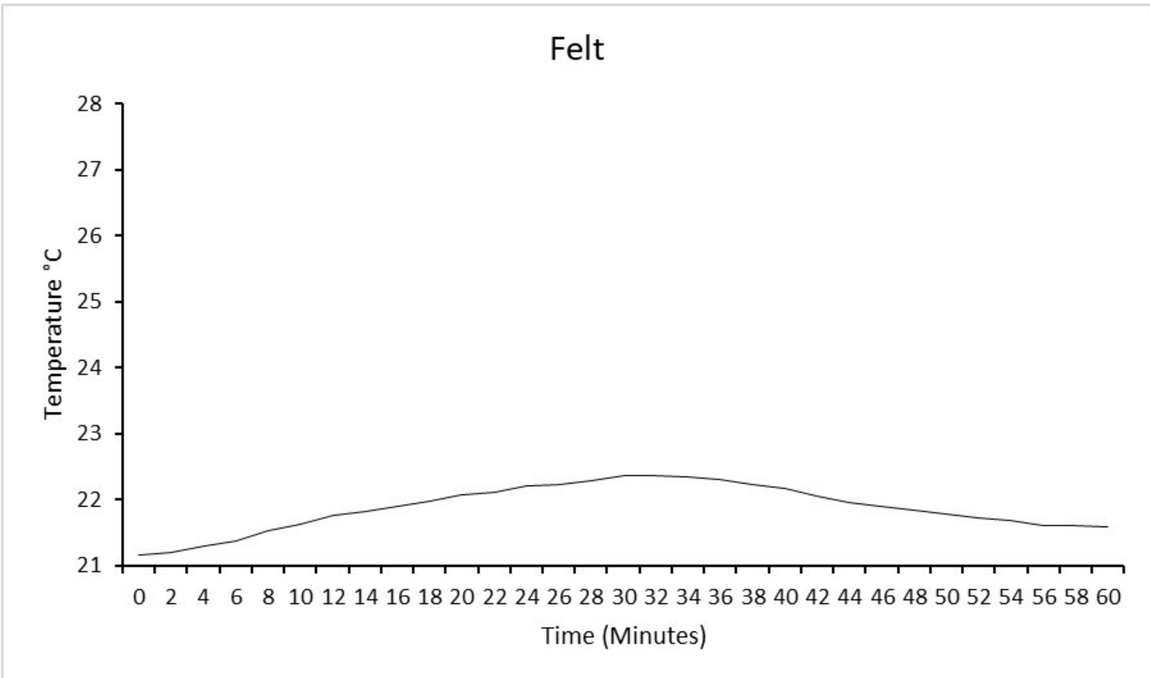

Figure 3c) temperature profile of carpet

After testing the most commonly used material we moved on to the new materials tested for the first time in these experiments. The first was Brass. Seen on figure 3d below, Brass initially had a swift increase in the first four minutes but stopped increasing after that until the heat bulb was turned off, where it slowly decreased in temperature. At eight minutes Brass had reached 23.94°C, and at twelve minutes had reached 23.96°C, the highest it would reach. It then slowly alternated between these two temperatures until the heating bulb was turned off. Its starting temperature was 23.32°C, meaning an average overall temperature increase of 0.64°C. Its final temperature was 23.72°C, still 0.4°C higher than its starting. This means that while it did not increase by much compared to other materials it still held 62.5% of its temperature.

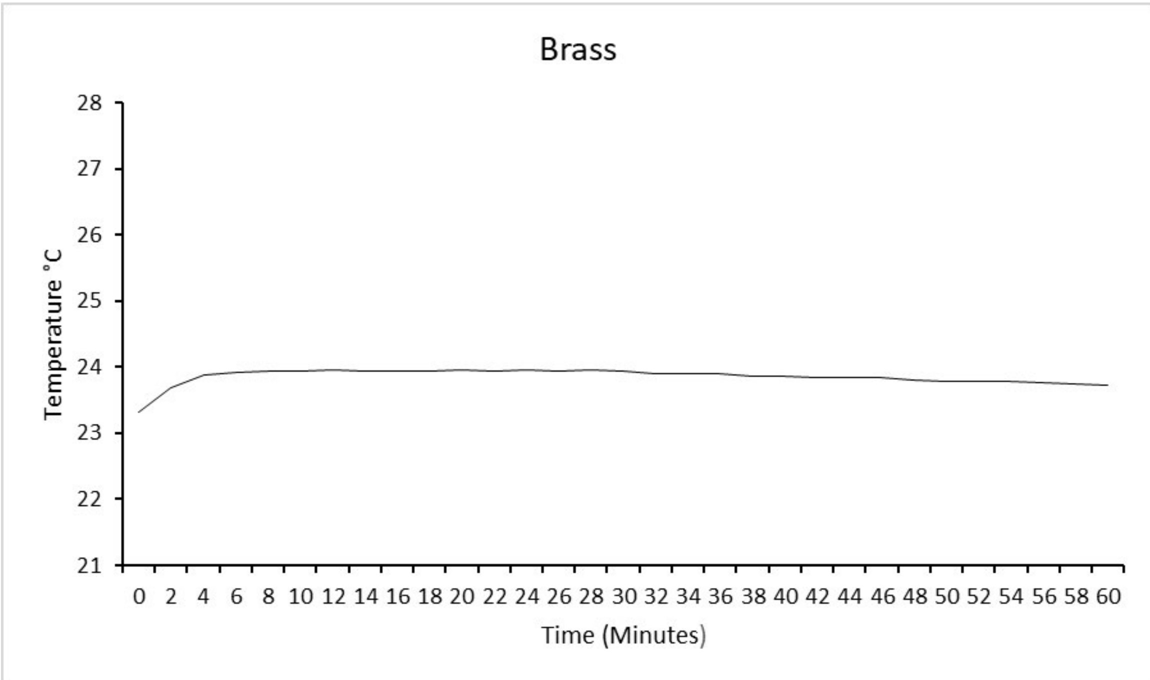

Figure 3d) temperature profile of brass

The second new material tested was aluminium, shown on figure 3e. Within the first four minutes the temperature rose to an average of 23°C from an average of 22.8°C. After that the temperature remained consistent throughout the experiment, neither heating nor cooling for the entire period. Overall, this means a total net heat gain of 0.2°C and no heat loss.

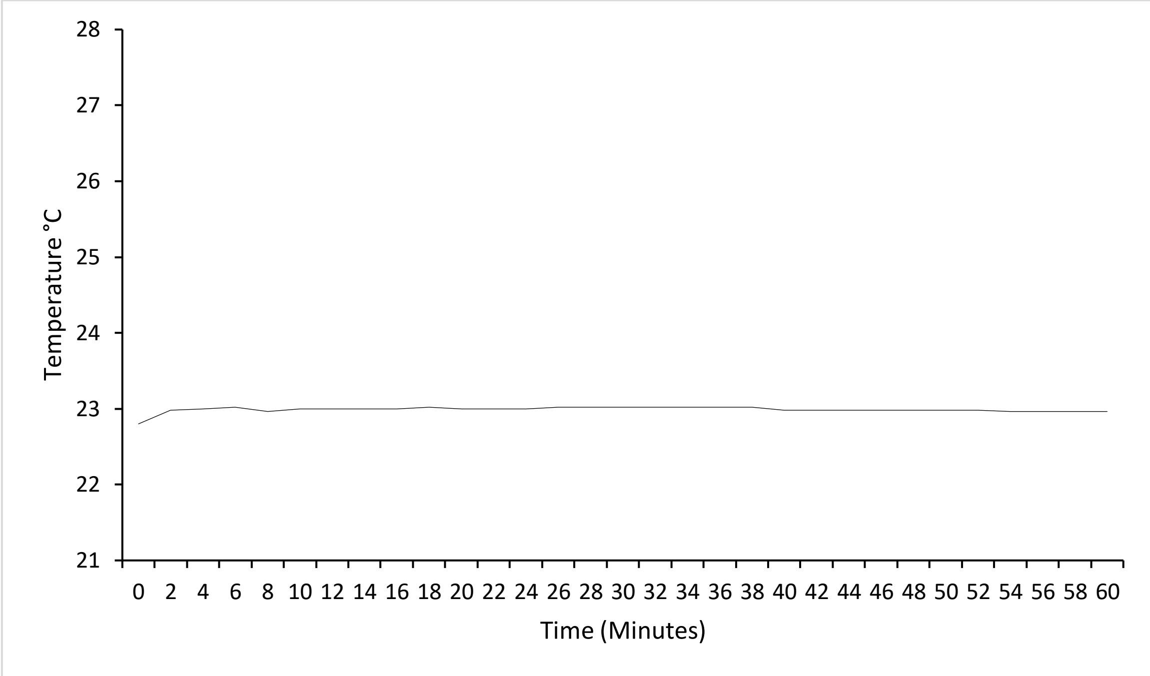

Figure 3e) temperature profile of aluminium

After testing all the materials in their original state, the metals were tested again after being painted black. Black in known to absorb heat better and we wanted to investigate the effect this would have on the materials properties. It was possible that the front runner at the time, bitumen, performed so well because it was already black in colouration. Figure 3f below shows iron painted black and the difference the black paint has made. Unpainted iron had a much less pronounced curve showing less temperature increase overall. Black iron increased by a total average of 0.92°C, compared the regular iron which increased by 0.28°C, meaning the black paint made iron increase by roughly three times as much. Still, it was only a quarter of the temperature increased shown by bitumen.

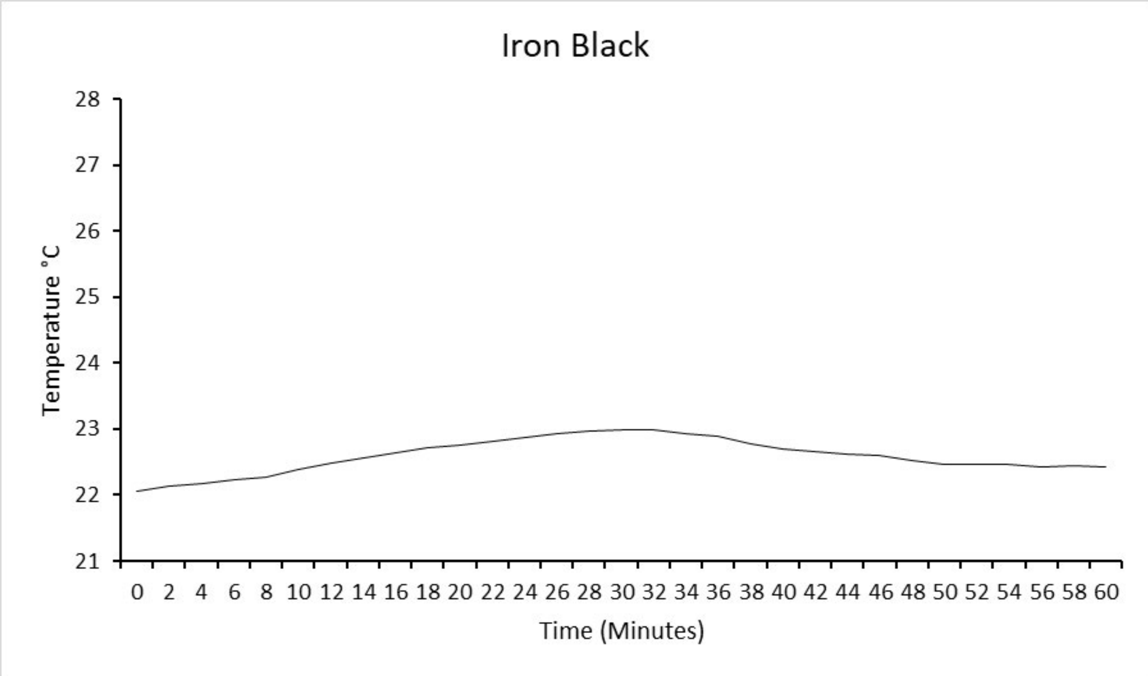

Figure 3f) temperature profile of black painted iron

After iron, brass was tested with black paint. Once again, the paint made a noticeable difference to the curve of the graph, shown in figure 3g below. When painted, brass shows a strong increase over the 30 minutes of heating. The total increase was 1.34°C, the second highest of all materials second only to bitumen. Peak average temperature was 23.72°C and the average temperature after 30 minutes of cooling was 22.74. This is a total temperature gain of 1.34°C against a temperature loss of 0.98°C, which shows that painted brass kept 27% of temperature gained.

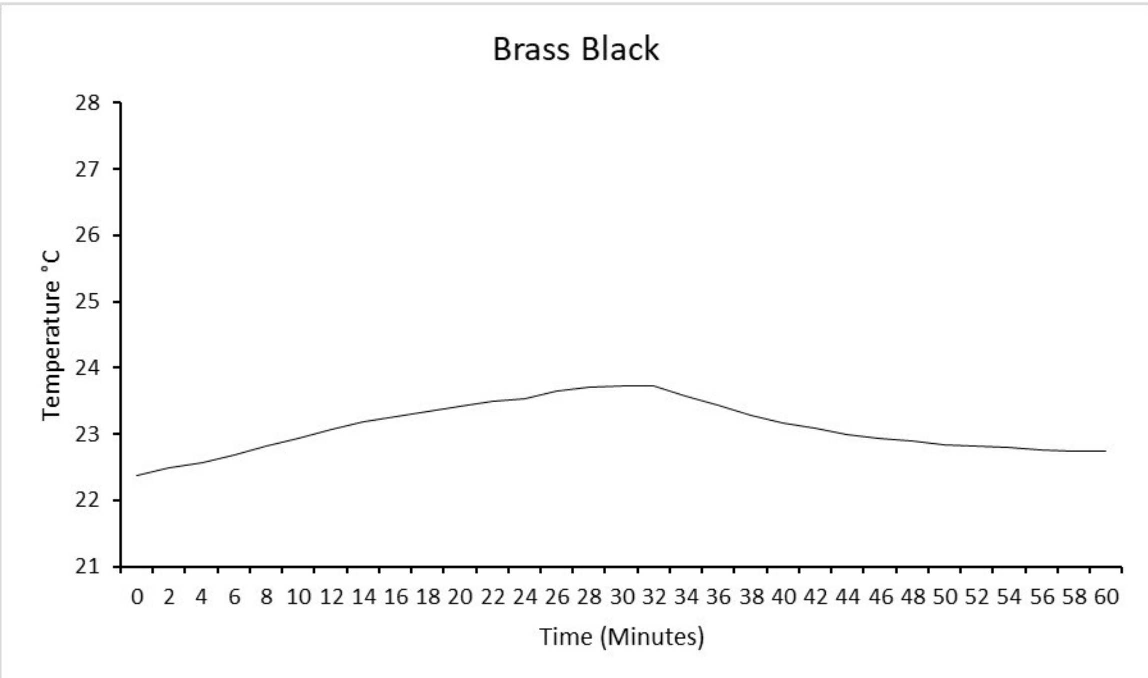

Figure 3g) temperature profile of brass painted black

The final material was Aluminium painted black which, like the other metals, improved with the paint. Previously, aluminium barely changed at all, but with the black paint it shows a much more pronounced curve as shown in figure 3h. Painted aluminium peaked at 23.52°C, showing an overall increase of 1.18°C. At the end of thirty minutes of cooling it had reduced to 22.68°C, decreasing by 0.84°C and keeping 29% of its gained temperature. This puts black aluminium fourth in terms of temperature gain, behind bitumen, black brass, and carpet. It still only shows a third the temperature increase bitumen does

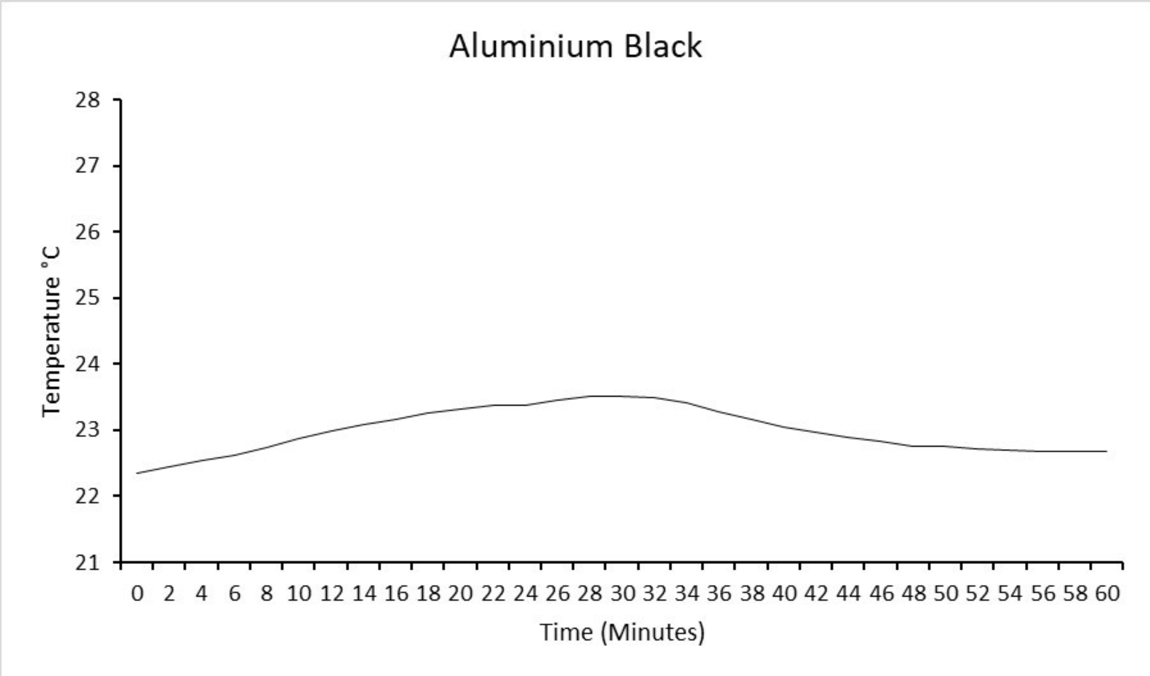

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Figure 3h) temperature profile of black painted aluminium

Figure 3i and table 3a below compare the total average temperature increases of every material. In order of most temperature increased it goes bitumen, black brass, carpet, black aluminium, black iron, brass, iron, and aluminium.

Table 3a) overall increases in temperature for each material

Material	Increase in temperature (°C)	Temperature Drop	Percentage of heat retained
Bitumen	3.86	2.16	56%
Brass B	1.34	0.98	27%
Carpet	1.2	0.78	35%
Alu B	1.18	0.84	29%

Iron B	0.92	0.56	61%
Brass	0.64	0.22	34%
Iron	0.26	0.28	107%
Alu	0.22	0.06	27%

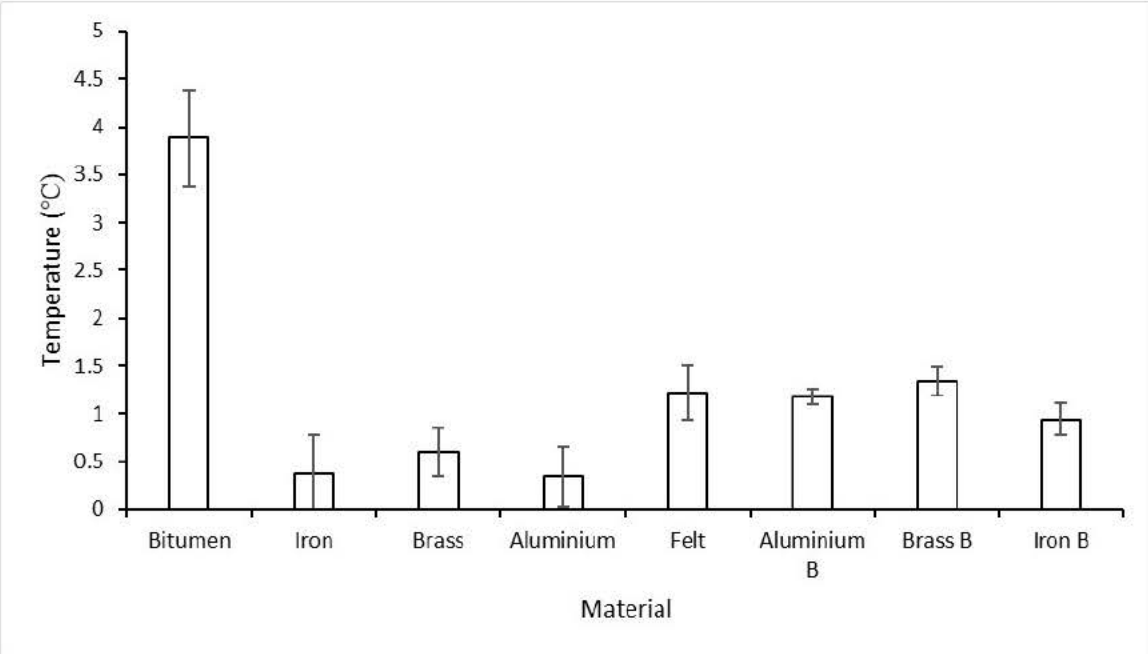

Figure 3i) the average temperature increases for each material over thirty minutes

The next step was to determine if the differences between the materials were statistically significant. If so, then we will be able to use those differences to determine which material might perform better in the field. A one-way ANOVA test was carried out between the different materials. Table 3b below summarised the differences or lack thereof.

Green: statistically significant difference

Red: no statistically significant difference

Table 3b) P values for significant difference between every material

P value	Bitumen	Carpet	Iron	Brass	Aluminium	Black Iron	Black Brass	Black Aluminium
Bitumen		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Carpet	<0.001		<0.001	>0.05	<0.001	>0.05	>0.05	<0.001
Iron	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001

Brass	<0.001	>0.05	<0.001		<0.001	>0.05	>0.05	<0.005
Aluminium	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
Black Iron	<0.001	>0.5	<0.001	>0.5	<0.001		>0.5	<0.005
Black Brass	<0.001	>0.5	<0.001	>0.5	<0.001	>0.5		<0.001
Black Aluminium	<0.001	<0.001	<0.001	<0.005	<0.001	<0.005	<0.001	

There were significant differences in temperature between the different materials (one-way ANOVA: $F = 229.658$, $P < 0.001$). Exceptions include Carpet vs Brass ($P = 0.946$), Carpet vs Black Iron ($P = 0.918$), Carpet vs Black Brass ($P = 1$), Brass vs Black Iron ($P = 1$), Brass vs Black Brass ($P = 0.983$) and Black Iron vs Black Brass ($P = 0.969$).

3.3.2 Second Experiment

Figures 3j and 3k and table 3c below show the average time taken in minutes to gain and then lose 0.1°C. We were looking for materials that heated up quickly and cooled slowly. In the field, this would allow the maximum survey window, as the materials got warm fast and stayed warm for longer. Bitumen, which increased the most in the previous test, increased the fastest in this experiment at an average of 1 minute and 44 seconds. However, it was one of the fastest to lose its heat at an average of 6 minutes 28 seconds. The material that held its heat the longest was black painted brass at 14 minutes and 32 seconds. This is roughly six times longer than the time taken to increase, which was an average of 2 minutes and 39 seconds. Unpainted iron was the worst performer, taking both the longest time to heat up at an average of 11 minutes and 4 seconds, and the quickest to lose that heat at an average of 3 minutes and 49 seconds.

Table 3c) time taken to gain and lose 0.1°C for each material

	Time (mins)	
Material	Increase	Decrease
Iron	11.04	3.49
Brass	10.18	7.04
Carpet	4.11	8.66
Alu B	3.24	11.85

Brass B	2.39	14.32
Alu	1.5	9.24
Iron B	1.48	11.41
Bitumen	1.44	6.28

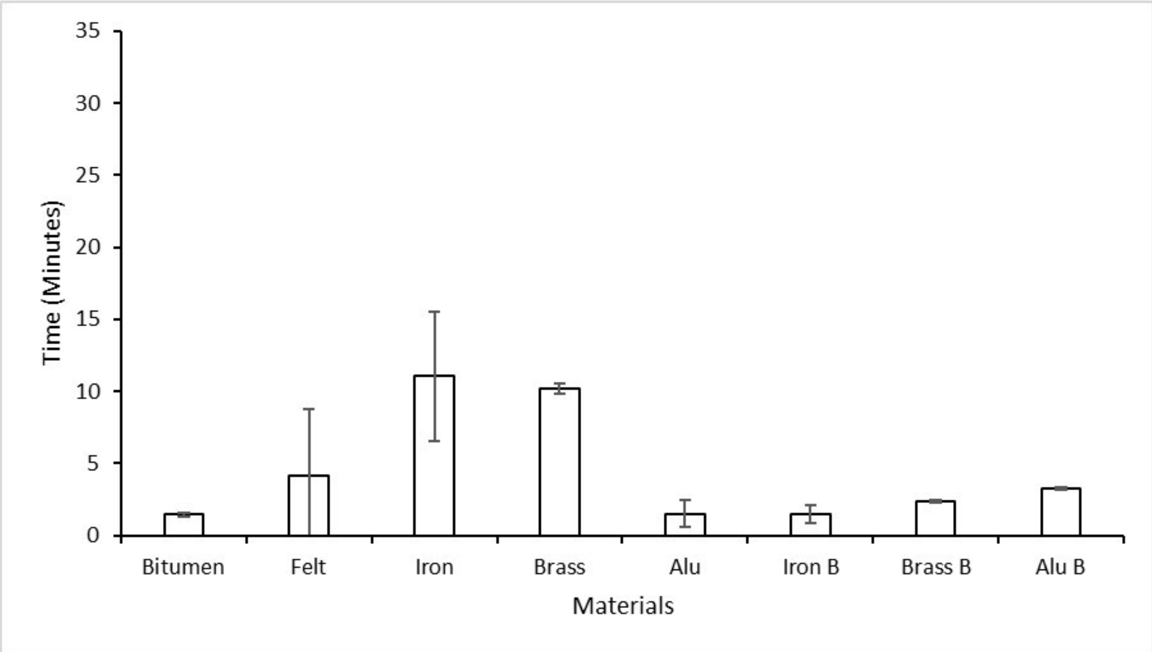

Figure 3j) time taken for each material to increase by 0.1°C

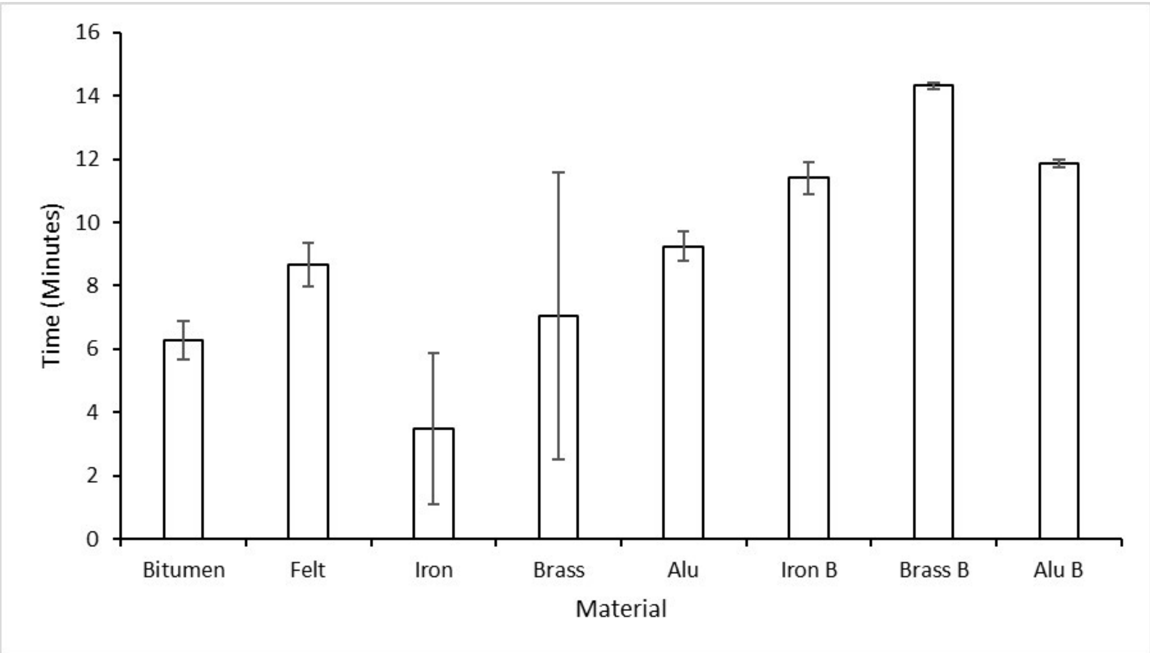

Figure 3k) time taken for each material to lose 0.1°C

3.4 Discussion

Of the eight different materials in the first experiment bitumen is the clear winner in terms of temperature increase, standing at an average of 3.86°C. this is consistent with bitumen being a popular material with surveyors. The lowest was aluminium at 0.22°C. Brass outperformed iron, the most commonly used metal in refugia surveying (Jofré and Reading, 2012; Cabuy, 2014), suggesting that it may be a viable replacement material and produce more results in the field. However, all the metals were outperformed by carpet tiles. This changes when we apply the black paint. With the exception of iron, the metals outperformed carpet when painted black. Black painted brass was the second highest performer out of the materials further supporting its viability as a replacement for iron.

In the second experiment black brass is one of the clear winners. It took the longest to lose heat implying that in the field it would keep its heat for longer than any of the other materials. According to Sewell *et al*., (2012) surveyors should not stay in the field during a reptile survey for longer than three hours. This means that any reptile survey could last up to this long and should a sudden change in weather occur that stops the materials heating then it would need to stay warm for up to three hours with the heat already gained. It was also among the fastest material to gain heat. The fastest material to gain heat in the second experiment was bitumen. According to Gaywood and Spellerberg (1995) reptiles start to increase their temperature by thermoregulation at roughly 8 o'clock, only a couple hours after sunrise. The guide to surveying reptiles by Froglife (1999) says a similar time. A refugia must reach optimum temperature or close to it at this time. This time is likely to vary depending on seasonal and daily variations in temperature and weather, but all reptiles begin thermoregulating in the morning, meaning refugia will only ever have a couple hours to reach temperature. When painted black, all of the metals held their heat for longer and heated up faster (with the exception of aluminium, which heated up slower when painted black). From this it was concluded that painting the metals black resulted in them being better materials overall for refugia surveying.

Bearing in mind the results of the various experiments, the hypothesis at that time was that bitumen will be the superior material. Its temperature profile shows strong heating and its rate of temperature decrease is much slower than its rate of temperature increase. The results imply that bitumen will swiftly heat up in the field and then hold its heat for an extended period of time, giving the reptiles in the field plenty of time to utilise the refugia. As well as performing well in every aspect of the test bitumen is also cheaper than all the metals and so is possibly the most cost-effective of all the materials and less likely to be targeted by thieves.

Chapter 4: Fieldwork

4.1 Introduction

Fieldwork consists of practical work conducted by a researcher in the natural environment of their research subject, rather than in a laboratory or office. In the field of ecology, fieldwork typically involves either watching the subject without any interaction, to avoid harming them or changing their natural behaviour, or surveying the area and its wildlife to gather data. Sometimes however, it is essential to gather specimens from the field, which can unfortunately be harmful to the local wildlife as animals are either killed (Pearce *et al*., 2005), or taken alive from the habitat (Smith and Tschinkel, 2009; Hettler, 1979; Paarmann and Stork, 1987). The importance of fieldwork comes from the fact that wildlife behaves naturally only in its natural environment, and so to understand them fully we must observe them in this natural environment. It is also important for many people to experience something first-hand rather that to simply hear or read about it, as it helps to deepen their understanding and put things into context (Hope, 2009). Within the education sector there are many people who are arguing the important role of fieldwork in the students learning as fieldwork is proven to increase the students understanding and enjoyment of the subject (Kent *et al*., 1997; Foskett, 1999; Gayford, 1985). In ecology however, fieldwork assumes that the behaviours we observe in the field are in fact natural, when in truth our very presence may be a disruption that causes changes to the animal's behaviour. Despite a scientist's best efforts their presence in the field may disturb the animals or cause them stress, forcing them to change their behaviour. The animals may seek to avoid them, denying them the chance to observe them, or they could try to drive the scientist out of their territory which is dangerous.

Despite this field research is often considered a necessity for ecology and so field methods will be employed in this research. However, in this research it is the fieldwork itself that will be studied. Refugia surveying is one of the most commonly used method for surveying reptile species (Hampton, 2007; Grillet *et al*., 2010). It consists of laying sheets of materials that absorb heat at a high rate, creating artificial refuges for them to take shelter and bask in. Once they are set up throughout the reptile's habitat they are checked periodically and the reptile species using them are recorded. The advantage the technique has over alternatives such as pitfall traps or transects include less maintenance and effort overall, however they do come with a smaller window for reptile encounters (Grant et al., 1992). Refugia are also known to have less of an impact on the animals whereas pitfall trapping can kill the animals and requires special modification to keep animals safe (Enge, 2001).

4.2 Methodology

To truly see which material would produce the best results a field test was required. The results of this test would be compared to the lab data to determine which material is the strongest and what factors determine this. For the first stage of the fieldwork a survey of reptile populations at 3 sites was conducted using the refugia materials studied in the lab (iron, black iron, brass, black brass, aluminium, black aluminium, bitumen, and carpet). All refugia were 50cm x 50cm. Figure 4a below shows a satellite view of the 3 sites. Detailed pictures of the sites can be found in appendix A. Each of the sites was less than a square acre meaning refugia density would be high throughout the surveys. The sites were chosen because they all had reported populations of Grass Snakes (*Natrix natrix*), a snake species that is common and frequently found near the water (Hutinec and Mebert, 2011). These would act as the focus species for this survey, but any instance of additional species would also be recorded. According to the National Biodiversity Network (NBN) there has been a single reported sighting of the Common Adder (*Vipera berus*) within a mile of the sites, but not on the sites, and no reported sightings of Smooth Snakes (*Coronella Austriaca*) within the entirety of Warwickshire. In terms of other reptiles, there have been no sightings of Slow worms (*Anguis Fragilis*), Common Lizard (*Zootoca vivipara*) or Sand Lizards (*Lacerta agilis*) within Leamington. Therefore, while it remains possible that a Common Adder or Common Lizard will be found in the survey it is far less likely than a Grass Snake. Steps would be taken in the survey to maximise the chances of finding Grass Snakes specifically, because they are the only species guaranteed to be at the site. All three of the sites were adjacent to the river Leam meaning that there would be a higher chance of a Grass Snake presence. The data recorded were the total number of reptiles and the number of each species and which refugia each individual was basking under. The purpose of this was to find whether one material outperforms any of the others in any measure, be that number of reptiles or abundance of species. Any knowledge of this kind will inform us of the relative strength and weaknesses of each material, which will further inform more rigorous studies of reptile populations. It was of particular interest if aluminium or brass has any advantages over the traditional materials, as that would set a precedence that there may be superior materials out there waiting to be tested.

Figure 4a) satellite view of the 3 sites, image taken from google maps

Welches Meadow

Site 1: 52° 17'20.8"N, 1° 31'16.9"W

The Welches Meadow reserve is a flood meadow on the south bank of the River Leam. The survey was focused on the far eastern edge of the site where the terrain was deemed more suitable. It features many tall grasses with the marshy areas dominated by Reed Sweetgrass (*Glyceria maxima*) and Reed Canary Grass (*Phalaris arundinacea*). The reserve is regularly walked along by the public but there are no formal paths. Next to the meadow is a reservoir. The refugia were placed on the boundary of the marsh areas next to the reeds and tall grasses due to the Grass Snakes being known to favour areas near the water. The area is a known floodplain which unfortunately did flood during the survey, submerging the refugia and preventing data collection from this site for a number of weeks.

Leam Valley

Site 2: 52° 17'10.9"N, 1° 30'47.3"W

Site 3: 52° 17'08.1"N, 1° 30'41.6"W

Leam Valley is a much larger reserve with a wider variety of habitats, including different types of grassland, marsh, and woodland. Within Leam Valley two different sites were chosen. The first was a wild, rarely managed grassland dominated by Meadow foxtail (*Alopecurus pratensis*), Common Reed Grass

(*Phragmites australis*) and Perennial ryegrass (*Lolium perenne*) on the north bank of the river Leam. The grass had been cut somewhat recently before the survey but was not cut again throughout the survey. This meant that the sward height gradually increased over the course of the survey, beginning at less than thirty centimetres, and growing to over a metre in height. The refugia were spread evenly throughout the grass a good distance away from the path that ran along the edge of the site. The second site was a much more managed site with regularly cut grass and a large hedgerow on the boundary between the grassland and a woodland. This site was also on the north bank of the Leam river. The refugia were placed along the hedgerow. The dominating grass species was False oat-grass (*Arrhenatherum elatius*)

An additional theory tested was the use of polystyrene refugia. Regular refugia are conductors and give the reptiles a place to bask when it's cold. The use of polystyrene refugia tested if the reverse could also be true and whether an insulator would give the reptiles somewhere to cool down when it's warm. Polystyrene was not tested in the lab as the point of the lab test were to find out how well each conductive material heated up, meaning it was pointless to test an insulator like polystyrene. This method being new and untested it was important to give it every chance to succeed. The standard refugia method in a reliable and often used one that is proven to draw in reptiles. Therefore, it was decided to place the polystyrene adjacent to the standard refugia to ensure that the reptiles would find them as well. This removes one of the possible causes for no results and helps ensure that if there really are none it will be because polystyrene is an unsuitable as refugia, not because they were never found in the first place. As an insulation material its purpose would be to shelter reptiles from heat. Therefore, it was decided to perform this first survey in the height of summer during July and August. This was not the most optimal time for the standard refugia, but it gave the polystyrene the best chance of working.

The sites chosen to be surveyed were sites where the Warwickshire wildlife trust had confirmed a reptile presence. These were however, near to human settlements and frequented by the public. Measures therefore had to be taken to prevent disturbances to the survey. Signs were printed of and attached to the refugia that stated they were part of a wildlife survey and requesting the public not to disturb them.

The purpose of the experiment was to test witch material was better and so all other independent variables had to be controlled. This included the chance that a refugia may get stronger results due to lucky placement. To combat this the refugia were all placed in close proximity to each other. No refugia was ever more that ten metres away from at least one other. By placing the refugia in a cluster it reduced the chance of any of them achieving a stronger result because it was in a better spot. A reptile finding one would not be far away from any other giving it the opportunity to make an active decision between the materials. Each material was also paired with a polystyrene refugia. This ensured that the two would be found alongside each other and also the weight of the traditional materials prevented the polystyrene from being blown away by the wind. The reptiles need time to locate the refugia and determine that they are safe to begin using them. During a survey carried out before this research began the surveyors stated that they had left theirs out for a week. Their survey got strong results so following their example the refugia were left on site for a week before any visits were made.

A paper by Sewell *et al*., (2012.) stated that for the most common reptile species at least four visits using artificial refugia and transect walks are required to establish presence. The refugia were visited every day for a week before they were moved to a new site and checked again every day again and finally moved to a third site. Refugia work by being strong conductors of heat and swiftly getting hotter than their surroundings. This provides the local reptiles with a basking site to warm up. Therefore, standard refugia work best at cooler temperatures, as long as the sunlight is strong. the standard refugia were visited in the morning between 7:30 and 9:30, depending on weather. Refugia surveying is ineffective during rain and so the sites were not visited during such weather. Rain has the effect of lowering ambient temperatures and making thermoregulation difficult (Lillywhite and Tu., 2011), therefore it was surmised that the animals would not emerge from their overnight shelters if it were currently raining and would not use the refugia. The theory behind polystyrene refugia is that they would provide the reptiles with a place to cool down when the temperatures were at their highest. Therefore, polystyrene refugia were checked between 13:00 and 14:00, when air temperatures had reached the day's peak.

There was concerns that the number of refugia and the frequency of visits was not enough to establish presence on the first survey. For the second survey a number of alterations to the methodology were carried out. First, the number of refugia on each site was increased to nine, three of each material and all sites were surveyed at the same time. Those materials were iron, bitumen and carpet. Polystyrene failed to produce a single result at the time of year expected to be the most successful and proved too fragile to survive in the field. Therefore, it was cut from later surveys. Aluminium and brass were also cut, due to the prohibitively high costs of acquiring more of them. They proved impractical as a survey material seeing as they are more expensive than the other materials and did not produce any stronger results.

The second survey took place over two weeks in October. This was not ideal survey times for reptiles as they typically are about to go into hibernation at this time of year (Reading and Davies, 1996) however it was still possible to find them active. Adjustments would still have to be made for the time of year. In an effort to maximise the chances of finding reptiles in a smaller window of opportunity the survey was made more intensive then would be practical during a longer survey. The sites were visited six times a day, three in the morning and three in the afternoon. The weather during this time of year is not ideal for reptile surveying and it was unclear what time of day would be best. In order to discover this for future surveys data loggers were placed under the refugia. The exact data loggers used were the DS1920 temperature iButtons which possess a temperature range of -55°C to 100°C and can measure in 0.5°C increments. They recorded temperature every thirty minutes creating a temperature profile of the refugia during the survey. The loggers were placed under the centre of each refugia where the vegetation cover from overhanging grasses would be the least. Isaac, (2003) found that grass snake (*Natrix natrix*) digestion was optimised between 25°C and 35°C, and that locomotion was optimised between 25°C and 38°C. Based on this it would be safe to assume that the ideal body temperature for grass snakes, and therefore refugia temperature, would be 25°C, a temperature where digestion and locomotion are optimal but requires the least amount of time beneath the refugia. Using the data loggers, we will be able to see what times of day if any are viable visitation times for reptile surveying. This also gives us another metric to compare the refugia with. Using the temperature profiles created by the data loggers we can see how often each material reaches the optimal temperature for reptile basking. The material that reaches this temperature the fastest and most often would be a better survey material. Similar to the first survey the materials were placed in clusters. This time each cluster consisted of one of each material to ensure that reptiles would have the opportunity to make an active choice between the materials. In an attempt to widen the range of the survey and potentially attract more reptiles the clusters were spread further apart that the previous survey, between 20 and 30 metres from each other.

During the survey in October the carpet tiles absorbed and held a great deal of moisture, far more than the other materials. It was deemed that in the event of rain it would be too damp to heat up to required temperatures and would stay that way for too long. Therefore, carpet tiles were cut from the study and did not feature in the third survey. The two materials left were iron and bitumen, the two most commonly used materials by other herpetologists. The third survey took place over five months, starting on April 15th and ending on August 31st. Spring is considered one of the best times to survey for reptiles, as they begin to emerge from hibernation in March (Reading and Davies, 1996) and so will be fully active in April.

Typically, when iron and bitumen refugia are used they are in a sinusoidal shape instead of flat. Until the third survey we were unable to use sinusoidal shapes as they were not available for the other materials and it was necessary to keep the independent variables consistent. Now that all materials other than iron and bitumen had been removed from the survey it was possible to add sinusoidal refugia to the study and compare their performance to the flat refugia used up until now. Part of investigating how the two material shapes differed involved measuring the temperature difference between them. A sinusoidal shape would likely have more air flow beneath it which would lower the overall temperature. Was this an advantage or a disadvantage? To find out, a temperature probe (specifically a Digital LCD psychrometer temperature-humidity meter) was taken on site visits and used to measure the temperatures under each refugia, the air and the ground. These results would be compared to each other and to the number of reptiles found under each material to determine which material and shape produced the best results and why. The sites were visited 84 times in total over the course of the survey. Like in the previous survey the refugia were placed in clusters of one of each material in an adjacent line with cluster spread far apart across the site. The practice of not visiting during the rain was continued during this survey and the previous one.

The survey was for the purpose of investigating which material produced superior results and so it was important to minimise the possibility of other factors affecting the result, such as location. Therefore, as in previous surveys, the different refugia materials were placed in a cluster, with one of each material type (flat iron, sinusoidal iron, flat bitumen, sinusoidal bitumen) being placed in a row. This reduced the likelihood of one material performing better due to lucky placement. Additionally, should a reptile find one refugia they also find the others. This gives the reptile a choice. Reptile are known to be able to determine the best basking sites out of any options presented to them (Kearney and Predavec, 2000; Law and Bradley, 1990) and so the material any reptiles are found under are likely to have been chosen deliberately by the animal. There had been a problem on previous surveys with locals disturbing the refugia and moving them off site. Because of this, efforts to discourage the locals were increased. The refugia were roped off and marked with a laminated sign that read: Do not disturb. Wildlife survey in progress. This survey is being carried out with permission from the Warwickshire Wildlife Trust and the Warwickshire County Council.

Figure 4b) a sign used to deter locals from disturbing the refugia

During the third survey there was a disturbance at the first site when unusually high rains brought a flood, submerging the refugia for roughly a month. The site in question was known to be a flood plain but was still surveyed because the local Wildlife Trust had confirmed a reptile presence. Grass snakes (*Natrix natrix*), the most common reptile in the local area, are known swimmers (Gray and Lissmann, 1950) and so the flooding does not likely pose a risk or obstacle to them.

Over the end of July and beginning of August data loggers were once again placed under the refugia to create a temperature profile. Just like October, the height of summer is not considered optimal surveying time for reptiles, this time due to the increased temperatures reducing the need for basking sites and increasing time spent seeking shelter. The data logger temperature profiles will allow us to see at which times the materials reach optimum temperature during this hotter time of year, how regularly and how quickly they do so.

4.3 Results

4.3.1 Field Temperatures

The interactions between the temperatures of the air and ground and the refugia are of great interest to this study. Are refugia temperatures dependant on air and ground temperatures and if so, how does it affect their function? If refugia temperatures are not significantly higher than their surroundings, then they serve no purpose. The interactions between air and ground temperatures are also relevant to this study as they can influence reptile behaviour. Grass cover at ground level can trap heat during colder months making it warmer, but also provide shade during the hotter months which helps to avoid overheating. The difference in temperature between them can determine whether the reptiles stay close to the ground amongst the grass or emerge from the grass into the open air. Figure 4c below shows air and ground temperatures plotted against each other. Throughout the study the ground temperatures stay consistent whereas air temperatures slowly rise throughout the year. Towards the end of the study average air temperature looks to overtake average ground temperature by a slight margin. Hypothetically, if the survey had continued air temperature could have risen even higher. This implies that depending on the time of year it may be beneficial to abandon grass cover and seek out the open air for more efficient thermoregulation.

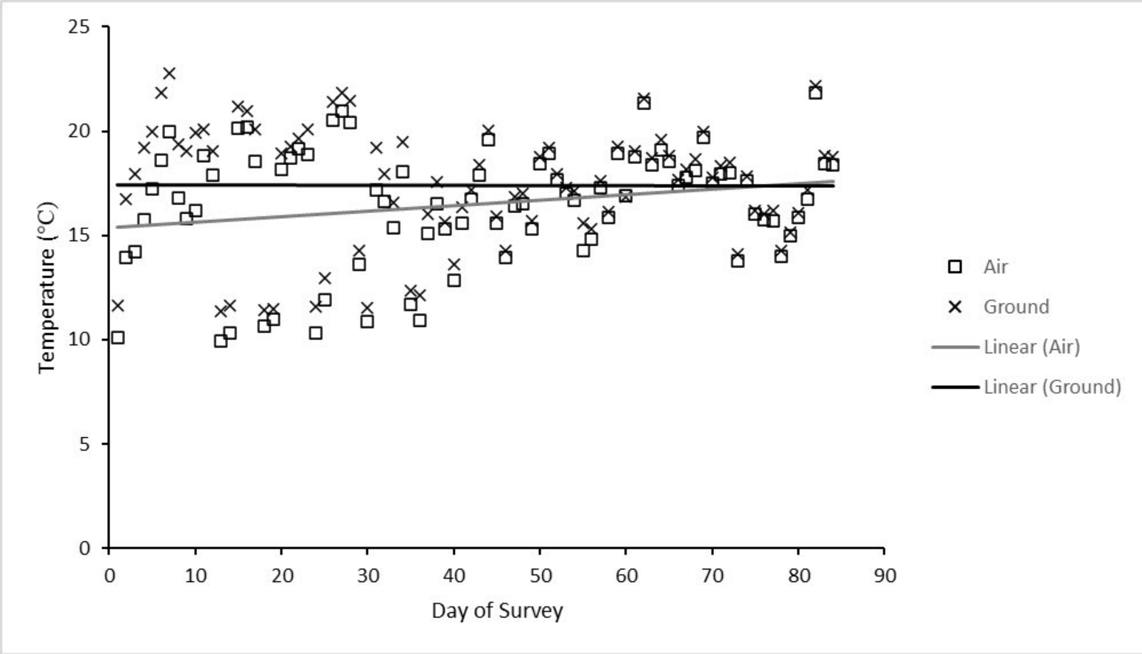

Figure 4c) average air temperature compared with average ground temperature over the course of the third survey.

When comparing air temperature to refugia temperature we see that at the beginning of the survey there was a significant gap between them, as shown in figure 4d below. During the survey however, this gap closes as average refugia temperature slowly decrease while air temperature rises. A rise in air temperature throughout the survey makes sense, as it began in spring and continued through to summer when overall temperatures are higher. The drop in refugia temperature average, however, is contrary to what one would expect. As temperatures rise throughout the year it would be safe to assume that refugia temperatures would rise as well. A possible explanation for this would be vegetation cover. Throughout the survey the flora around the refugia grew. When the refugia were first placed they were set atop the vegetation and had little to no cover but throughout the year the vegetation grew until it cast shade over some of the refugia. This implies that it may be important to manage the vegetation around refugia during a survey to prevent this from interfering with the results. As air temperature rises and refugia temperature drop the refugia offer diminishing reward to the reptiles, although refugia temperature did manage to stay ahead of air temperatures throughout the survey.

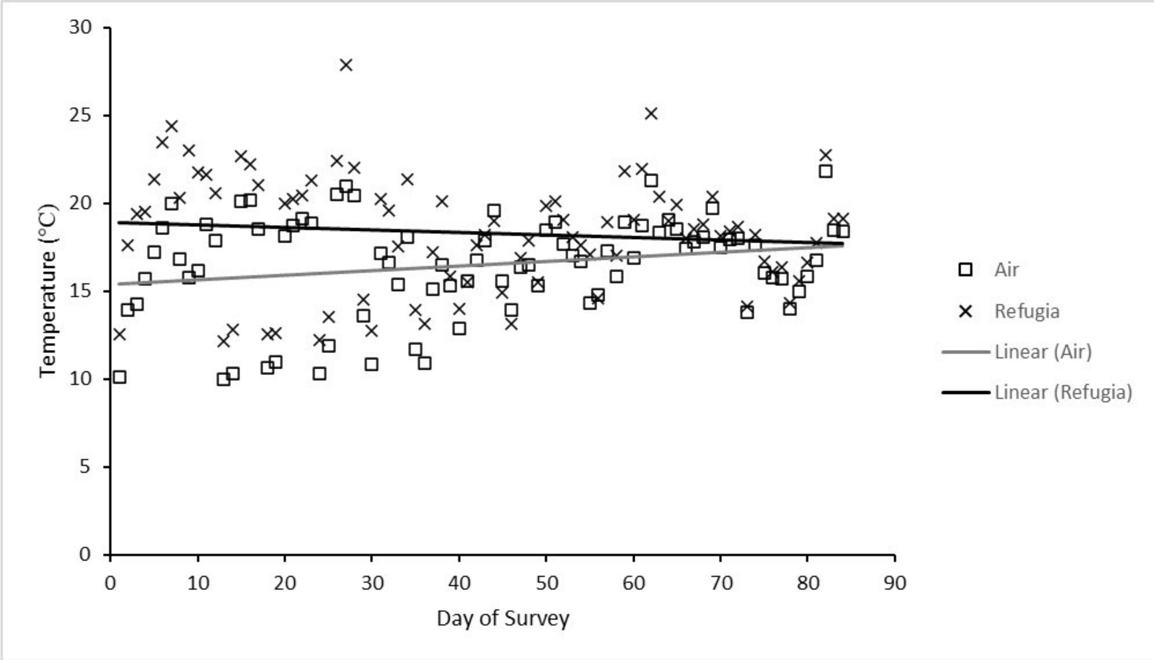

Figure 4d) average air temperature compared with average refugia temperature over the course of the third survey.

When comparing refugia temperatures to ground temperatures in figure 4e below we once again see how the ground stayed stable throughout the survey whereas the refugia declined. This continues to imply that the refugia would be more effective earlier in the year when overall temperatures are lower and there is less vegetation to cast shade over the refugia and reduce their effectiveness. Overall, this implies that refugia are less effective in warmer times of years, due to there being less of a difference between refugia temperatures and their surroundings and the increased vegetation growth impacting their ability to absorb heat from the sun.

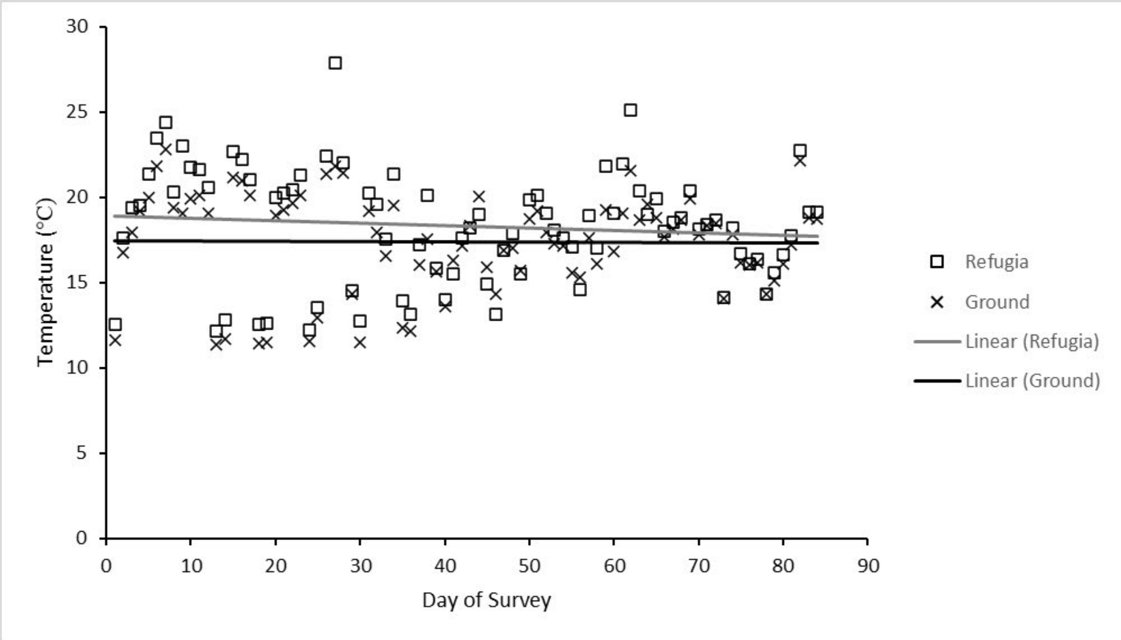

Figure 4e) average refugia temperature compared with average ground temperature over the course of the third survey.

The temperature of each material, the air and the ground were averaged so they could be compared. Figures 4f, 4g and 4h below show these temperatures. At first glance it looks like all the materials were significantly higher than the air and ground temperatures, which is in keeping with the purpose of the refugia. It also looks as though the sinusoidal materials had higher temperatures that the flat ones. There is always at least a 1°C temperature difference between the air temperature and the refugia temperatures and sometimes as much as a 3°C difference. There is less of a difference between the refugia and the ground temperatures but the refugia are still always higher.

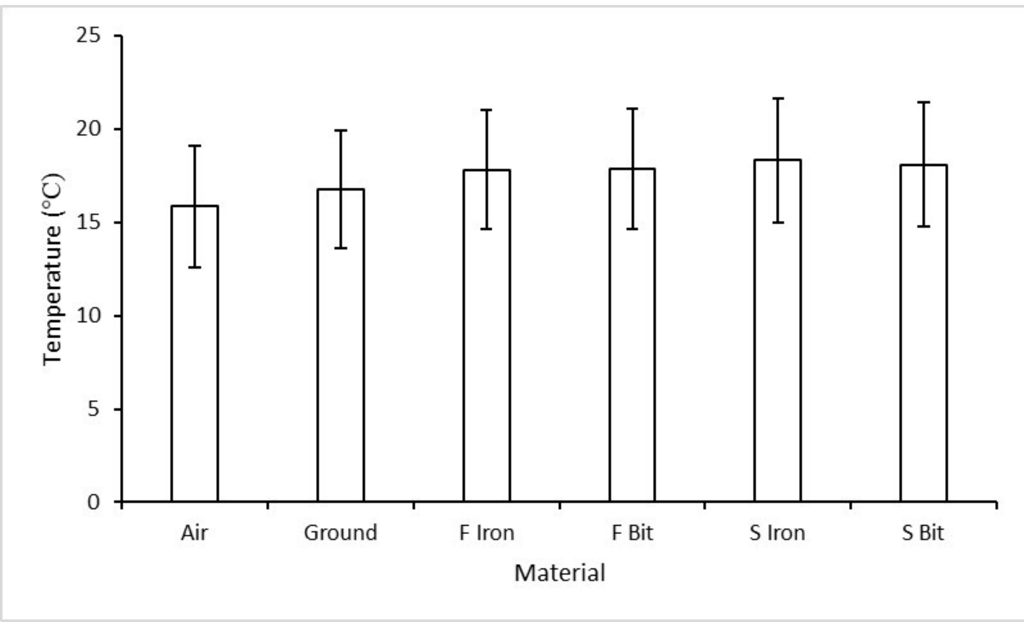

Figure 4f) average temperatures of air, ground, and each material at habitat 1

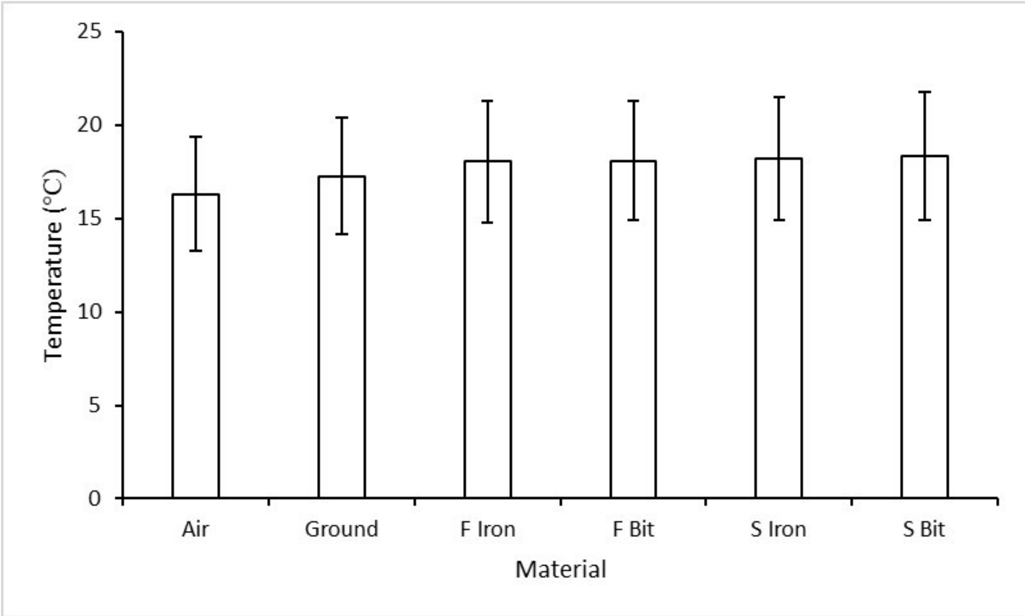

Figure 4g) average temperatures of air, ground, and each material at habitat 2

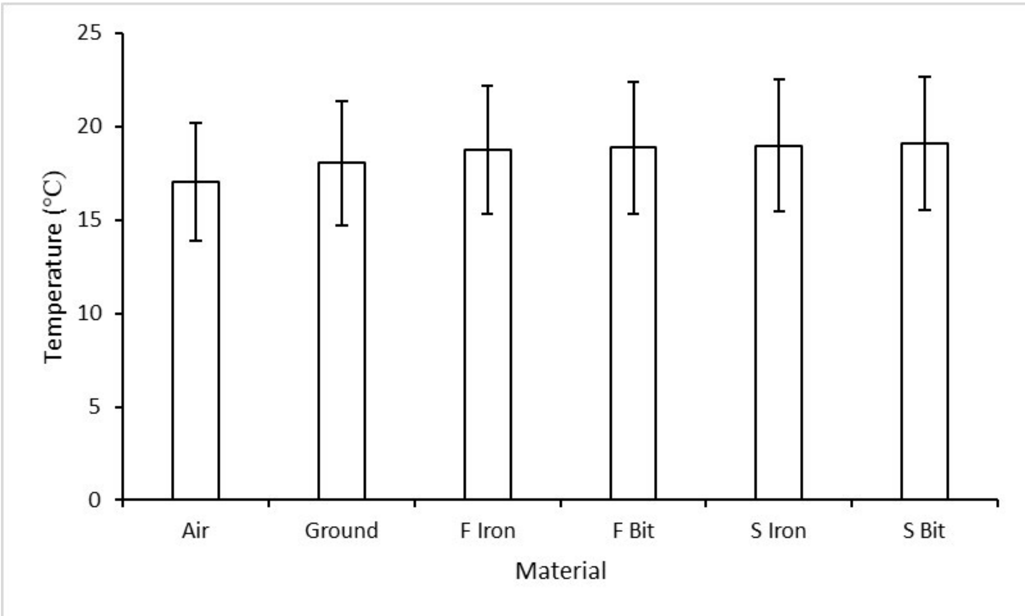

Figure 4h) average temperatures of air, ground, and each material at habitat 3

To further explore the data taken from the fieldwork statistical tests were carried out. Before that the data was tested for normalcy, skewness, and kurtosis, all of which were found to be within acceptable ranges.

The next test was a general linear model conducted to explore the relationships and interactions between the different factors involved and the temperatures of the refugia. Included in this test were two specific interactions the first being ground temperature and refugia cluster. This interaction was examined to see if there was significant variation in temperature within a single habitat. The other interaction was material and site, included to explore the possibility that the differences in habitat could favour different materials over the others. The results of this test are summarised below in table 4a.

Table 4a) summarised results of the general linear model

	df	f	p
Material	2	33.127	<0.001
Air	3	57.598	<0.001
Ground	2	59.689	<0.001
Site	81	11.449	<0.001
Cluster	87	61.252	<0.001
Ground/Cluster	208	2.625	<0.001
Material/Site	6	0.572	0.753

There was a statistically significant relationship between all the different factors and refugia temperature meaning that all of them should be considered when conducting a refugia survey. Additionally, there was a significant interaction between ground temperature and refugia cluster meaning that the ground temperature within a habitat is not homogeneous and subject to internal variations. There was, however, no significant interaction between material and site meaning that all the different materials performed roughly the same across the different habitats.

4.3.2 Data Loggers

The average temperature over time for each material during the October survey is displayed below in three temperature profiles (Figures 4i, 4j and 4k). Optimal temperature for the grass snake (*Natrix natrix*), the only species found in our survey, is 25°C. With these profiles we can see exactly how many times over fourteen days the refugia reached the optimal temperature threshold of 25°C.

Bitumen reach optimal temperature nine days out of fourteen and had the highest overall temperatures. Conversely, it dipped into the negative figures five evenings out of fourteen, also more than the other materials. This implies that bitumen is prone to wider variations of temperature in the field achieving both the highest and the lowest. This may or may not be an advantage as higher temperatures are good for surveying, but it is possible to reach too high temperatures. The upper threshold for optimum grass snake temperature according to Isaac, (2003) is 38°C, beyond that and the snake is no longer performing optimally. Therefore, beyond 38°C will be the point this survey considers dangerously hot. On the third day bitumen exceeded that threshold, becoming too hot. This is especially interesting at this was during the October survey where temperatures are much lower on average.

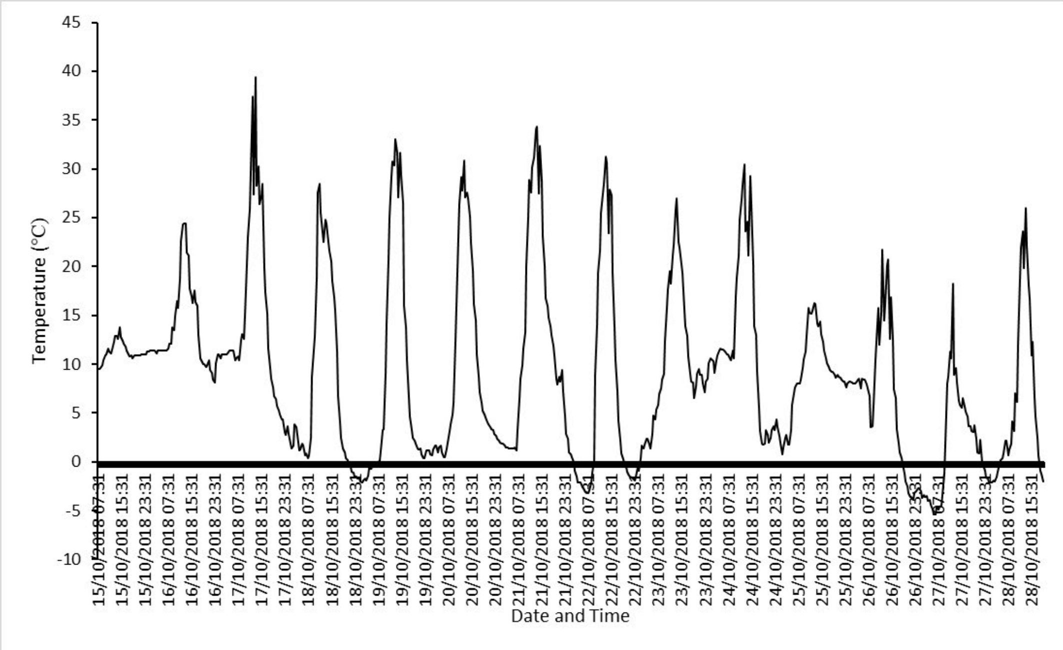

Figure 4i) temperature underneath bitumen refugia over time

Carpet performed similar to bitumen, but with less extreme variations. It reached optimal temperature less often that bitumen, seven out of fourteen days, but went into negative figures less as well, only four evenings. Carpet can therefore be considered slightly more thermally stable then bitumen and therefore may be the better performing material. At no point in the survey did carpet temperatures exceed the 38°C threshold. In the field however, carpet was noted for its tendency to withhold moisture from morning dew and rainfall, making the underside of the refugia frequently damp. It is possible that this lowered the average temperatures over the course of the survey

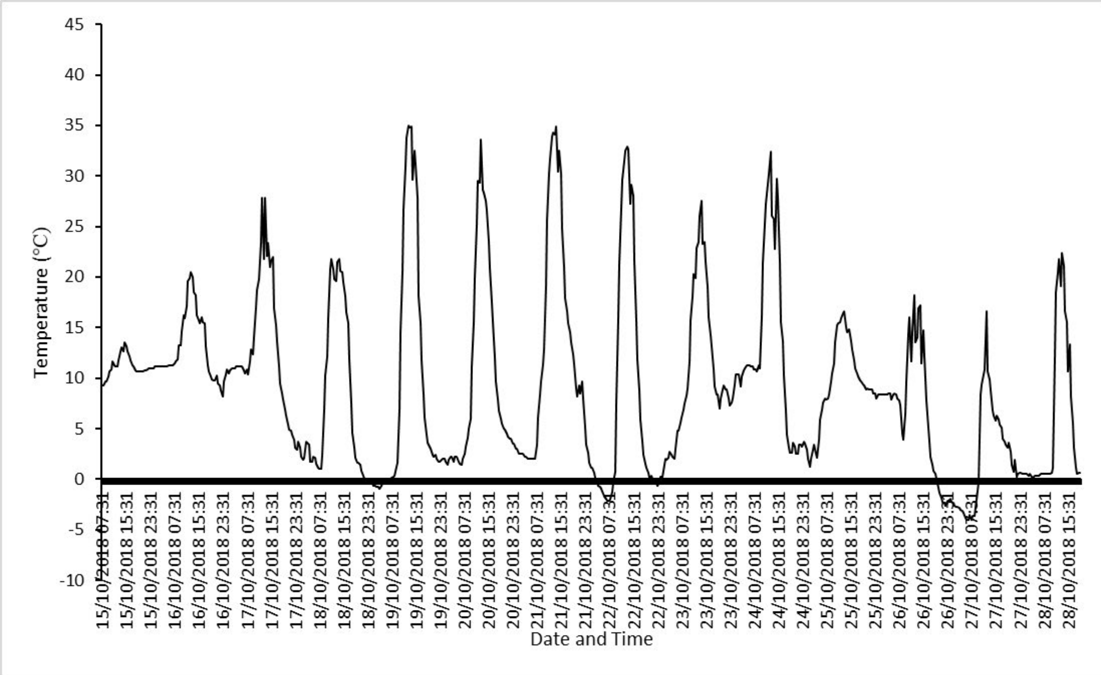

Figure 4j) temperature underneath carpet refugia over time

Over the fourteen days iron reached optimal temperature once. At the same time, it only reached negative figures once. These much fewer extreme variations in temperature may be to the material’s credit in warmer months, but during October it can be definitively said that iron refugia are not suitable. The iron refugia seem to take longer to increase and decrease in temperature. If conditions were hotter this means that it would reach optimal temperature slower, but also stay at temperature longer and have less chance of exceeding the optimal threshold. During colder months however, iron cannot keep up with other materials that heat up quicker.

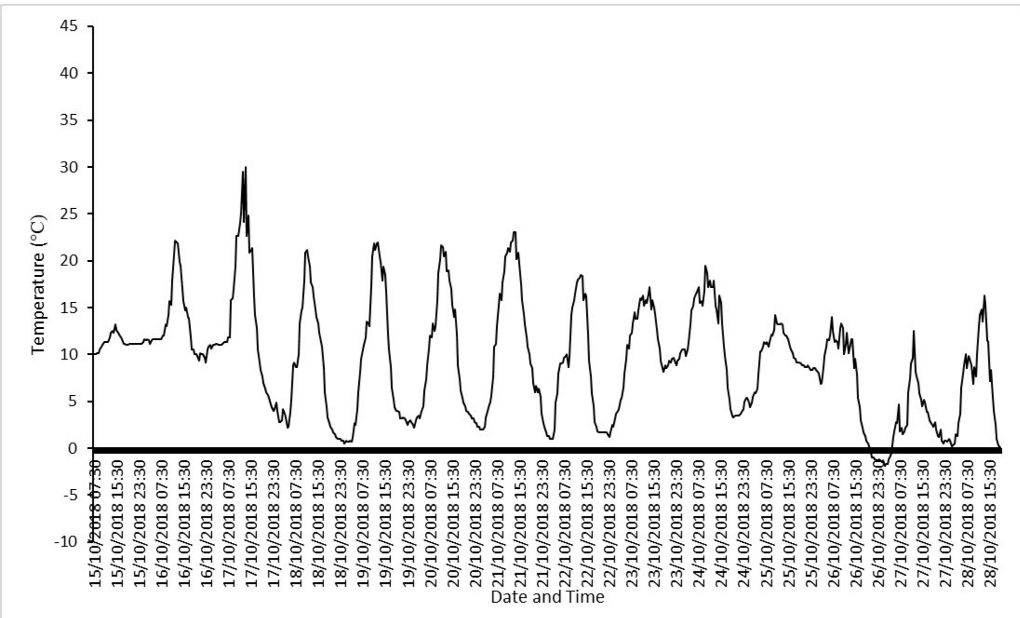

Figure 4k) temperature underneath iron refugia over time

During the summer survey temperatures were exceptionally higher than the October survey. The refugia frequently reached temperatures exceeding 60°C with the exception of sinusoidal bitumen. These temperatures are possibly the result of a fault in the equipment as they are abnormally high even for the height of summer. Another possibility is the data loggers were in direct contact with the material and so logged the temperature of the material rather than the air beneath the refugia. Below are the temperature profiles for the summer survey (figures 4l, 4m, 4n and 4o). Flat Bitumen began regularly reaching or exceeding 70°C but eventually stopped reaching these excessive temperatures. At no point during the survey did the material fail to reach the 25°C optimal threshold, performing well during the survey. However, on most days it exceeded the 38°C cap on the optimal threshold. Only once during the survey did the material drop into the negative figures at night. The material therefore performed well, regularly reaching the optimal temperature threshold but not reaching the excessively high temperatures as often as the other materials.

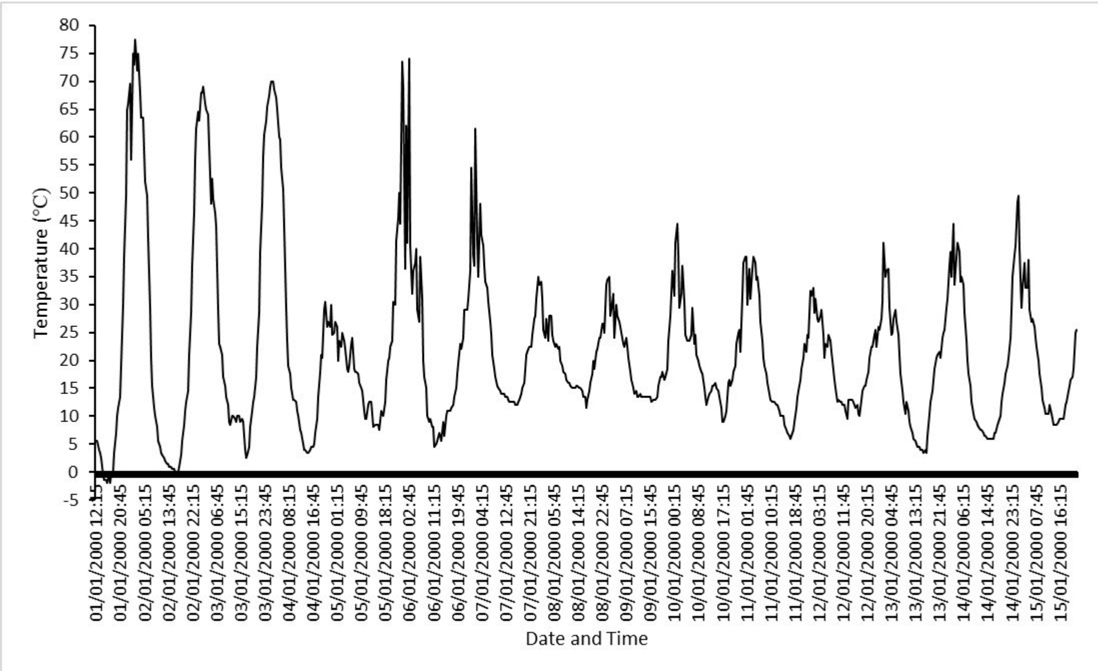

Figure 4l) temperature underneath flat bitumen refugia over time

Flat iron exceeded 60°C more often than flat bitumen and had higher temperatures overall. At no point during the survey did it drop into negative figures. Flat iron reached the optimal temperatures every day but exceeded them by a higher margin that flat bitumen, spending more time outside that threshold. The higher temperatures also pose a threat to reptiles as temperatures that high might be lethal. Overall, flat iron did not perform as well as flat bitumen.

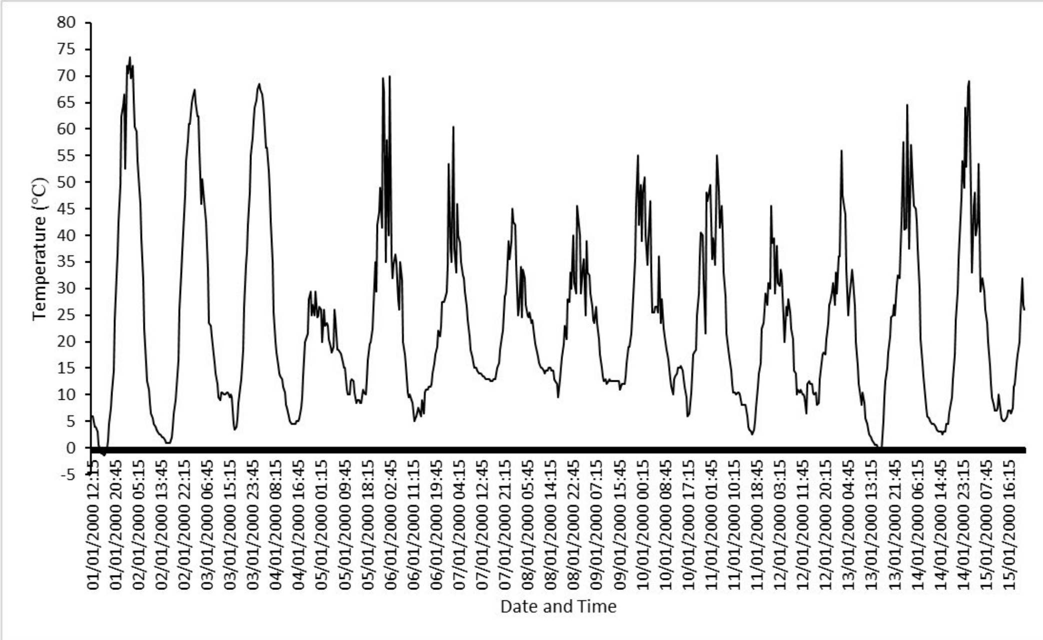

Figure 4m) temperature underneath flat iron refugia over time

Sinusoidal bitumen did not reach the excessively high temperatures the other materials reached, meaning it did not become dangerously hot at any time. It still reached the optimum temperature threshold every day except for one and never went into negative figures. On some days, its highest point was still within the optimum temperature threshold meaning that it spent longer than any other material at optimal temperature. This implies that sinusoidal bitumen might be the best performing material, however the one day it failed to reach temperature proves that in unfavourable weather it may not be able to keep up. It was the only material to have a day that didn’t reach temperature.

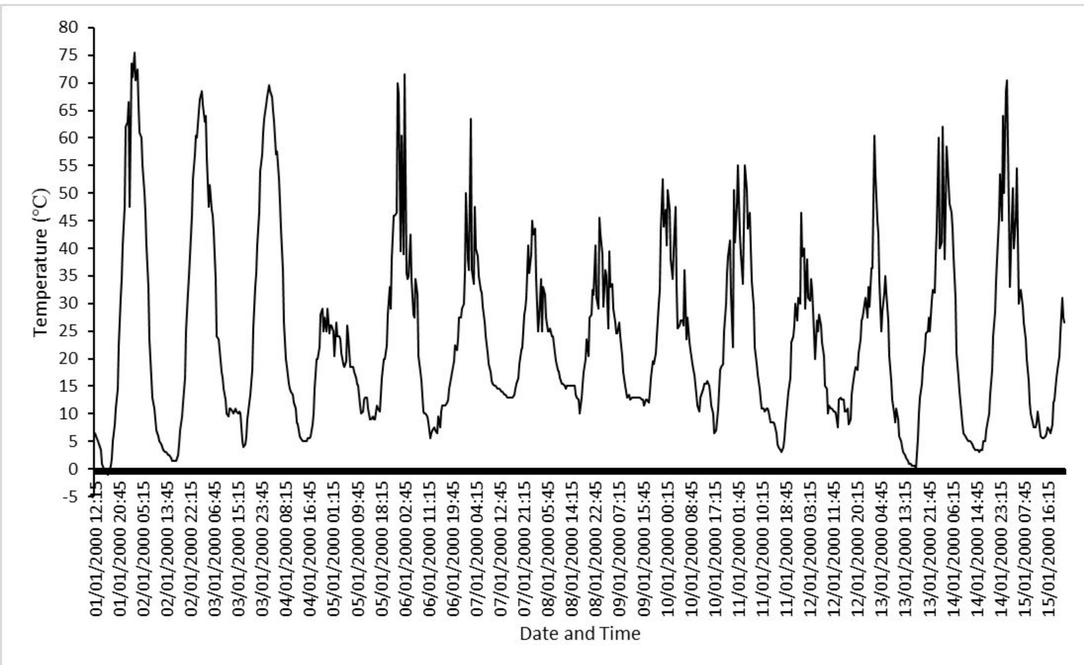

Figure 4n) temperature underneath sinusoidal bitumen refugia over time

The temperature profile for sinusoidal iron is almost identical to flat iron, calling into question whether or not the difference in shape makes a difference in temperature. Just like flat iron sinusoidal iron reached excessively high temperatures on most days. It never failed to reach the optimal threshold and only went into negative figure once. It spent a lot of time at temperatures too high for reptiles and so did not perform as well as bitumen. Overall, the temperature profiles of all four materials were very similar, sometimes only having a fraction of a degree difference. This implies that material might not be the most important factor when determining refugia temperature. Air and ground temperature might have a greater effect. Whether or not material makes a difference could depend on how sensitive the reptiles are to small variation in temperature.

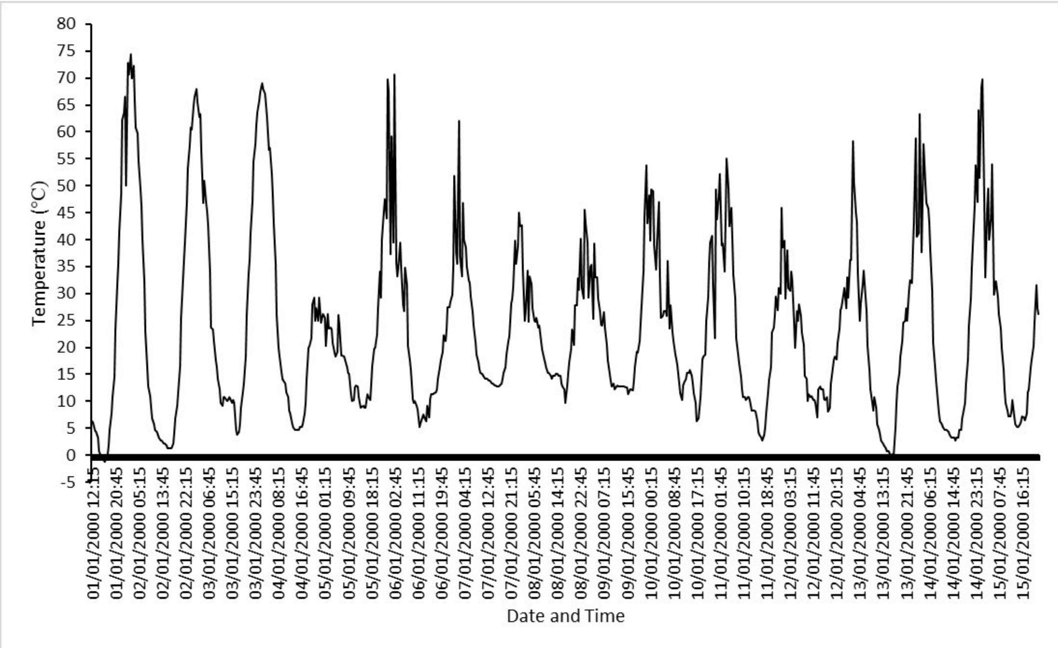

Figure 4o) temperature underneath sinusoidal iron refugia over time

4.3.3 Reptiles Found

During the first and second surveys no reptiles of any kind were found under the refugia. Refugia surveying is a tried and tested method and the sites being surveyed were known to support a reptile presence. Therefore, there must have been a flaw in the specific methodology of the first two surveys, most likely the weather being too hot during the first survey and too cold during the second. The alternative is that the reptiles are no longer present at the three sites. Assuming the former, the lack of results was attributed to the use of experimental materials, the sub-optimal time of year and the possible less effective shape. These were only assumptions however as there was no data to draw conclusions from. Therefore, the different materials were treated to have performed equally with none being better or worse that the rest. The decision to remove certain material came down to cost and survivability in the field.

Over the course of the third survey eight snakes were found under the refugia, all of them grass snakes (*Natrix natrix*). Shown in figure 4p below, six snakes were under sinusoidal Iron, two were under flat iron and none were found under either shape of bitumen. This displays an obvious preference for iron over bitumen and a possible preference for sinusoidal shapes over flat. With only eight snakes providing the data however, these results are dubious. This is not enough data points to provide a reliable conclusion and so we cannot say for certain that these conclusions are accurate.

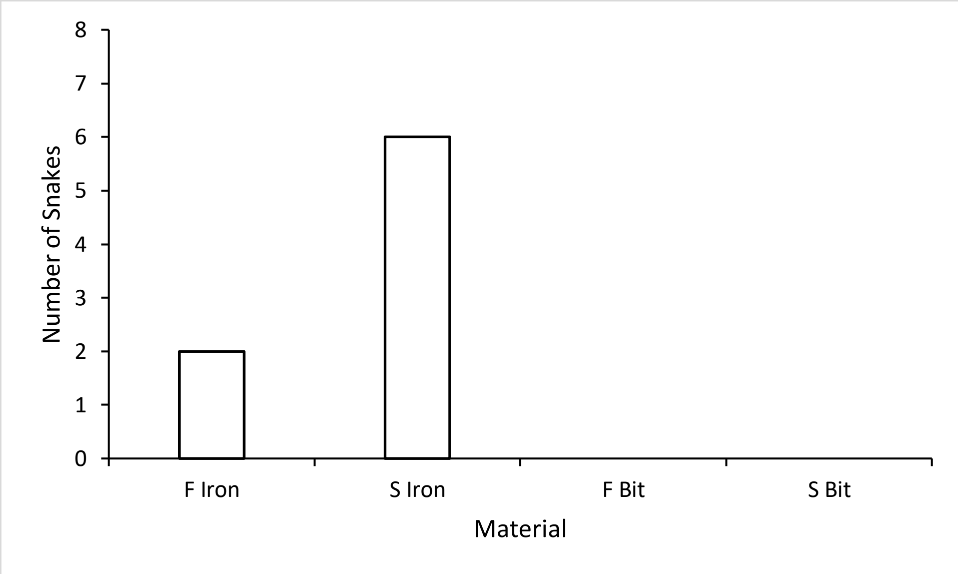

Figure 4p) number of snakes found under each refugia material

4.4 Discussion

According to the data there was a statistically significant relationship between every factor, material, site, air and ground temperature and location within the site. This means that everything must be considered when conducting a refugia survey. A significant relationship between material and temperature confirms that each different material creates a different microclimate beneath it and therefore it is true that one can be more suitable than the others. However, there was no statistically significant interaction between material and site meaning that the differences in habitat did not affect the performance of each material. While the different sites did affect the temperatures of the refugia it did not affect any material differently meaning none perform better at different habitats. It remains possible that if a wider range of habitats were surveyed there could have been an interaction but between the three used in this survey all refugia performed the same. The interaction between ground temperature and cluster means that temperatures vary within a single habitat and that has an effect on the temperature of the refugia. When preparing to conduct a survey it could be important to analyse how different areas within the site vary in temperature because that can have an effect on refugia performance and if refugia placements are chosen correctly it could be a positive effect.

Based on data from the data loggers in October, Bitumen looks to be the best material so far. Of the three materials in that survey it reached optimum temperature (25°C, Isaac, 2003) more often, whereas the next most popular material, iron, only reached optimum temperature once. Carpet was discounted as a candidate because of its tendency to withhold excess moisture making its underside damp for extended periods of time. There remained the possibility that iron would be more suitable in warmer months, as during the October survey it went into negative figures less than the other materials. However, during the July/August survey the data loggers reported abnormally high temperatures for both types of iron. During this survey, the materials regularly exceeded 60°C or even 70°C. These figures, while potentially anomalous and the result of equipment failure, represent excessively high temperatures that could be fatal to any reptiles sheltering underneath them. Reptile performance is known to decrease beyond 38°C (Isaac, 2003) and body temperatures greater than 40°C are considered to be lethal (Gaywood and Spellerberg, 1995). Flat bitumen also reached these abnormally high temperatures but not as regularly. The only material that did not reach these temperatures was sinusoidal bitumen, which frequently reached optimum temperature without exceeding it by a dangerous margin. Therefore, based on the data logger data on both surveys, bitumen still looks to be the most effective material. It reached high temperatures during the colder months but didn’t reach temperature too high in the hotter months, staying usable at both times of year.

However, despite all of the data up until this point suggesting that bitumen would be the best material, not one reptile was found sheltering underneath any bitumen refugia during the survey. While there have been studies where iron refugia were favoured over other materials (Hodges and Seabrook, 2016) even then the other materials produced some results. All reptiles in our survey were found sheltering under an iron refugia, two under flat and six under sinusoidal. This result contradicts the previous hypothesis and all previous data and suggests a fundamental misunderstanding on what makes a refugia effective. As stated earlier the refugia were placed in a cluster, all refugia adjacent to one another and one of each material present in each cluster. the purpose of this arrangement was so that when a reptile found one refugia they also found the others. The reptiles had a clear choice of refugia to use. Assuming that reptiles are capable of selecting which microclimate is most optimal for them (Kearney and Predavec, 2000; Law and Bradley, 1990) then the refugial they choose to shelter underneath is the refugia that provides the best microclimate.

There are two possible explanations for the contradictory data. The first is that all the previous hypotheses are correct, and bitumen is the superior material, but grass snakes (*Natrix natrix*), the only species found in the survey, are not capable of distinguishing this fact. Unlike other reptiles that are capable of determining the most optimal microclimate grass snakes may lack that capability and so simply went to whichever material they encountered first. This is unlikely as Luiselli *et al.*, (1997) found that pregnant grass snakes were capable of selecting the warmest microclimate during oviposition. The second theory is that temperatures first thought to be dangerously high may actually be better for basking. It would be in a reptile's best interest to reach temperature as fast as possible to give it more time to be active. The hotter the refugia the faster a reptile sheltering underneath it would heat up. It is possible that the reptiles are risking overheating under an exceptionally hot refugia to reach optimum temperature faster. During a survey of Galapagos Land Iguanas (*Conolophus pallidus*) by Christian *et al*., (1983), the animals were found to maintain a body temperature of roughly 32°C despite microclimate temperature reaching or exceeding temperatures of 60°C. This supports the idea that reptiles will choose the microclimate with the highest temperature, even if those temperatures are dangerously high, and simply vacate the microclimate when they have reached optimal body temperature.

The reptiles also displayed a preference for the sinusoidal material over the flat ones. It is possible that the reason flat materials were less favoured is that, due to their shape, more of them is in contact with the ground. This facilitates heat exchange between the two surfaces meaning that as the refugia is heated by the sun that heat is gradually leeched away by the ground. Sinusoidal materials do not have as much material in contact with the ground and so conserve more of their heat. From this experiment we can conclude that sinusoidal refugia are likely to perform better than flat ones. There are in fact many studies that specify the use of corrugated materials in their methodology (Michael et al., 2012; Hampton, 2007; Ballouard et al., 2013). This implies that corrugated materials are known to perform better than flat ones. The only potential advantage to flat materials is ease of transport due to them taking up less space.

Throughout the survey only eight reptiles were encountered. There also exists the possibility that the same reptile was encountered multiple times. Either way the sample size is small, and any conclusions drawn are uncertain. A likely cause of this is the sub-optimal times of year that the first two surveys took place in. The temperatures during July and August are often much higher than is recommended for reptiles surveying, as seen in the data logger readings where the temperatures often reached lethal extremes. The weather in October is also not ideal, being much colder than recommended. It is understandable then, that the first two surveys turned up no results. The high density of the refugia placement could also have been a factor in reducing the number of reptiles found, as it meant less overall space was covered. During the third survey, all 12 refugia on each site were concentrated on an area less than an acre. This was a consequence of the clustered style of placement that was done to ensure the reptiles had a choice of the different materials and also of budget constraints. It could well be that if more snakes were encountered a different pattern would have emerged. Eight data points is not enough to draw a statistically significant conclusion from. With the data available to us we must conclude that a) sinusoidal shapes are superior to flat ones, and b) iron outperforms bitumen when painted black.

Chapter 5: Conclusion

5.1 Survey Constraints and Possible Improvements

The surveyor for this project did not possess a valid driver’s licence. Acquiring one and running a car was outside their means. As a result of this the choices of available habitats to survey was extremely limited; only sites that the surveyor could reach via public transport were an option. One of these sites was a known floodplain that did in fact flood during the survey, making data collection impossible for several weeks. This also meant that ability to reach the sites was reliant on there being no disruptions to the transport network. Delays and cancellations could and did cost survey days. Additionally, the timetables of public transport dictated what times it was possible to survey the sites. For the most part the surveyor was able to reach the sites early enough but due to reduced services on Sundays it was impossible to reach the sites during the survey window on those days.

While the sites used in this survey did have a reptile population and provided results, they were frequented by the public and this could have led to the reptiles being more sensitive to disturbance and more likely to flee or avoid people. Additionally, the only reptile species recorded to be at these sites were Grass Snakes which made up the entirety of the species found. In order to maximise the chance of the refugia producing a result the survey was focused on Grass Snakes, choosing sites or areas within sites that were close to water. This may have helped the survey, or it may have excluded other reptile species that were present but not recorded meaning that the results and conclusions are biased towards a single species. It is possible that the surveys would have had a stronger result if they took place in a more rural area with less disturbance from people. A surveyor who possessed a valid driver's licence would have been able to choose from a wider array of sites that could have had a larger population of reptiles and produced a stronger result. Other surveys into this subject would benefit from recruiting more than one surveyor so that a larger number and wider variety of habitats can be studied.

Budget was an additional limiting factor in this survey. During the early field surveys only two brass and aluminium refugia were purchased, one of each painted black. This meant that there was only one of each material during the first survey, which the surveyor decided was not enough for later ones. Due to the high price of these materials no more could be purchased with the budget possessed. While this is a limiting factor that makes these materials potentially unsuitable for refugia surveying it remains possible that if more had been purchased, they would have eventually provided a stronger result. Theoretically, while those materials are not feasible in large scale refugia surveys due to their cost they might have been in smaller scale ones due to better performance. This is a hypothesis that could not be tested thoroughly due to budget restraints.

As mentioned in section 1.5, a weakness of refugia is that the reptiles are free to leave the refugia before the surveyor arrives, a weakness that could have been mitigated by camera traps. The addition of camera traps would have given a better idea of the number and species of reptiles visiting each refugia which could have resulted in a different conclusion than the one formed by visiting refugia alone. Small modifications to the refugia would have been needed in the form of an open margin around them, so the reptiles cannot hide in the undergrowth when they approach. Another technique that could have improved the survey was mark-recapture. While estimating population size was not the aim of this survey it would have been useful to know for sure if different individuals were visiting the refugia or if it was the same found multiple times. With the high density of the refugia placement this is a possibility and finding the same individuals over and over might have prompted a widening of the survey area.

5.2 Key Findings

The purpose of this research was to prove empirically which material produces the best results for reptile surveying with artificial refugia. Additionally, it was an attempt to discover new suitable materials and show that surveying techniques can always be improved upon. In the first regard, a conclusion on the best performing material has been reached. In the second regard, an alteration on traditional methods first attempted in this study has shown to be more effective than the original. This thesis had succeeded in its aims.

From the lab work we drew three conclusions. We believed that bitumen was likely to be the best performing material in the field, with the highest rate of temperature increase in the first experiment and a strong difference between heating time and cooling time in the second. We also found that painting metals black made them perform better as carpet initially outperformed the non-painted metals but was in turn outperformed when paint was applied. Brass was found to be a stronger conductor of heat than iron and so we believed it could serve as a replacement material in the field. Therefore, at the end of the lab work we had two hypotheses.

1. Bitumen will be the strongest performing material in the field
2. Brass will perform better than iron in the field and can serve as a viable replacement

These hypotheses were supported in our survey work. Of all our materials bitumen was voted the most popular among herpetologists by a wide margin, especially by the organisations that carried out more surveys. Iron was next most popular. If a material as commonly used as iron could be replaced by a better material than it would greatly improve reptile surveying as a whole. During the survey we also found out how important material cost is during a survey, with many herpetologists employing refugia in large numbers. Going forward cost became a factor in determining the best material.

Our hypotheses were tested with the field research. All materials previously tested were used in a reptile survey at three locations noted by the local Warwickshire Wildlife Trust to have a reptile population. However, our hypotheses were challenged by this first survey as all refugia failed to produce a single result. No reptiles were found sheltering underneath them. With this lack of data, the only conclusion we could draw was that all materials had performed equally; bitumen was not the strongest performer and brass did not outperform iron.

During the second survey the number of refugia used was increased. However, due to our findings in the online survey we now knew the importance of material cost in a survey. Therefore, we discounted aluminium and brass due to the prohibitively high expenses of ordering them in the quantities we needed. We therefore rejected out hypothesis that brass could replace iron as a survey material. This survey took place at a less effective time of year for reptile surveying and so data loggers were used to track temperature throughout the survey and see whether or not the refugia could get to optimal temperature in the colder weather. While no reptiles were found in this survey either the data loggers showed that of the materials left bitumen was most consistently reached the temperature required for reptiles. Our first hypothesis, that bitumen would be the best performer, was still viable.

The third survey was the largest and most comprehensive. It began in April and continued until October. The beginning of this survey was in peak reptile activity time, increasing our odds of getting results. Temperature was also taken from the refugia during visits to see exactly how warm they were during the survey window. These temperatures would be compared later to help determine which material performed better. According to statistical tests none of the materials were significantly different from one another in regard to their temperature, but only the sinusoidal materials were significantly different from the ground temperature. Given that the purpose of a refugia is to be warmer than its surroundings we drew from this the conclusion that sinusoidal materials do perform better than flat ones. Data loggers were also used during late July/early August, when temperatures would be at their highest. Like the previous survey, this was not ideal survey time and so the loggers were used gauge how well the materials performed throughout the higher temperatures. This data further supported our hypothesis that bitumen was the strongest material. While none of the materials showed a statistically significant difference in temperature taken during peak visiting hours, the data loggers revealed that every material other than sinusoidal bitumen was reaching dangerously high temperatures outside of those hours. Sinusoidal bitumen even had days where its highest temperature was within the optimal temperature threshold, meaning it stayed at optimal temperature longer than any other material.

However, despite all our data pointing toward our hypothesis that bitumen was the superior material, the most important factor of out survey, number of reptiles found, was not in favour of bitumen. Eight reptiles were found under the refugia, all of them grass snakes (*Natrix natrix*) and all of them sheltering under iron. This suggested a fundamental misunderstanding on our part about what makes a refugia suitable. Alternatively, it could be a behaviour unique to grass snakes specifically and other reptiles may have favoured bitumen.

With the data from this survey we made two conclusions:

1. Sinusoidal materials outperform flat ones
2. When painted black, iron outperforms bitumen

5.3 Implications of Findings

The importance if this result is that, as we saw in the survey, bitumen is more popular than iron but according to our results a simple alteration can make iron out-perform bitumen. Iron is already widely used and is cheap enough to be used in large numbers making it cost effective (McMillan, 2019; Stiles, 2012). The addition of black spray paint does not add a large amount to cost and has a strong positive effect on the performance of iron. Depending on the type of paint used a single can will paint up to ten refugia. We have also shown that sinusoidal shaped refugia will outperform flat ones in the field. Therefore, our research may have found the most optimal material for refugia surveying; corrugated iron painted black. This information will no doubt be of value to herpetologist across the country; the material that produces stronger results will make conclusions they draw from their own surveys more reliable. Reptile surveying, and by extension reptile conservation as a whole, can be improved with this information.

A precedent exists for iron being the superior survey material. Reading (1997) proposed a standard surveying methodology for reptile surveying in dry lowland heaths and in that methodology, they used galvanised steel painted black, similar to our own methods. No other materials were suggested. Different methodologies suggest different materials. Some surveyors even claim that iron may not be a suitable material (Stiles, 2012). According to different sources, there are differing reasons why on material may or may not be suitable. Langham (2011) found a preference among different species for different materials, claiming that adders prefer corrugated iron whereas lizards prefer roofing felt (bitumen). However, a different study by McInerny (2019) exclusively used bitumen for a survey of adders and still gained strong results. In another survey by McInerny (2017) who used only bitumen they found that only slow worms use the refugia, whereas adders and lizards that were found on site did not always use them. Having not found either species in our own survey we cannot support or refute this claim, but we can suggest the possibility that grass snakes (*Natrix natrix*) have a preference to iron. It is possible therefore, that our results show not a single superior material but a species-specific preference. When Hubble and Hurst (2006) conducted their study of slow worm translocation they used exclusively bitumen refugia. Since their research was focused on a single species it is possible that their choice was based on a known preference among the species for that material.

The survey protocols for British Herpetofauna suggests using a mix of iron and bitumen (referred to as tins and roofing felt), possibly due the preferences by different species, but does not specify either colour or shape (Sewell *et al*., 2013). It says that because of the differing thermal properties of the two materials a mix will perform better that any one alone. We cannot discount this based on our conclusions; it may be possible that our methodology carried an innate bias towards iron we were unaware of. However, we can dispute, or at least add to this piece, with our conclusions that corrugated shapes perform better and black improves the performance of iron.

Sewell *et al*., (2012) suggested at least thirty refugia should be laid out to determine presence or absence. Our own survey had responses of up to 300 refugia. It was from this survey that we realised the importance of cost to the ideal refugia. The website we used to supply our bitumen refugia (https://www.nhbs.com/corrugated-reptile-survey-refugia) sells them as £3.95, meanwhile an iron refugia of the same dimensions sells for slightly higher at £5.22

(https://www.wildcare.co.uk/reptile-profile-tin-500x500mm.html). When fielded in large numbers iron can reach a cost significantly higher than bitumen. This is likely a factor in why bitumen was more popular than iron in the survey. Additionally, at those numbers it is important to note that the increased weight of iron over bitumen would make transportation harder. Even if iron is the superior material it could be that bitumen is just more convenient for a surveyor.

Thermoregulation might not be the only reason for species-specific material preference. Grass snakes (*Natrix natrix*), the only species found in our survey, is known to be a generalist predator that feeds mainly on anurans but also on small mammals (Gregory and Isaac, 2004). Two such potential prey species were found underneath iron refugia, the preferred material of the grass snake. An unidentified population of small mammals had fashioned a burrow in the dead grass underneath an iron refugia, the same refugia that was frequented by snakes. Figures 5a and 5b below show a burrow underneath a refugia and one of the snakes found under the same refugia. Additionally, an amphibian, likely either a common frog (*Rana temporaria)* or a common toad (*Bufo bufo)*, was found beneath an iron refugia on the same site. While no snakes were located underneath that particular refugia it still proves that amphibians can be found underneath refugia. Figure 5c below shows the individual beneath the refugia. Amphibians being found under refugia designed for reptiles, specifically iron ones, is a known occurrence (Hampton, 2007). Many small mammals are known to make use of artificial nest boxes (Hoffmeyer, 1973; Marsh and Morris, 2000), which implies that they might be inclined to take shelter underneath other artificial cover like a reptile refugia. This opens up the possibility that prey items can be located underneath refugia designed for reptiles and this could influence reptile refugia choice. Therefore, the preference if these potential prey animals for iron or bitumen is another contributing factor to which material performs best.

Figure 5a) a burrow found underneath a refugia in the field containing a population of unidentified small mammals

Figure 5b) grass snake (*Natrix natrix*) found underneath same refugia as the burrow in figure 5a

Figure 5c) unidentified amphibian found sheltering beneath an iron refugia in the field

5.4 Contributions to Professional Practice and Recommendations to Practitioners

One of the original aims of this study was to improve on professional practice by finding new materials to use as artificial refugia. Unfortunately, none of the new materials proved effective meaning we failed in that respect. Our findings support the idea that iron and bitumen, the two most common materials used by professionals, are the best performers. We did find however, that painting an iron refugia black has a marked effect on its temperature profile and in our fieldwork black iron outperformed bitumen in terms of how many reptiles it attracted. There is no way of knowing however, if that is a universal improvement or specific to Grass Snakes. Beyond that, this study did not make any significant discoveries that will improve professional practice. One method of our study that we believe could improve professional practice is the use of data loggers underneath the refugia to track the temperature of the refugia. The data loggers allowed the creation over time of a temperature profile that would tell a surveyor at roughly what times the refugia reach the optimal temperature. This would allow surveyors to carry out their site visits at the best possible time. This practice would however be very dependent on air and ground temperatures witch as we explained in section 4.3 does have an effect of refugia temperature. We discovered during the analysis of our data that there is a great number of factors that affect refugia temperature, all of which could dictate their ability to attract reptiles. Practitioners could do well to consider each of these factors when preparing to conduct their own refugia surveys. Temperatures are not homogenous throughout a site, as we found when we looked at the interaction between cluster and ground temperature is section 4.3, meaning that a surveyor should examine the ground temperatures of every location they plan on putting a refugia down as it could impact that refugia's performance. We also found that different material performance was not affected by each site being a different habitat. Surveyors should therefore not need to consider whether different refugia would be unsuitable for specific habitats and simply use whatever materials have given the best results no matter where they survey. However, we drew this conclusion from a survey across only three different habitats, all of which were different types of grassland (marshy, wild, and regularly managed). While this brings up the possibility that habitat does not affect material performance is not affected by habitat this hypothesis could be disproved by surveys across a wider variety of habitats.

Our conclusions were, regrettably, formed from a small sample size. Only eight reptiles were found over a five-month period. We have formed what conclusions we can from these results but with such a small set of data points our results are not as reliable as would be ideal. To truly prove whether or not black iron is better than bitumen additional surveys would be needed over a longer time period. Most importantly at sites which support a larger reptile population so a larger sample size can produce more reliable conclusions. We would recommend any surveyors looking to use iron refugia to trial painting it black to see how it performs beyond this study. With the information taken from the National Biodiversity Network and the results of our survey it is likely that there are no other reptiles species present at our sites.

We believe that species-specific material preference warrants more detailed study. The purpose of this study was to find a single material that performs the best in the field and our results have pointed towards a single material, but the idea that individual species might have their own preferences suggests that such an aim might be impossible, especially in light of the fact that all our data came from one species. Circumstances forced the survey to be focused on a single species, but similar surveys conducted on different species could yield results indicating specific preferences. If the preferences of each species could be identified than it is possible to find what attributes of that material exactly attracts that species. A single material that incorporates all those attributes could be the single ideal material for surveying British reptiles.

Additionally, the ability of a refugia to attract non-reptile prey animals is a potentially unknown or under-represented factor in refugia surveying. Thermoregulation is known to decrease time spent hunting and make predatory behaviour less efficient but on the other hand digestion is improved at higher body temperatures (Avery *et al*., 1982). There are costs and benefits to thermoregulation (Huey and Slatkin, 1976; Herczeg *et al*., 2006; Sears and Angilletta, 2015) but if artificial refugia can make thermoregulation more efficient and simultaneously provide hunting opportunities than the costs will be drastically offset.

In conclusion this thesis has initially achieved it aims but in the process revealed new factors that call into question whether or not those aims are actually possible. The concept of species-specific material preference introduces the possibility that our conclusions are incorrect, and one material will never be ideal for all reptiles. Indeed, as has been previously stated in this study, some surveyors claim that a mixture of materials is better for this reason (Sewell *et al*., 2013). With the data we have we cannot confirm or deny this, we would need results from multiple species and to perform a statistical analysis of species preference for each material. Without reasoning beyond our data, we have concluded that corrugated iron painted black is the optimal material, but we would encourage any other surveyors to study material preference between different British reptiles to determine how pronounced this effect is and what it means for reptile surveying.

Appendix

Appendix 1: Introduction

<u>1.1.2 Physiological Adaptations for Thermoregulation</u>

One of the few physiological adaptations reptiles have that can aid thermoregulation is the ability to adjust their heart rate. Reptiles can exhibit differential heart rates in response to heating or cooling, with heart rates increasing when warming and decreasing with cooling (Kik and Mitchell, 2005). This is referred to as heart-rate hysteresis (Millard and Johansen, 1974; Hicks, 2002; Seebacher and Franklin, 2003; Grigg and Seebacher, 1999). Hysteresis is where the system's response to an outside influence depends not only on the magnitude of the influence but also on the system's previous history. Laboratory studies on lace monitor lizards (*Varanus varius)* conducted by Seebacher and Grigg, (2001) show that heart rate in reptiles is faster while heating than cooling. This is due to changes in peripheral blood flow, which is the blood flow in the extremities. The purpose of this higher heart rate and peripheral blood flow is to accelerate heating by convective heat transfer, the transfer of heat through the movement of fluids (Seebacher, 2000).

Metachromatism is the ability to change skin colour (Rosenblum *et al*., 2004). This adaptation allows any reptiles that have it to regulate body temperature. When temperatures are low, they turn dark and when temperatures are high, they turn lighter (Rosenblum, 2005). Darker colours increase heat absorption whereas lighter colours reflect heat. This phenomenon was examined by Walton and Bennett, (1993) on three species of chameleon in Kenya (*Chamaeleo dilepis, C. jacksonii, and C. ellioti*). They found that darker colouration reduced the amount of basking time required to reach optimal temperature. While Chameleons (family *Chamaeleonidae*) are most famous for their dramatic colour changes they are not the only reptiles who change colour to suit their environment. Boback and Siefferman (2010) discovered regular cycles of colour change in Boa constrictors (*Boa constrictor*), a daily cycle wherein the animal becomes lighter at night and darker during the day, and a seasonal cycle where the animal becomes lighter in the wet season and darker in the dry season. The snakes are suspected to change colour based on a hormone cycle, which is fundamentally different to the use of chromatophores in chameleons (Teyssier *et al*., 2015). Multiple different mechanisms have evolved separately in reptiles to change colour, showcasing how much of an advantage it gives. The ability to willingly change colours to suit their thermal needs allows them to spend less time basking or sheltering, giving them more time to pursue other needs like food water and mating.

1.2.3 Disease

In recent times there has been an increase in infectious diseases threatening wildlife throughout the globe (Daszak *et al*., 2000; Smith *et al*., 2006). Fungal diseases in particular are on the rise and contributing to many species decline (Fisher *et al*., 2012). Two particular examples of these damaging fungal diseases are *Chytridiomycosis* which is responsible for a global loss of amphibian diversity, and the so-called white-nose syndrome which caused massive population declines of some bat species (Berger *et al*., 1998; Blehert *et al*., 2009; Turner *et al*., 2011).

One example of diseases threatening wild reptile populations is mycoplasmosis in desert tortoise (*Gopherus agassizii*) (Brown *et al*., 1994). *Mycoplasma agassizii* is a bacterial pathogen that leads to upper respiratory tract disease (URTD) in tortoises with symptoms including nasal discharge, lesions or necrosis in the respiratory tract, conjunctivitis, and edema of the eyelids and ocular glands (Brown *et al*., 1999). Factors that are believed to contribute to URTD outbreaks include environmental stress, human impacts, exposure to heavy metals and other toxicants, and the escape or release of captive tortoises (Jacobson et al., 1991, Brown et al., 2002, Sandmeier et al., 2009, Sandmeier et al., 2013). Mercury especially is known for having a variety of toxicological effects when wildlife is exposed to it (Rice *et al*., 2014). Jacobson et al., (1991) surveyed seventeen desert tortoises infected with URTD, thirteen of which had to be euthanised due to severe necrosis. They found that mercury concentrations in the livers of affected tortoises were significantly higher than those of the control specimens; the controls contained an average of 0.0287 parts per million, ppm, whereas infected wild tortoises had 0.326 ppm. Other stress factors can compromise the immune system of tortoises, making them more susceptible to the disease. Berry *et al*., (2006) surveyed 21 plots for desert tortoise. The abundance of URTD was negatively correlated with distance from human settlement, implying that the stresses associated with proximity to humans which, in this particular instance, included surface disturbances, trash, military ordnance, and proximity to paved roads, make the tortoises more vulnerable to the disease.

The most obvious threat diseases pose is the increase in mortality rates that come from an outbreak. However, diseases can also have indirect negative effects. Some conservation organisations rely on the public to carry out their work, either in the form of donations or volunteers. In order to gather this support, they must maintain a good public image. Many organisations use charismatic flagship animals to promote conservation, typically large-bodied mammals of significant conservation concern (Clucas *et al*., 2008). Negative public opinion can be a large detriment to conservation efforts as the public view species as pests or even threats. In recent years there has been a rise in reports of reptile transmitted salmonella from both captive and wild reptiles. A study in Italy by Corrente *et al*., (2017) found that the average reptile owner was unaware of basic hygiene measures for prevention of reptile-associated salmonellosis (RAS). Lukac *et al*., (2015) conducted a survey of 200 individual reptiles belonging to private owners or housed at the Zagreb Zoo and detected salmonella in 13% of the animals. Whitten *et al*., (2015) found that 3.5% of salmonella patients in Minnesota during 1996–2011 reported reptile exposure and that children where the ones primarily effected. This shows that reptile husbandry carries with it an increased risk of infection, even is well cared for and healthy animals. Wild animals could carry an even greater threat of infection. A study of wild snakes in Japan collected 87 individuals and examined them for presence of Salmonella. They found that over half (58.6%) of snakes were carriers. Hilbert *et al*., (2012) suggested four pathways in which salmonella carried by wild reptiles could infect humans:

1. Via contact with domestic animals as transmission or accumulation vectors
2. By direct contact with humans
3. Through meat of wild animals
4. By contamination of food or food producing units

Cases of RAS are on the rise and if it continues it could shift public opinion against reptiles. This in turn could have a negative effect on reptile conservation as it loses support in favour of reptile extermination.

Snake fungal disease is a disease causing a severe decline in snake numbers throughout the USA (Lorch *et al*., 2016). It was first noticed in 2006 in New Hampshire, associated with a decline in a timber rattlesnake (*Crotalus horridus*) although Clark *et al*., (2011) noted it was not the only cause for the species decline. Clark *et al*., (2011) found that the populations that suffered from a lack of genetic diversity due to genetic bottleneck were more susceptible and other, non-inbred populations, were unaffected. The year in question also had exceptionally high summer rainfall which would increase the local humidity and create an environment where fungi could more easily thrive. This emphasises how multiple other factors can combine to increase the vulnerability of a population to diseases that were otherwise not a threat. In 2011 Sutherland *et al*., (2011) published a horizon scan of global conservation issues, and in their paper, they listed snake fungal disease as a major conservation concern in North America.

An important factor in disease resistance in population genetics. Hosts and their pathogens are involved in a co-evolutionary arms-race, with each evolving new strategies to overcome the other (Clay and Kover, 1996). New mutations and adaptations to a disease can appear anywhere at any time and as a result, there are often special variations in resistances within a population (Laine *et al*., 2011). An example would be viral eye disease in juvenile Swedish common lizards (*Lacerta vivipara*). A study by Uller *et al*., (2003) found that there was a difference in resistance to the disease between lizards in the north of Sweden and lizards in the south, with the south being more resistant due to increased selection pressure. Hypothetically, if the southern population were damaged by an unrelated event then the species as a whole would lose its resistance to the disease because the individuals that have inherited a genitive resistance would not be able to pass it on to future generations. Loss of genetic diversity can make a species more vulnerable to disease (Spielman *et al*., 2004). Therefore, any factor that reduces populations, and so also reduce genetic diversity, can have the secondary effect of increased disease susceptibility. This opens up the possibility of a vicious cycle, wherein the other threats to reptiles (climate change, habitat loss, pollution, or invasive species) cause a significant population loss that is later exacerbated by subsequent disease outbreaks. Loss of habitat is one of these factors and one that has been increasing in recent years (Pavlova *et al*., 2017; Jackson and Fahrig, 2016; Browne *et al*., 2015).

1.2.4 Pollution

Human activity does not only cause habitat loss, but also habitat pollution. Manmade pollutants include plastic, heavy metals, herbicides and pesticides, sewage, and radiation. All pollutants have negative effects on the health of ecosystems and the wildlife within them, by definition. One example of a toxic pollutant is mercury, which is released into the air when fossil fuels are burned and falls into the water and land (Joensuu,1971; Pacyna and Münch, 1991; Pacyna *et al*., 2006). Mercury released into the environment is especially dangerous due to the phenomenon called biomagnification, the increasing concentration of a substance at successively higher levels in a food chain (Poste *et al*., 2015).

Sea turtles have been subjected to increasing levels of plastic pollution in the ocean. Plastic debris in the oceans is accidently ingested by the animals, often due to mistaking the plastic for natural prey items (Schuyler *et al*., 2014). A study by Bjorndal *et al*., (1994) which examined the digestive tract of 51 turtles found that 25 out of 51 had ingested some form of plastic; 24 of 43 green turtles (*Chelonia mydas*), and 1 of 1 loggerhead (*Caretta caretta*). The debris found by Bjorndal *et al*., (1994) was not only limited to plastic, and included fishhooks, rubber, aluminium foil, and tar. The risks to animals posed by plastic debris include entangling and subsequent drowning, starvation or predation, wounds from abrasion or cutting from debris and blocked digestive tracts or damaged stomach linings from ingestion (Laist, 1987). It is worth noting however, that while ingestion of plastic has often been reported to cause injury or death (Nelms *et al*., 2015), it is not always the case. Clukey *et al*., (2017) conducted a study on plastic ingestion in pacific sea turtles. While 91% of turtles examined had some kind of plastic in their digestive tract, most commonly in the large intestine, Clukey *et al*., (2017) did not find signs of any adverse health effects directly caused by plastic in any of the 55 turtles examined. An analysis by Wilcox *et al*., (2018) found that the accumulation of plastic in the digestive track results in an increased chance of death by plastic. Simply put, a single item of plastic is unlikely to result in death, but as more plastic items are ingested the likelihood of death rises at a commensurate rate. Wilcox *et al*., (2018) found that 14 items corresponds to a probability of mortality of 50%. It is a safe assumption therefore, that as levels of plastic pollution in the ocean increase so too will mortality in sea turtles.
The industrial use of pesticides has caused a variety of negative effects to non-target species in the areas they are used, including local reptiles (Mingo *et al*., 2016). A study by Weir *et al*., (2016) found that the reptile skin is permeable to pesticides in their environment; the permeability of reptile ventral skin is roughly equivalent to mammals. However, it is rare that pesticides taken through the skin are in high enough concentrations to be lethal (Weir *et al*., 2015) but they can

cause health issues and stresses to the animal. Chang *et a*l., (2018) identified a number of sub lethal effects in native Chinese lizards caused by local pesticides absorbed through the skin. They found that an increased body burden of diflubenzuron and flufenoxuron caused liver lesions and altered the transcription of genes in the hypothalamus-pituitary-thyroid (HPT) axis and the metabolism, disrupting both systems. Pesticides can also enter a reptile's body through the food it eats. If pesticides enter any local species, they will inevitably enter into the food web as the exposed animals are eaten by predators. If the reptile's prey species are contaminated, then there will be increases in mortality and decreases in food consumption leading to overall lower health, an effect that will increase the higher the concentration of pesticide consumed (Chen *et al*., 2019). It is difficult however, to determine an overall global trend in the threat's pesticides pose to reptiles because there is huge variability in the pesticides themselves, the dermal permeability of individual reptiles, reptile species and the likelihood of reptiles coming into contact with pesticides. Realistically, the threats posed can only be considered on a case by case basis based on the exact pesticide being used and the species present in the area.

Appendix 2: Site Photos

Site 1 (Welches Meadow)

Figure A2.1: An image of the area within Welches Meadow that was surveyed

Figure A2.2: An image of some of the grass species common to site 1

Figure A2.3: An image of some of the grass species common to site 1

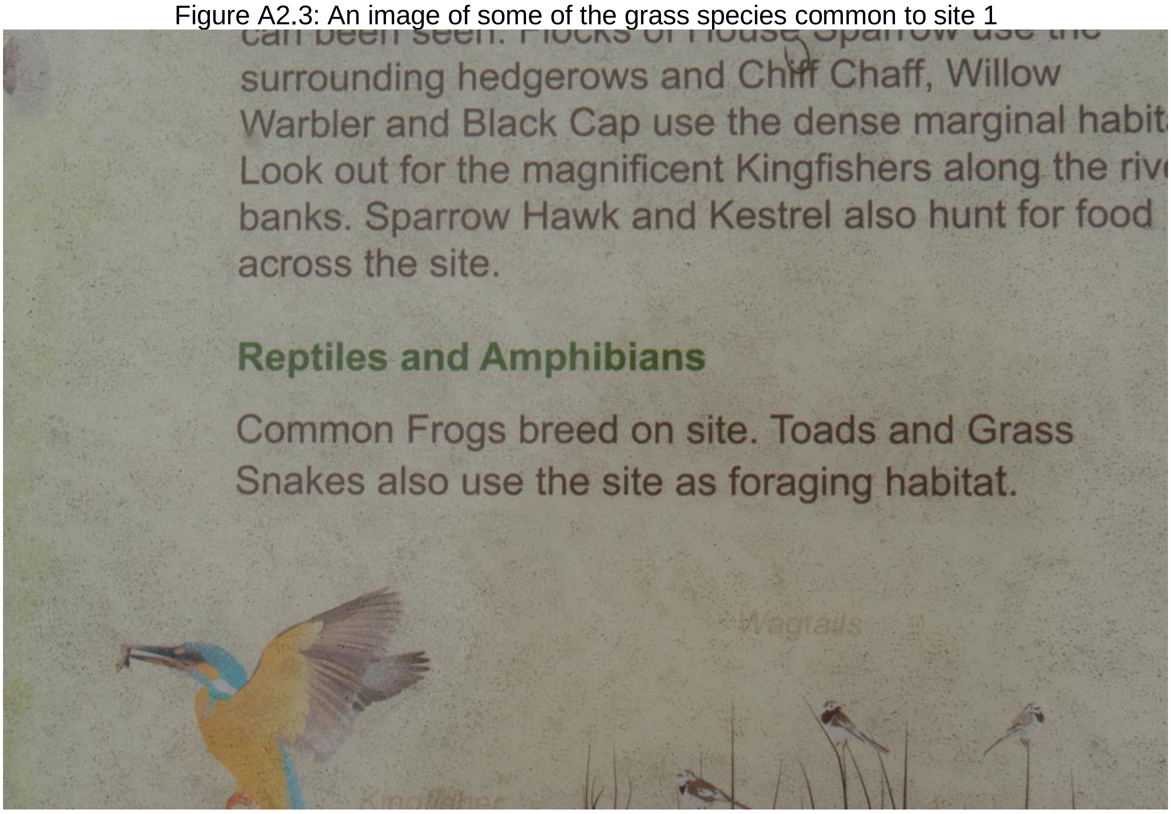

Figure A2.4: An image of the onsite information screen provided by the Warwickshire Wildlife Trust confirming the presence of Grass Snakes

Site 2 & 3 (Leam Valley)

Figure A2.5: An image of the area within Leam Valley that made up site 2

Figure A2.6: An image of the area within Leam Valley that made up site 3

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