Evaluating survey design and long-term population trends in slow-worms (*Anguis fragilis*)

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"The fact is that no species has ever had such wholesale control over everything on earth, living or dead, as we now have. That lays upon us, whether we like it or not, an awesome responsibility. In our hands now lies not only our own future, but that of all other living creatures with whom we share the earth."

Sir David Attenborough

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It would not have been possible to complete this project without the help and support of so many people.

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Abstract

Habitat loss and fragmentation from increased development and land use change, are major threats responsible for national declines in slow-worm numbers. The legal protection and priority status afforded to this species has increased the need for species-specific surveys and monitoring to be undertaken. Current reptile survey guidance is outdated and unstandardized, which has the potential for survey results to vary significantly, especially relating to the levels of survey effort needed to obtain meaningful results. Consequently, such survey results are used to inform important ecological decisions, particularly surrounding slow-worm mitigation and conservation. This study was undertaken to determine if and how the number and distribution of artificial refugia impact on slow-worm detectability and additionally, compare slow-worm populations over time between two sites within King's Wood, Challock, UK. Tin size and layout, as well as tin density were identified as key factors that impact slow-worm detectability. Consequently, more slow-worms were recorded using tins 0.25 m^2 in size at a density of 40 per hectare compared to using tins 0.5 m^2 in size at a density of 20 per hectare. Doubling the number of tins at the site resulted in a doubling of the number of slow-worms, but no change in the number of slow-worms captured per tin. There was no difference in captures between tins laid down for a year and tins laid for a few weeks. Long-term population monitoring suggested that vegetation change is a major factor contributing to declines in slow-worm numbers within a local population. The findings documented in this study, emphasise the need for existing reptile survey guidance to be updated to account for the significant impact refugia density and refugia size has on slow-worm detectability. In addition, slow-worm conservation should be determined on a site-specific level, to ensure the best outcome for slow-worm populations.

Key words: slow-worm; artificial refugia; detectability; long-term monitoring

Chapter 1: General Introduction

1.1 Global biodiversity

"Biological diversity, or biodiversity, is the term given to the variety of life on Earth." (CBD 2006)

The current number of species described only constitutes a small proportion (between 1 to 10%) of Earth's total species (Mittermeier et al. 2011; Novotny et al. 2002). Estimations of species diversity for major vertebrate groups have suggested approximately 5,644 mammals, 11,121 birds, 7,696 amphibians and 10,450 reptile species may be found worldwide (IUCN 2017a; IUCN 2017b). Currently, species are facing a number of threats, which are contributing to accelerated rates of global biodiversity loss (Cardinale et al. 2012).

Threats impacting the majority of species include; habitat destruction, climate change, habitat fragmentation and emerging infectious diseases, where anthropogenic activities are increasing threat levels to species and causing increased extinction rates (Pimm et al. 1995; Sala et al. 2000). According to the International Union for Conservation of Nature (IUCN) in 2017, the most threatened vertebrate group is Amphibia (amphibians), followed by Mammalia (mammals), Aves (birds) and Reptilia (reptiles). Approximately 63% of the total described reptile species found worldwide have been evaluated for the IUCN Red List; therefore, it is estimated that the total number of threatened reptile species is greatly underrepresented in comparison to the number of mammals and birds, which have been fully assessed (IUCN 2017c).

One hundred and fifty one species of terrestrial and freshwater reptile are native to Europe, approximately 48% of which are endemics (Cox and Temple 2009). European reptile distribution follows a latitudinal gradient, with higher diversity in the

Mediterranean (Figure 1.1) (Sillero et al. 2014). Habitat loss, fragmentation and degradation are major drivers in the decline in 98 European reptile species. The levels of sensitivity to the threat of habitat loss are correlated with species specialization (Henle et al. 2017). Specialist species, which are restricted to a small number of habitats, are more vulnerable to the effects of habitat loss, compared to generalist species, that occupy a broader range of habitats (Segura et al. 2007).

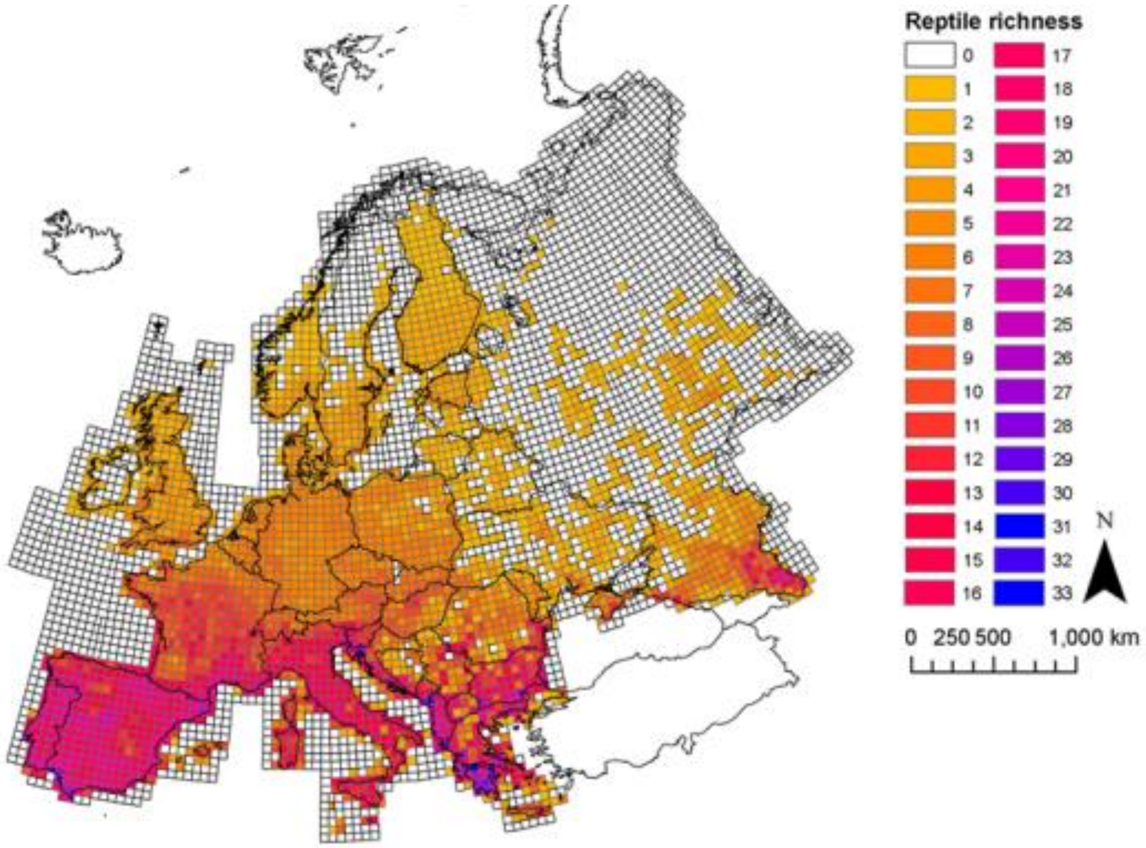


Figure 1.1: Map of reptile species richness in Europe (Sillero et al. 2014).

Species with extremely restricted ranges, include island endemics, such as the critically endangered Canary Island giant Lizard (*Gallotia bravoana*). In 2009, the wild population of *G. bravoana* consisted of 90 individuals that were known to occupy an area no larger than 20 km^2 in La Gomera, Spain (Miras et al. 2009). Long-term isolation and increased ecological pressures, contribute to endemic species being increasingly more sensitive to habitat loss, fragmentation and degradation (Gonzalez et al. 2014).

Habitat loss, fragmentation and degradation impact reptile species differently, dependent on species distribution, ecology and habitat preferences (Henle et al. 2004). Specialist species with restricted ranges, especially within temperate areas where diversity is lower, are generally more vulnerable to ecological threats, compared to generalist species with wide distributions (Henle et al. 2017).

1.2 Local biodiversity

In the United Kingdom, habitat loss (from management practice, agriculture, woodland/forestry or drainage/abstraction), infrastructure development, climate change, invasive/non-native species, human disturbance and pollution (freshwater or land) are amongst the threats impacting native reptile species (Natural England 2010). Six native reptile species are found in the UK: adder (*Vipera berus*), sand lizard (*Lacerta agilis*), common lizard (*Zootoca vivipara*), slow-worm (*Anguis fragilis*), grass snake (*Natrix helvetica*) and smooth snake (*Coronella austriaca*) (Froglife 1999; Inns 2009). In general, reptiles occupy an array of habitat types, which include: heathland, scrub, grassland, allotments and suburban brownfield sites (Froglife 1999; Inns 2009). Microhabitats within larger habitat areas may possess key attributes which increase their suitability to support reptiles. Features associated with suitability relate to prey abundance, the proportion of vegetation cover for refugia and dispersal, and, connectivity between other suitable habitats to aid with dispersal (JNCC 2004; Platenberg 1999).

1.2.1 Reptile distributions

Slow-worm, common lizard, grass snake and adder are widespread species within the UK. However, the sand lizard and smooth snake have isolated distributions, localised within Surrey, Dorset and Hampshire (Figure 1.2) (Inns 2009). The widespread distributions for common species, such as slow-worm and common lizard, illustrate adaptability to reside within an array of habitats throughout the country (Inns 2009).

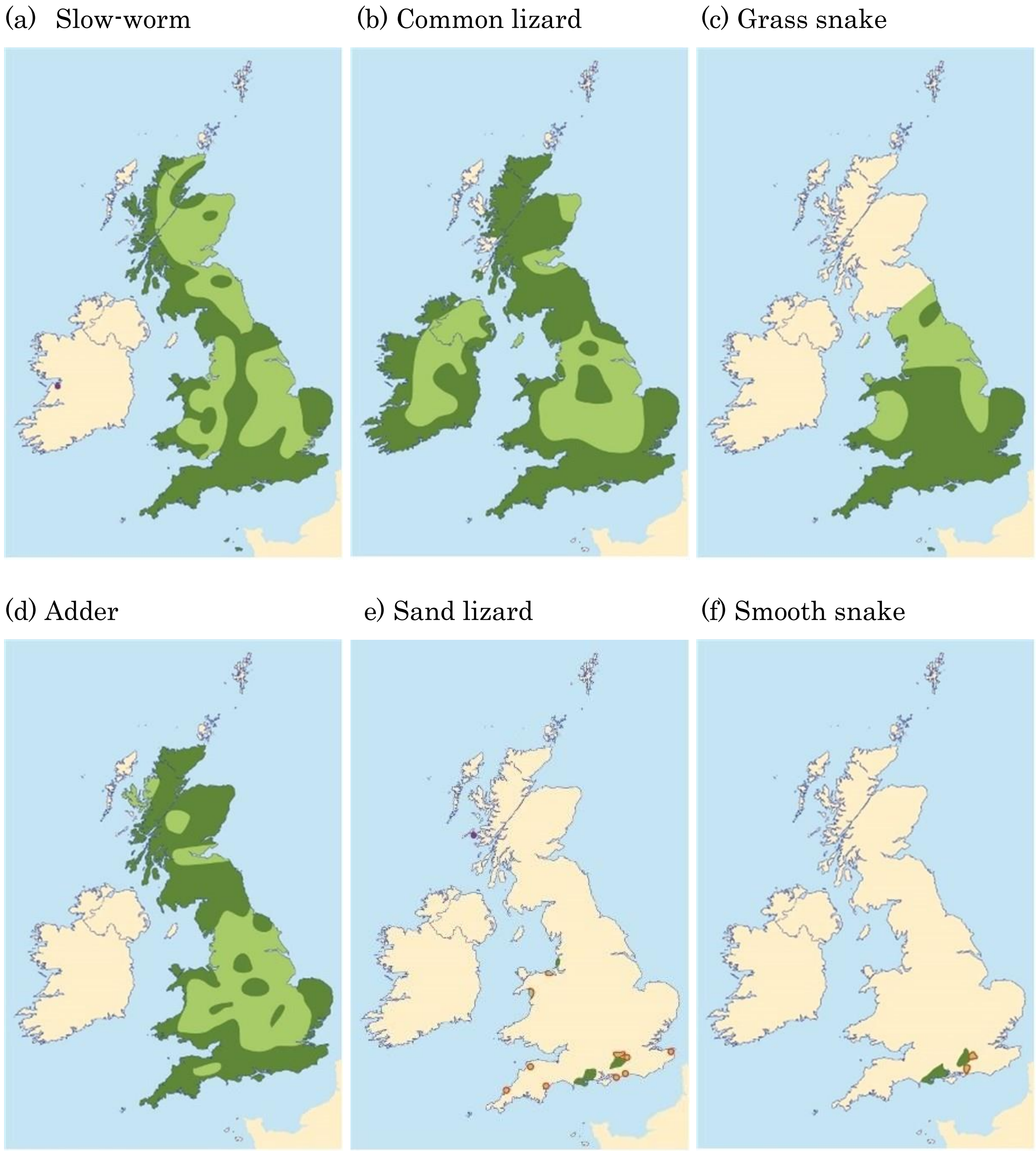


Figure 1.2: Distribution maps indicating the current natural range of six native reptile species. (Dark green – Species recently recorded in most 10 km squares; Light green – Few recent records but area is within the species' range; Orange dots – re-introduction area; Purple dots – Non-native species introduction) (Arc Trust 2017).

1.3 Slow-worms

The slow-worm, (*A. fragilis* (Linnaeus, 1758)), a legless lizard from the Anguidae family, is a semi-fossorial reptile species with a widespread distribution throughout the UK and Europe (Inns 2009). Slow-worms are predominantly thigmothermic (absorbs heat from utilising warm objects within the environment) and only partially heliothermic (gain heat from the sun), preferring to maintain a low body temperature (thermal gradient 25.3 – 26.4°C) (Brown and Roberts 2008; Spellerberg 1976). To maintain such temperatures slow-worms reside under natural refugia, typically: flat rocks and log piles, and will utilise artificial refugia (e.g. corrugated iron) if present within the environment (Inns 2009).

Slow-worms tend to have semi-nocturnal activity patterns, however, activity patterns throughout the day follow an irregular structure (Capizzi et al. 1998). Slow-worms are typically active during first daylight hours, (between 07:00 and 10:00 am), after periods of rainfall and during twilight hours (between 1830 and 2130 hours) (Luiselli 1992). Slow-worm activity is partially related to prey activity and abundance within the environment, where prey species including: spiders, earthworms, insects and slugs / snails, are often nocturnal or active on the surface after periods of rainfall (Luiselli 1992).

Slow-worms are widespread in the UK (Figure 1.2a) and utilise a variety of habitat types, which results in them being one of the most frequently encountered animals within sites proposed for development. Consequently, development poses a threat to populations throughout the UK (English Nature 2004; Inns 2009; JNCC 2004; Platenberg 1999).

1.4 Infrastructure development and slow-worms

Development across the UK can occur in high densities throughout urban and rural areas, which in turn increases levels of human-wildlife conflict. Common lizards and slow-worms are commonly encountered on proposed development sites, due to their widespread distributions and ability to reside in a multitude of habitats, which increases the likelihood of such species being impacted by a development (Platenberg 1999). There are many impacts that pose significant threats to reptiles during the development process, predominantly throughout pre-construction, construction and operation phases. Some threats include vegetation clearance (to a low height), ground clearance, tracking machinery over reptile suitable habitat and removing rubble or other debris (English Nature 2004).

1.4.1 Protection and mitigation

Together with other European fauna and flora species, in 1982 slow-worms were afforded protection both internationally (Bern Convention) and locally (The Wildlife and Countryside Act). All British reptiles are protected under the Wildlife and Countryside Act (1981) (sections 9(1) to 9(5)) (as amended) which protects against intentional or reckless injuring, killing or sale of any individual. Two species - smooth snakes and sand lizards - have additional protection which makes it illegal to capture, handle or disturb animals without a licence, and there is additional protection to habitats used for breeding, shelter or resting (JNCC 1981). Breaches of the legislation can result in confiscation of equipment, machinery or vehicles used to commit the offence, six months imprisonment or an unlimited fine per animal (if the offence was committed on or after 12th March 2015) (JNCC 1981; Natural England and DEFRA 2014; Sentencing Council 2018).

Ecological consultants, developers, planning authorities and conservation bodies all have crucial roles to play throughout all stages of development to protect and conserve protected species and to prevent any unlawful act from occurring. The role of an ecological consultant within a development project involves assessing how a development may impact protected and priority flora and fauna within a particular area and determining measures to minimise the impacts posed by the development (CIEEM. 2017a). If reptiles are present within a proposed development site, avoidance, mitigation, compensation and/or enhancement measures are generated to limit impacts posed to reptiles during the development (Natural England and DEFRA 2015). Mitigation planning can include displacing reptiles from sensitive areas by decreasing the suitability of vegetation within the area, changing the timing of work and/or liaising with developers to change the development design layout to utilise areas not used or occupied by reptiles (Natural England and DEFRA 2015). Alternatively, reptiles can be translocated to a receptor area either off or on site, but this mitigation should only be conducted as a last resort, as the effectiveness of translocations for conserving populations for the long-term is highly under recorded (Natural England and DEFRA 2015).

1.4.2 Surveying

Prior to development proposals reaching the planning stage, surveys must be conducted to gather important ecological information. The level of survey required for a site is always dependent on the nature of the project and the information that has been provided by the client. Initially, a Preliminary Ecological Appraisal is undertaken, which involves identifying the site's potential to support priority and protected species (e.g. reptiles, great crested newt and bats) and conclude whether any additional survey work is required and, where possible, determine the avoidance, mitigation, compensation and/or enhancement measures that may be required to facilitate the development (CIEEM. 2017b; Froglife 1999).

Additional surveying may be required if suitable reptile habitat is present, if the development will fragment suitable reptile habitat or if historical records and the current distribution of reptiles suggests a likely presence (Natural England and DEFRA 2015). Such methodologies may be required to inform an Ecological Impact Assessment (EcIA) which is a process used to identify, quantify and evaluate potential effects of a development on priority and protected habitats, species and ecosystems (CIEEM. 2016). Such documents can be submitted to planning, and the findings allow planning authorities to develop an understanding of ecological issues relating to a proposed development site when determining applications for consent (CIEEM. 2016).

1.5 Reptile surveys

There is currently no standardized guidance for carrying out reptile surveys in the UK. Various organisations, including Froglife and the Amphibian and Reptile Conservation (ARC) Trust, have produced their own guidance, but these are outdated, vary in the level of detail provided and are poorly underpinned by scientific evidence (Froglife 1999; Sewell et al. 2013). In addition, updated reptile mitigation guidance (including survey guidance) was released in 2011 by Natural England (the statutory government body responsible for the protection of the natural environment in England) but was withdrawn shortly afterwards (Natural England 2011). The only guidance available from Natural England and DEFRA are the basic principles set out within the reptile survey and mitigation standing advice, consequently, no detailed guidance has been published to replace the 2011 guidance (Natural England and DEFRA 2015).

Research undertaken by Reading (1996, 1997), prior to Froglife reptile guidance being published, evaluated existing reptile survey methodologies and proposed a standard method for surveying reptiles within dry lowland heath. Reading (1996) suggested corrugated sheet steel approximately 76 cm by 65 cm in size (0.49 m^2) should be used, within a hexagonal array with a 10 m spacing between refugia. Surveying using a hexagonal array is effective for large sites with uniform habitats such as heathland but is more difficult to execute on small sites with linear or mosaic habitats, which are more randomly distributed (Hill et al. 2005). The proposed methodologies suggested in Reading's studies, were recommended as a baseline for achieving a 'standardised' methodology for surveying reptiles and are likely to have informed the Froglife (1999) reptile guidance.

Research concerning British reptiles is biased towards population ecology, refugia use, refugia occupancy and mark-recapture. Platenberg (1999) and Hubble and Hurst (2006) undertook studies to gain an understanding of slow-worm ecology and population structure. A study undertaken by Fish (2016) assessed slow-worm and common lizard populations and investigated whether species exhibit a preference for specific artificial cover objects of different materials, felt and corrugated roof sheeting. Literature specifically relating to survey protocols for surveying for slow-worms and comparing the effectiveness of different methodologies is sparse and outdated.

1.5.1 Current reptile survey methodology

Methods of survey for reptiles accepted by Natural England include the use of directed observation, searching for basking animals along a transect and under artificial refugia (Natural England and DEFRA 2015). Slow-worm surveys require a more prominent use of refugia over searches for basking animals. Consequently, artificial cover objects (ACOs) are the only method recommended by Natural England

and DEFRA (2015) to survey slow-worms. Surveys should be conducted between the months of March and October when reptiles are active and out of hibernation, but, the most optimal survey months are April, May and September (Froglife 1999; Sewell et al. 2013). Most commonly, surveys are directed towards suitable reptile microhabitats e.g. grassland and scrub areas (Froglife 1999). General information recorded during a survey includes: number and type of species, age class, sex, location where animal/s were sighted, date and time and weather conditions (Froglife 1999). The level of detail recorded depends on the type of survey being conducted.

Reptile surveys are undertaken to gather information relating to presence / likely absence; population counts, densities and estimates. To gather information on species persistence and population dynamics within a site, a presence / likely absence survey is conducted (Mackenzie and Nichols 2004; Pollock 2006). Seven survey visits within suitable weather conditions are recommended as the minimum requirement to obtain adequate information to determine site occupancy (Froglife 1999; Sewell et al. 2013).

Population count and population density (detailed) surveys are recommended to determine species distribution within a site, whilst also gathering data to estimate relative abundance and density (abundance per area or search effort). At least 20 survey visits are recommended to generate "accurate" estimations of population size and to identify primary reptile areas within a site (Froglife 1999; Sewell et al. 2013).

Population estimate surveys are conducted to measure changes in reptile abundance or population densities through time. Generally, capture-mark-recapture techniques are recommended for population estimation, where individual markings are noted, with the aim of identifying recaptured individuals during subsequent surveys (Sewell et al. 2013). Some reptile species have more distinguishing features than others which aids with identification to an individual level. Adders can be easily identified from their head and neck markings, whereas grass snakes, slow-worms and viviparous lizards can be more difficult to distinguish from one another (Sewell et al. 2013).

1.5.1.1 Detectability

Reptile detectability can be influenced by a number of factors, including: geographical location, habitat characteristics, temperature, date (e.g. year or season), survey area, the observer and survey effort (techniques used and the number of survey visits conducted) (Kéry 2002; Kéry et al. 2009; Sewell et al. 2012). During a survey the detectability of a species or individual is dependent on 'availability' - whether an animal is available for detection at a given time of a survey (Kéry and Schmidt 2008). Species detectability is influenced by availability due to the 'iceberg' principle (only a small amount of information is available or visible at a given time), therefore, animals who are underground or outside of the survey area when a survey is conducted will go undetected (Morgan 2008; Sewell et al. 2012).

Survey effort and the information obtained from surveys is influenced by the detectability of the species being surveyed (Sewell et al. 2012). Conducting presence / likely absence survey for rare or elusive species can be categorised by a high proportion of zero observations, where some of these observations are 'false zeros' when a species is present but has not been detected (Mackenzie et al. 2002; Martin et al. 2005). Therefore, species presence on a site can be confirmed with a high degree of certainty, however, only a degree of probability can be used to prove a species is absent (Kéry 2002). Species detectability can also decrease the reliability of population count data. Only a portion of all individuals present within a site are 'available' during a survey, therefore it is likely that results obtained from such surveys will be smaller than the true abundance of species and individuals within a site (Kéry and Schmidt 2008).

Furthermore, detectability influences results obtained during reptile surveys and therefore sites and species are being imperfectly assessed (Kéry and Schmidt 2008).

Occupancy modelling can be used to correct imperfect detectability. Sewell et al. (2012) conducted such modelling to develop a survey design for reptile monitoring that takes into account detection differences between species. The study deduced that combining the use of ACOs and directed transect increases the detectability of slow-worms, and that increasing the number of ACOs used would increase detectability further (Sewell et al. 2012). There is a positive relationship between the number of survey visits conducted and the confidence of detecting species presence at an 'occupied' site (Sewell et al. 2012). Between three and four survey visits are required to be 95% confident that slow-worm would be detected if present on a site (Sewell et al. 2012).

Current Froglife guidance states that at least seven survey visits must be conducted during presence / likely absence surveys. This increases the likelihood of detecting species if present within a site, however results will only provide indicative results of abundance and distribution of species within a site.

1.5.1.2 Guidance implications

Natural England and DEFRA (2015) standing advice for reptile surveys and mitigation provides information for local planning authorities (LPAs) to enable them to assess the impacts of a proposed development on reptiles. This standing advice indicates that ecological consultants are responsible for determining appropriate survey methods and mitigation measures to address impacts posed to reptiles by a proposed development (Natural England and DEFRA 2015). Currently, the reptile survey and mitigation standing advice relies on Froglife (1999) guidance which specifies technical survey methodologies and protocols. Consequently, practitioners are using the outdated Froglife (1999) guidance to inform reptile surveys undertaken to support planning applications, as these are the only ones that exist. Consequently, LPAs are basing their judgements, when determining planning applications, on data obtained through surveys where outdated, unstandardized survey methodologies have been used to inform survey effort and results.

1.6 Study aims

Important development and conservation decisions are being decided based on outdated reptile survey guidance backed by minimal scientific evidence (Griffiths et al. 2015). The potential uncertainty associated with slow-worm population assessments based on existing reptile guidance has not been investigated in detail. In addition, increasing efforts in population monitoring are key in developing our understanding of slow-worm population ecology further. However, there are few long-term studies comparing population trends in this species.

The focus of this study is to explore whether slow-worm detectability is influenced by using refugia of differing sizes; whether variability in refugia density significantly affects population assessments of slow-worms; and whether refugia placed out for different lengths of time affect slow-worm detectability. As well as analysing trends in slow-worm abundance over time within sites specifically managed for reptiles.

This research has the potential to:

- Improve and refine current survey guidance;
- Increase the effectiveness of reptile surveying;
- Increase certainty and reliability of data used to inform decisions made by LPAs and the measures proposed in relation to development schemes;
- Improve the understanding of slow-worm population ecology on a local scale; and
- Influence future conservation efforts and site management.

Chapter 2: Effects of artificial refugia characteristics on slow-worm detectability

2.1 Abstract

Reptile surveys are conducted for a multitude of reasons but are primarily undertaken within the commercial sector for proposed development projects, where survey results are used to influence ecological decisions and inform planning applications. Existing reptile survey guidance are currently outdated and lack appropriate standards, especially concerning how artificial refugia characteristics impact on reptile detectability. This study was undertaken to determine how different artificial refugia characteristics, specifically tin size, tin density and tin age impact slow-worm detectability and capture rates. Slow-worms were studied at Soakham Down, King's Wood, Challock and at a control site known as Earthworks site, also within King's Wood between 2014 and 2016. More slow-worms were recorded per visit under single tins (tins 0.25 m^2 in size) spread out across the site than under paired (doubled-up) tins (0.5 m^2 in size) covering the same total area. Equally, more single tins were occupied by slow-worms when they were spread out across the site than when they were doubled-up. More slow-worms were recorded per visit when total tin density was doubled from 20 tins to 40 tins. Tin age did not influence the number of slow-worms recorded per visit. Tin size and tin density notably influenced slow-worm detectability. Therefore, using tins 0.25 m^2 in size and at a density of 40 per hectare increases slow-worm detectability. This emphasises the need for existing reptile survey guidance to be updated and modified, in light of these findings.

2.2 Introduction

Surveying for British reptiles, most commonly involves active survey methods of visual encounter surveys and/or the use of artificial cover objects (ACOs) henceforth, known as "refugia" (Froglife 1999; Wilkinson and Arnell 2013). Visual encounter surveys are highly dependent on the biology of the study species. Most surveys combine the use of active survey techniques by walking a directed transect through suitable habitat containing natural or artificial refugia.

Slow-worms are elusive animals, naturally attracted to cover objects from surrounding catchment areas within nearby vegetation (Christian et al. 2016; Froglife 1999; McInerny 2016). Refugia provide a safe and sheltered environment to rest, forage and aid thermoregulation (Christian et al. 2016; Inns 2009; McDiarmid et al. 2012). Natural refuge objects, such as logs, leaf litter, scrap metal and other discarded rubbish, generally vary in size, number and distribution between sites (McDiarmid et al. 2012). Artificial refugia, such as pieces of wooden board, roofing felt sheeting, corrugated iron sheeting and other flat materials, in general, are a more effective tool for surveying reptiles compared to searching under natural cover objects already present within a site (McDiarmid et al. 2012). Deploying artificial refugia is relatively inexpensive in terms of time and costs (McDiarmid et al. 2012). In addition, artificial refugia do not require daily checking, require minimal maintenance, can be checked with little training and fundamentally, experimental designs can be replicated and altered between sites (Christian et al. 2016; Englestof and Ovaska 2000; Kjoss and Litvaitis 2000; McDiarmid et al. 2012). Recent research has suggested that slow-worm captures increase when the distance between refugia decreases and the most effective inter-refugia spacing for slow-worm surveys is approximately 28 m however, this value can fluctuate depending on the type of habitat (Schmidt et al. 2017). The deployment of between 5 to 10 artificial refugia, approximately 0.5 m^2 in size for every hectare of a site being surveyed, is the currently accepted method of surveying for reptiles (Froglife 1999; Hill et al. 2005).

Results from reptile surveys allow the surveyor to develop an understanding of local scale reptile distribution, generate population assessments and identify how reptiles utilise the site (Froglife 1999; Natural England and DEFRA 2015; Wilkinson and Arnell 2013). Minimum capture efforts required for translocations generated by (Herpetofauna Groups of Britain and Ireland 1998) provide rules-of-thumb, which are influenced by population size (Table 2.1). Overall, reptile survey results are essential in informing avoidance, mitigation and compensation methods appropriate for the site and species in question.

Table 2.1: Minimum mitigation capture effort for slow-worm projects (adapted from Herpetofauna Groups of Britain and Ireland 1998).

Species	**Population size (adult density)**	**Tin density (tin number/ha)**	**Minimum number of suitable trapping days**
Slow-worm	High population (> 100 ha^{-1})	100	90
	Medium population (> 50 ha^{-1})	100	70
	Low population (< 50 ha^{-1})	50	60

Current outdated Froglife (1999) guidance is primarily based on anecdotal information, with little scientific evidence supporting the methodology provided. The lack of up-to-date standards for commercial reptile surveying, especially concerning refugia characteristics (e.g. size, density and age) and impacts on reptile detectability, have potential to significantly influence ecological decisions associated with proposed development projects (Edgar et al. 2010; Griffiths et al. 2015).

Over the last decade, research on British reptiles has primarily focused on the thermal properties of refugia, and comparing survey sampling methods (Lettink and

Cree 2007; Thierry et al. 2009). The prominent gap in the literature increases the level of anecdotal evidence and expert opinion used within current guidance. A suggestion that the higher the density of refugia used on a site the higher the number of reptiles observed is a key example of an untested hypothesis (Christian et al. 2016; Froglife 1999).

Currently, the Froglife (1999) guidance does not fully address the following issues: imperfect detectability of species and individuals; the relationship between count, abundance or density of animals within a given area and how these are interpreted; and the lack of standardisation relating to ACO layout, size or density used for surveying. Research is required to address and develop an understanding into how the issues highlighted above impact reptile population assessments. Findings from such research could be used to influence and increase the effectiveness of survey guidance and promote evidence-based conservation. Fundamentally, reptile survey guidance should be supported by valid scientific results, to ensure that ecological practitioners have access to up-to-date resources and planning related decisions are reinforced by more robust and reliable survey data.

This chapter sets out to explore how slow-worm captures are impacted by using artificial refugia of differing sizes; whether variability in refugia density and layout significantly affects population assessments of slow-worms; and whether refugia placed out for different lengths of time affect slow-worm detectability.

2.3 Methodology

2.3.1 Study areas

2.3.1.1 Soakham Down

The study site, Soakham Down, King's Wood, Challock (approximate central OS grid reference: TR035492), is a *c.* 0.7 ha mosaic of dense scrub (predominantly bramble (*Rubus fruticosus agg.*)), tussocky calcareous grassland with scattered trees; largely silver birch (*Betula pendula*), surrounded by 1,500 acres of ancient woodland. Soakham Down lies on a SE-facing slope of the North Downs and is located *c.* 7 km north east of Ashford, UK, and *c.* 14 km south west of Canterbury, UK.

King's Wood has been owned and managed by the Forestry Commission since the early 1930s (Forestry Commission 2016). Previous and current management of the site has increased the levels of diversity for both flora and fauna, and subsequently made Soakham Down increasingly more suitable for reptiles.

2.3.1.2 Earthworks

Earthworks is the control site within King's Wood (i.e. survey protocol and refugia kept the same over the survey period), approximately 0.3 ha in size and exhibits similar vegetation characteristics to Soakham Down (Figure 2.1). The same management strategy is also undertaken on site but surveying has occurred within this site since 2005.

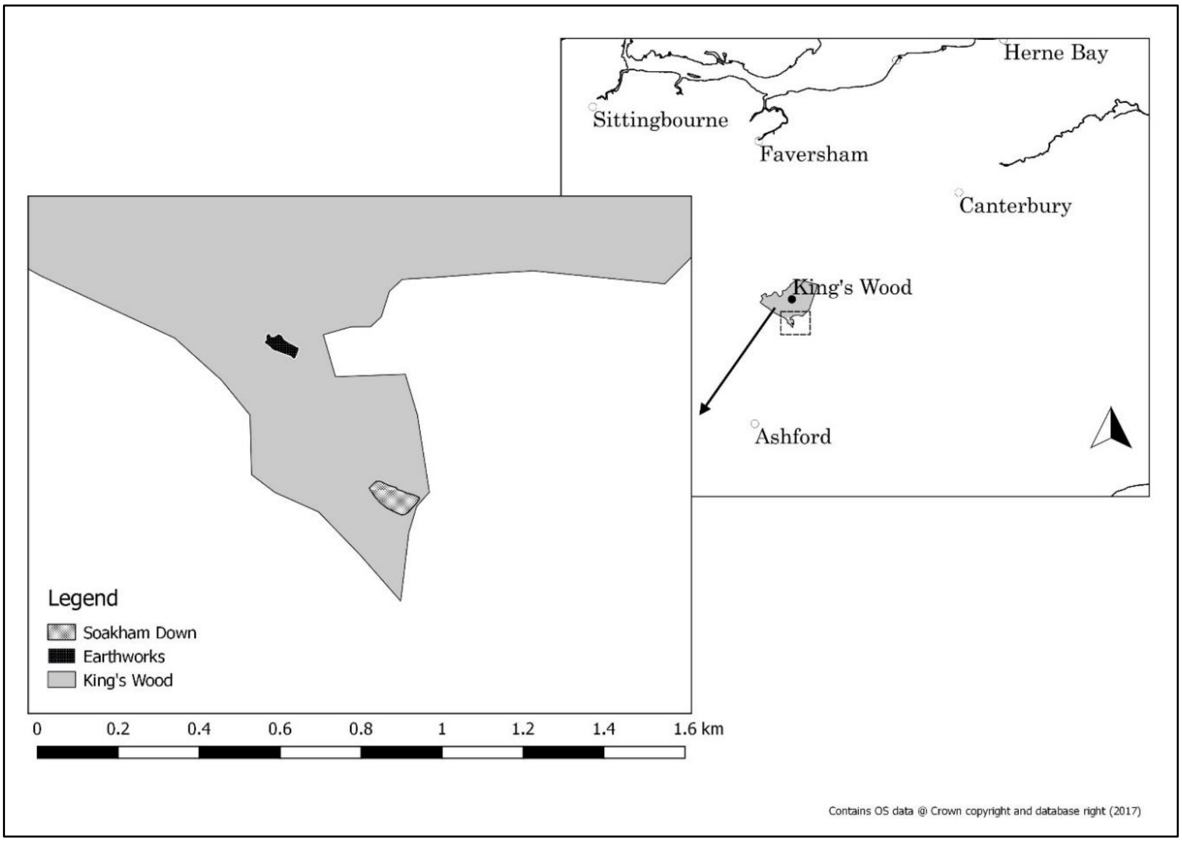


Figure 2.1: Map illustrates the geographical location of the study site Soakham Down, King's Wood, Challock.

2.3.2 Study species

Previous studies and continual long-term monitoring undertaken by University of Kent students and Kent Reptile and Amphibian Group (KRAG) have highlighted the presence of four native reptile species within King's Wood; grass snake, adder, common lizard and slow-worm. No studies were undertaken at Soakham Down prior to 2014.

Slow-worms are the target species for this study due to their high abundance throughout King's Wood. Slow-worms are more easily detected using artificial refugia than the three other reptile species present within King's Wood.

2.3.3 Experimental design

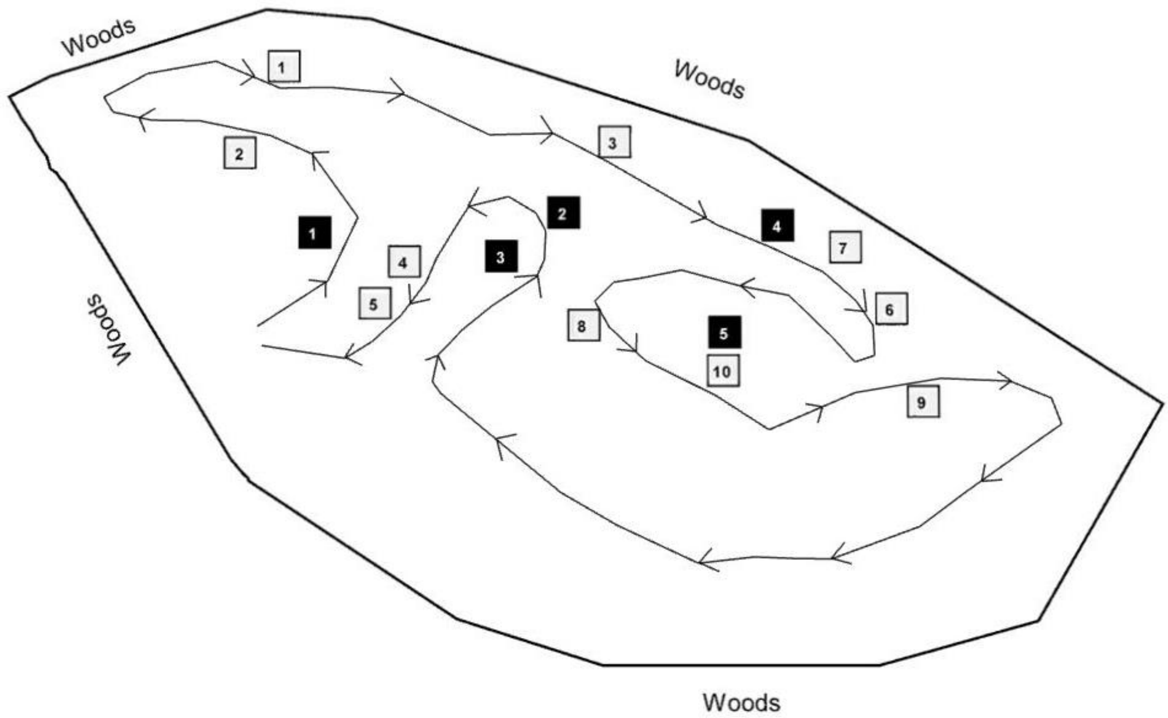
A full factorial repeated measure design (fully crossed design) was used to investigate how refugia size, refugia density and refugia age (length of time left in situ) affect slow-worm detectability. Multiple survey visits were conducted every 1-4 weeks between the months of March and October to account for fluctuations in slow-worm captures expected throughout the year.

Sheets of lightweight corrugated iron sheeting (henceforth known as tins) were used as artificial refugia to attract slow-worms from the surrounding vegetation. To investigate how tin size affects slow-worm captures tins were split into two category types: single tin and double tin. An individual tin, 0.25 m^2 in size, is denoted as a single tin whereas a double tin, consists of two individual tins of the same size placed directly next to each other (Figure 2.2).

Figure 2.2: Tin size scale comparison. **Left:** Single tin, **Right:** Double tin. Scale: one square signifies a 0.5 m x 0.5 m sheet of tin.

Tins were placed at least 2.5 metres apart in areas identified as potential reptile habitat, i.e. areas that provide shelter from predators but, allow reptiles to absorb heat from the refugia surface to thermoregulate. Such ideal vegetative cover included edges of dense scrub patches, areas within open tussocky grassland and along woodland edges. Tins were distributed in open areas adjacent to vegetation throughout the site (Figure 2.3a,b).

a)



b)

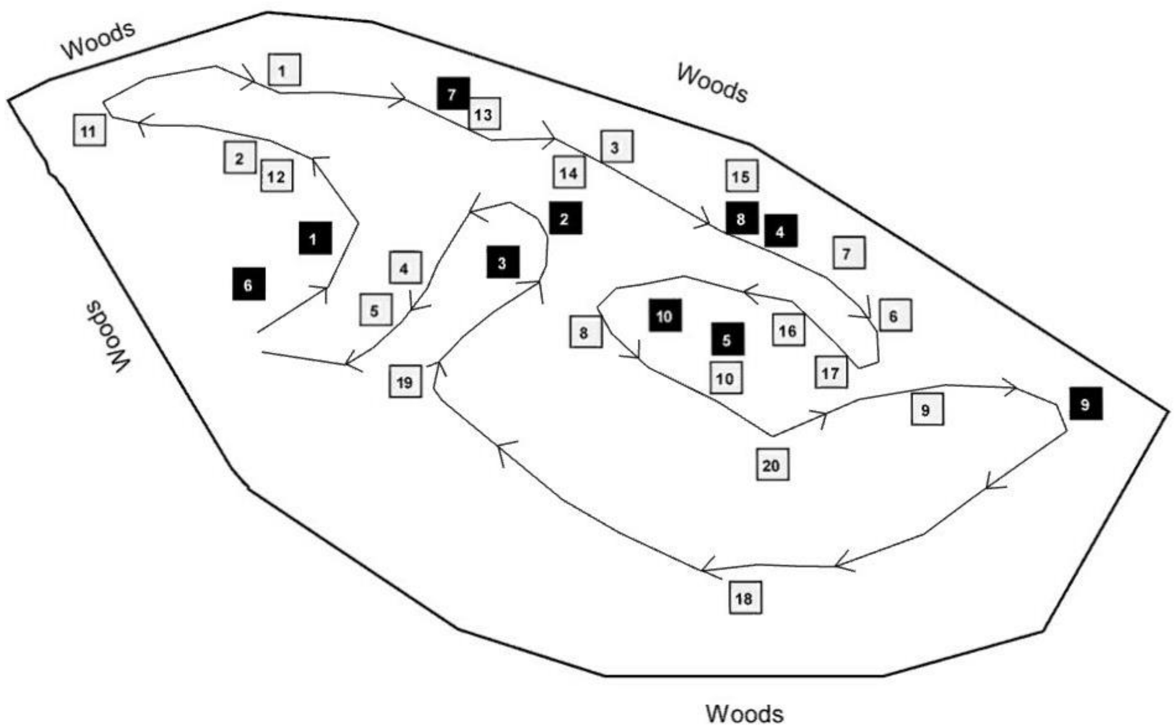


Figure 2.3: Tin distributions at Soakham Down, King's Wood: **a)** 2014 **b)** 2015. (The white squares illustrate the location and number of the single tins. The black squares indicate the location and number of the double tins. The black arrowed line indicates the transect walked).

On 20th April 2014, 20 tins were distributed across the site. Ten tins were distributed as single tins and ten tins were dispersed to form 'five' double tins (Figure 2.3a). Single and double tins both covered 2.5 m^2 of the site in 2014. One year later on 25th April 2015, the total tin density on site was doubled to 40; where ten new single tins and 'five' new double tins (formed by the ten new tins) were distributed across the site (Figure 2.3b). After the number of tins doubled on site, the total surface area covered by each sized tin doubled to 5 m^2. Therefore, in both years the surface area covered by single tins was the same as that covered by double tins.

To determine whether any changes in slow-worm numbers at the Soakham Down site were solely dependent on the changes in refugia characteristics between years rather than changes in reptile abundance between years, a control site, known as the Earthworks, was used for comparison. Here the refugia size and density was kept constant across both survey years, with, 12 tins and 12 roofing felt sheets, both 0.25 m^2 in size used in both 2014 and 2015.

2.3.4 Data collection

Prior to the start of each survey visit general weather conditions were recorded. Weather parameters documented were percentage cloud cover (range from 0% when clear to 100% when completely overcast), minimum and maximum air temperatures (°C) over the duration of the survey, wind speed (categorised by none, light, moderate, strong), wind direction and ground conditions (recorded as dry, damp or wet) (Appendix 1). The start and end times of the survey were also recorded.

Surveying combined a visual encounter survey along a directed transect and checking tins (Figure 2.3a,b). Tins were lifted to check for reptile presence. The directed transect walk allowed for any reptiles basking in the open to be recorded. Surveys involved walking slowly and carefully, scanning the vegetation at least 3 to 4m in front and to the side of the path. When slow-worms were present the following variables were recorded: tin number (no slow-worms were found out in the open), tin size (single or double), the number of individuals, age class and sex (Appendix 1 and Appendix 2).

2.3.4.1 Soakham Down

Surveys were initiated in May 2014, giving the tins placed during April 2014 time to bed in and for animals to find them. Surveys continued until the end of September 2014 and recommenced in February 2015, continuing up until April 2015. After the new tins were laid the survey schedule followed a similar pattern to the previous year, with surveys beginning in May continuing until the end of September and including the early months of 2016.

Furthermore, the datasets for both years begin in April and contain all survey records up to and including the following April. Therefore, the two survey years are identified by the year in which surveys began: 2014 and 2015.

Overall, a total of 22 surveys were conducted between 6th May 2014 and 12th April 2016 with an average of two surveys undertaken per month. Visits were made at various times during the day, between 0930 and 1800 hours. Each survey took between 0.5 hrs to 1.5 hrs to complete, which was dependent on the number of slow-worms recorded.

2.3.4.2 Earthworks

A total of 17 surveys were undertaken between 6th May 2014 and 11th April 2016. On average, two surveys were conducted per month. Visits were undertaken on the same day as the Soakham Down surveys, with the order of the surveys alternating between the two sites. Each survey took between 20 and 45 minutes to complete, dependent on the number of slow-worms recorded.

2.4 Data Analysis

Prior to analysis, datasets were organised and manipulated from the raw data sheets using Excel. Datasets were standardised to contain data from May to September, and April of the following year. The total number of slow-worm captures per visit, the number of slow-worms per tin, and total number of tins occupied by slow-worms were used as the dependent variable for each analysis.

These variables were calculated for each tin size (single and double), at each tin density level for each survey year. To allow a direct comparison with single tins, each dependent variable was calculated for each of the two tins used to form each double tin e.g. 10 individual tins used to form 5 double tins in 2014.

Tin density levels were categorised as follows: original (2014), increased (2015) and original (2015). Firstly, the original (2014) dataset contains data collected in 2014 for the 10 single and 5 double tins (10 individual tins) originally laid in April 2014. Next, the increased (2015) dataset contains data collected in 2015 for the 20 single and 10 double tins on site. Finally, the original (2015) dataset only includes data collected in 2015 from the 10 single and 5 double tins, originally laid in 2014.

For the tin age comparison only data collected in 2015 for single and double tins was used. Age levels were categorised as old tins, the originally laid tins *c.* 1 year old, and new tins, recently laid tins *c.* < 1 year old.

For the Earthworks, the total number of slow-worm captures per visit, number. of slow-worm captures per refugia and total number of refugia occupied by slow-worms was calculated for each survey year to compare with Soakham Down.

For both sites, there are instances where multiple survey visits were undertaken within a given survey month. The replications within the survey month allow for variation in detectability within a given month and throughout the survey season.

Assumption tests and statistical analysis were undertaken using R (Appendix 4). The assumption test findings determined whether data could be appropriately analysed using parametric test. Where required, data was transformed to comply with the assumptions (Appendix 4).

In the results section, only statistically significant patterns are highlighted within the text. For example, higher or lower slow-worm captures are only mentioned within the text if statistically significant ($P < 0.05$). The results of each statistical analysis, whether statistically significant or not, are included on the graphs included within the results section.

2.4.1.1 Tin size comparison

To compare slow-worm captures between single and double tins several two-way univariate ANOVAs were performed. Tin size (i.e. single or double) and date (month) were used as independent, fixed factors. The analysis was undertaken twice, first with the 2014 data and then repeated with the 2015 data. The ANOVA tested whether slow-worm captures differed between single and double tins. Interaction effects determined whether slow-worm captures for single and double tins across survey months were exhibiting similar trends.

2.4.1.2 Tin density comparison

Two-way univariate ANOVAs were undertaken to investigate whether increasing the number of tins in 2015 affected slow-worm captures. Tin density and date (month) were used as independent, fixed factors. Two comparisons were undertaken per tin size, between (1) the “original (2014)” and “increased (2015)” tin density, and (2) the “original (2014)” and “original (2015)” tin densities. This investigation examined whether increasing tin density increases the number of slow-worm captured. Similarly, the comparison examined whether slow-worm captures remained the same under the originally laid tins. Interaction effects illustrated whether slow-worm captures for single and double tins under different tin densities across survey months exhibited similar trends.

2.4.1.3 Tin age comparison

Multiple univariate two-way ANOVAs were conducted to compare slow-worm captures for tins placed out for different lengths of time. This analysis only used data from 2015 when ‘old’ (i.e. laid the previous year) and ‘new’ tins (i.e. laid in 2015) could be compared. Tin age and date (month) were used as independent, fixed factors. The analysis tested whether there were significant differences in the dependent variables influenced by tin age. Interaction effects determined whether slow-worm captures for single and double tins for different tin ages across survey months were exhibiting similar trends.

2.4.1.4 Control site comparison

Univariate two-way ANOVAs were undertaken on the Earthworks control site dataset. The size, density and age of tins used on site remained constant throughout the study. Date (month) and date (year) were used as independent, fixed factors. The ANOVAs tested for any differences in slow-worm captures between years. This analysis highlighted whether any differences in the independent variables observed at Soakham Down could be due to changes in numbers between years rather than changing the number of tins. Additionally, interaction effects were analysed to determine whether slow-worm captures in 2014 and 2015 across survey months were exhibiting similar trends.

2.5 Results

During the 10 survey visits conducted at Soakham Down in 2014, a total of 209 slow-worms were captured, where 9 ± 5.129 slow-worms were recorded per visit. In 2015, 489 slow-worms were captured throughout the 12 visits conducted, where 24 ± 9.327 slow-worms were recorded per visit. Slow-worm numbers fluctuated between months throughout both survey years. At Soakham Down, on average, the highest captures were recorded in August and September and the lowest in July.

Data from the 17 surveys undertaken at the Earthworks site across the two-year survey period, indicate that a total of 239 slow-worms were recorded in 2014 (8 visits), and 312 were recorded in 2015 (9 visits). On average, 30 ± 22.7 slow-worms were recorded per visit during 2014, whereas 35 ± 15.9 were recorded per visit in 2015. At the Earthworks site, the highest captures were recorded in September, whilst the lowest were recorded in April. No difference in slow-worm numbers was recorded per visit at the Earthworks site. The total number recorded per visit, the number per tin and the number of tins occupied by slow-worms remained the same over time (Figure 2.4a,b,c).

The analysis controls for seasonal variation, therefore, the results focus on the main effects influenced by tin size, tin density and tin age.

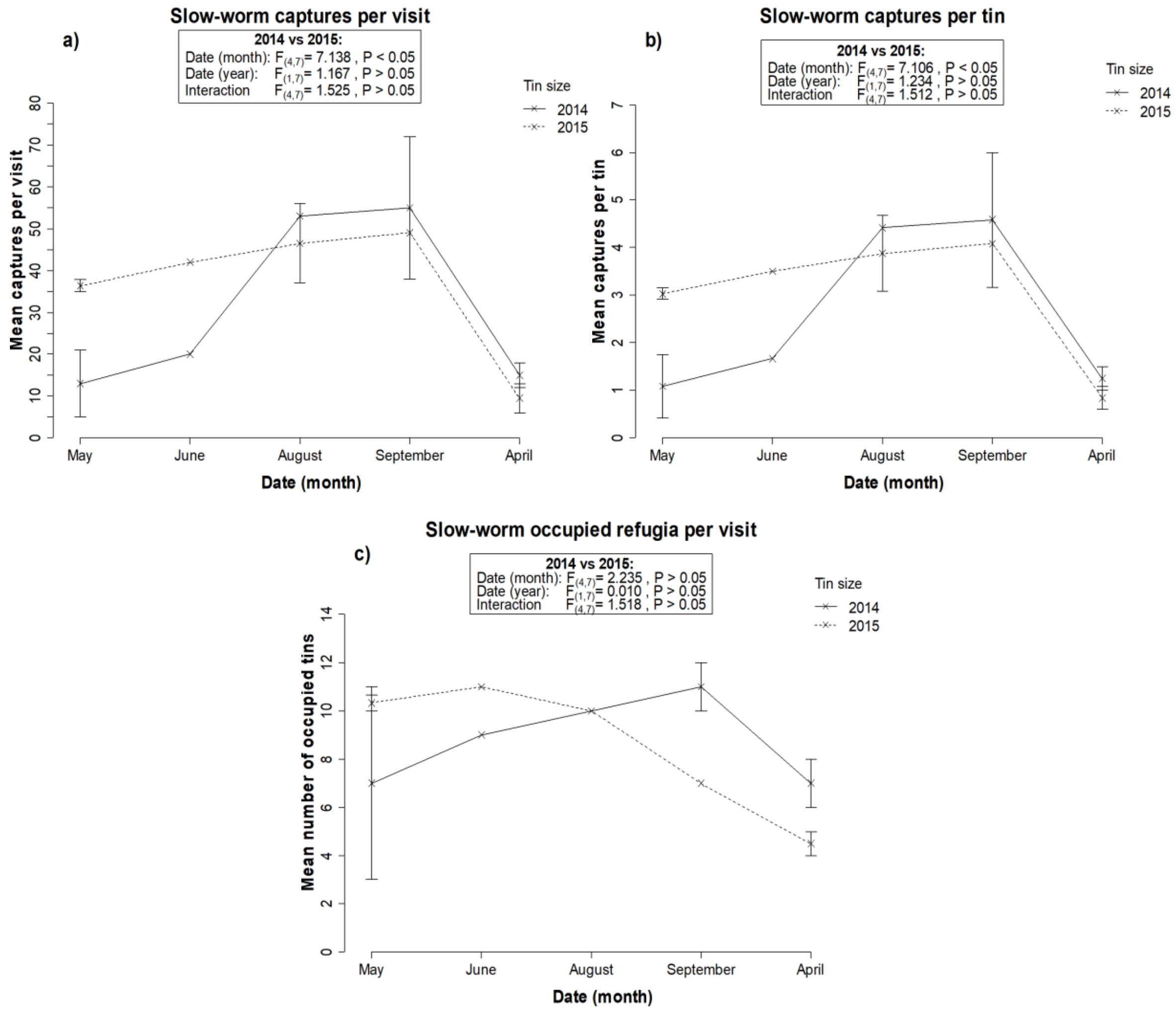


Figure 2.4: Variation in slow-worm numbers at Earthworks site between 2014 and 2015. a) Slow-worm captures per visit, b) slow-worm captures per tin and c) slow-worm occupied refugia per visit. Data grouped by week; data points are illustrated by slow-worn number per visit, per tin and number of slow-worm occupied refugia ± S.E. Standard error bars are absent for months where only one visit was conducted. Results of the two-way ANOVA show the differences between tin size.

2.5.1 Tin size comparison

Single tins spread out across the site resulted in higher slow-worm capture rates than an equivalent number of tins doubled-up at fewer location. This was reflected in higher total numbers recorded under single tins, higher numbers per tin under single tins, and a higher number of single tins occupied by slow-worms (Figure 2.5). There were no significant tin size x date interactions indicating that slow-worms did not change their distribution between the two tin types over time.

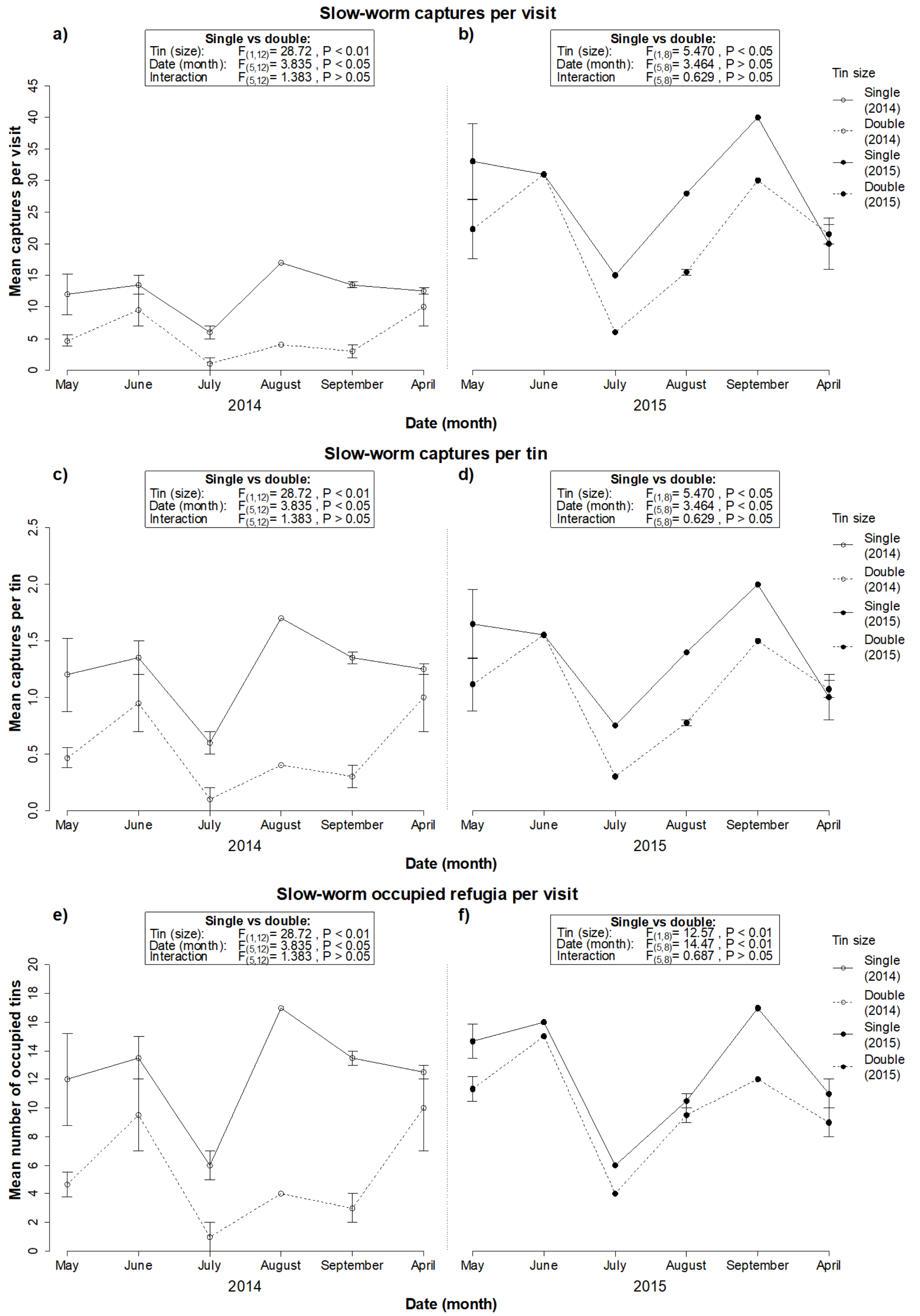


Figure 2.5: Variation in slow-worm numbers influenced by tin size. 2014: a, c and e; 2015: b, d and f. Data grouped by week; data points are illustrated per tin size ± S.E. Standard error bars are absent for months where only one visit was conducted. Results of the two-way ANOVA show the differences between tin size.

2.5.2 Tin density comparison

More slow-worms were recorded per visit after the tin density was doubled. This was reflected in higher total numbers recorded under single and double tins (Figure 2.6a, Figure 2.7a). However, no change in slow-worm numbers was recorded per tin under single and double tins, and the number of single and double tins occupied by slow-worms remained the same after tin density was doubled.

No difference in slow-worm numbers was recorded under the original single tins over time. The total number recorded under the original single tins, the number per tin under original single tins and the number of original single tins occupied by slow-worms remained the same over time (Figure 2.6d,f).

More slow-worms were recorded per visit under the original double tins over time (Figure 2.7b). However, no change in slow-worm numbers was recorded per tin under original double tins, and the number of original double tins occupied by slow-worms remained the same over time.

There were no significant tin density x date interactions, indicating that slow-worms did not change their distribution with respect to tin density, over time.

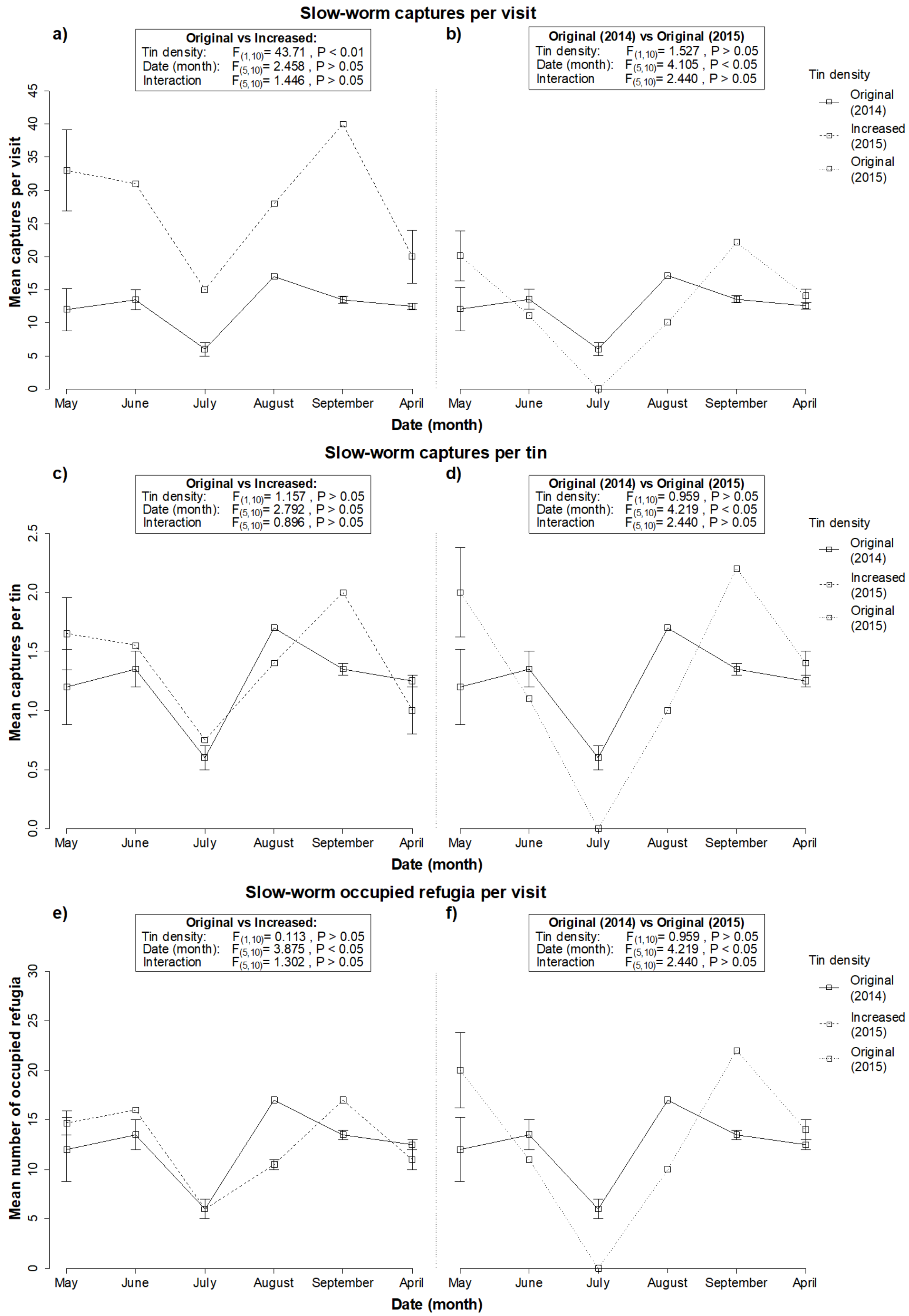


Figure 2.6: Variation in slow-worm numbers influenced by tin density: Single tins. Data grouped by week; data points are illustrated per tin density ± S.E. Standard error bars are absent for months where only one visit was conducted. Results of the two-way ANOVA show the differences between tin size.

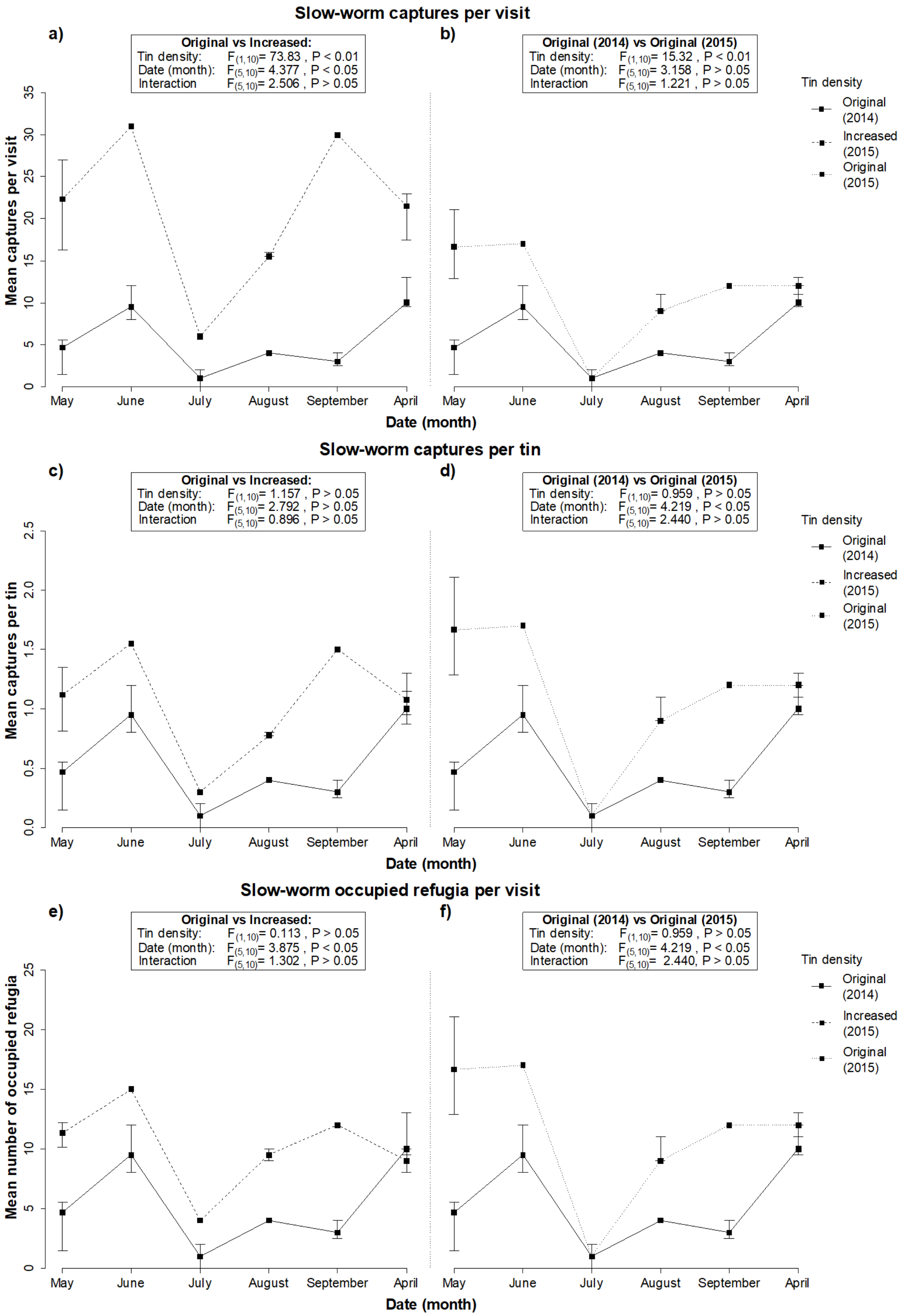


Figure 2.7: Variation in slow-worm numbers influenced by tin density: Double tins. Data grouped by week; data points are illustrated per tin density ± S.E. Standard error bars are absent for months where only one visit was conducted. Results of the two-way ANOVA show the differences between tin size.

2.5.3 Tin age comparison

Tin age did not influence the number of slow-worms recorded per visit under single and double tins. This was reflected in no difference between the total number recorded under single and double tins, no difference between the number recorded per tin under single and double tins, and no difference between the number of single and double tins occupied by slow-worms over time (Figure 2.8).

There were significant tin age x date interactions, for total number of slow-worms under single tins and number per tin under single tins. This indicted that slow-worms changed their distribution between the two aged single tins over time.

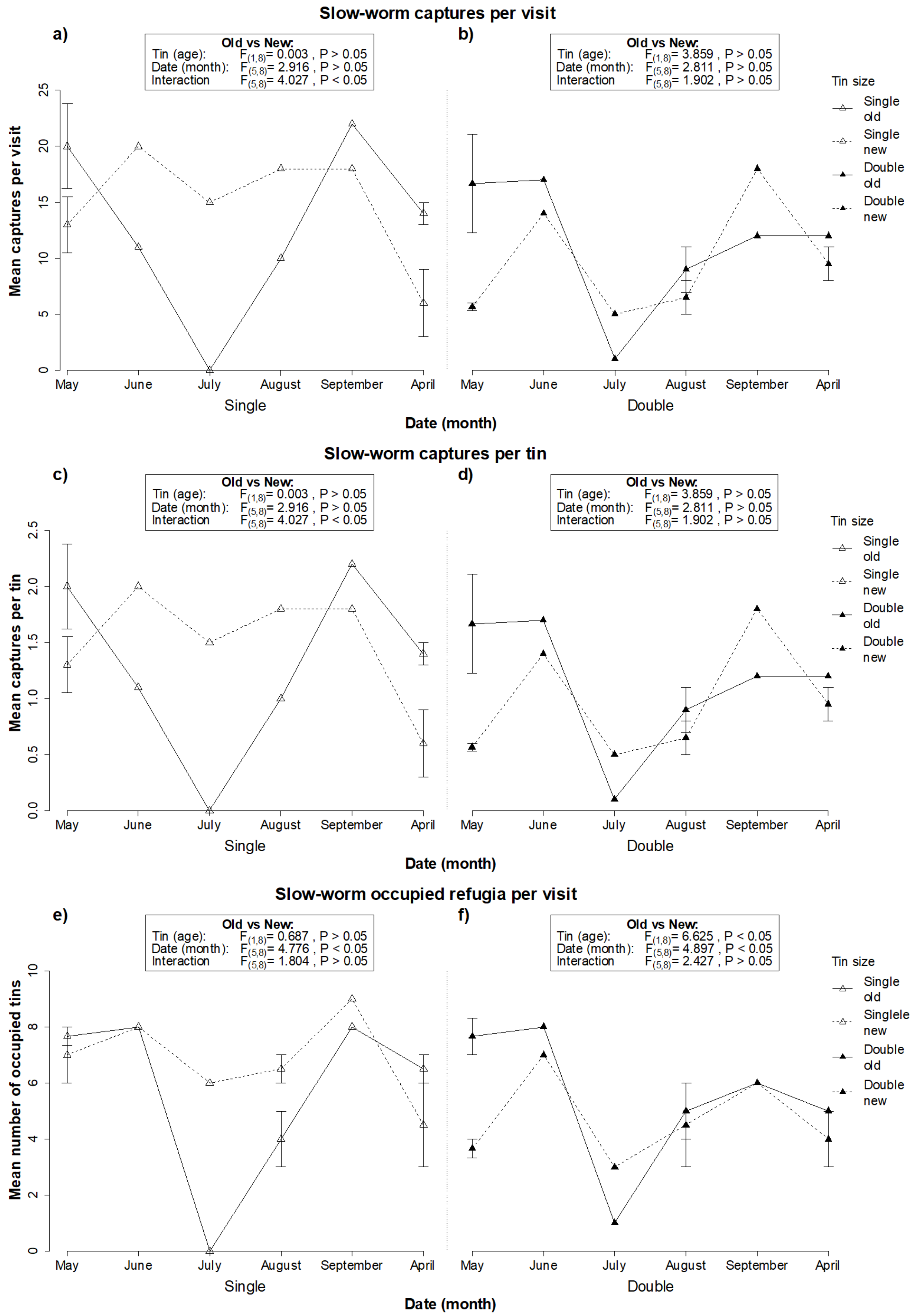


Figure 2.8: Variation in slow-worm numbers influenced by tin age. Single: a, c and e; Double: b, d and f. Data grouped by week; data points are illustrated per tin size ± S.E. Standard error bars are absent for months where only one visit was conducted. Results of the two-way ANOVA show the differences between tin size.

2.6 Discussion

Tin characteristics; size, density and age, remained constant at the Earthworks site. The total number of slow-worms recorded per visit, the number recorded per tin and the number of occupied tins did not change between survey year at the Earthworks site. Therefore, all the subsequent differences in slow-worm numbers between years at nearby Soakham Down are likely to be due to tin manipulation than natural changes in population size.

2.6.1 Tin size comparison

More slow-worms were recorded under single tins, 0.25 m^2 in size, per visit than tins that were doubled-up. These results contradict the view that refugia, 0.5 m^2 in size are most effective for surveying slow-worms (Froglife 1999), but indicate the importance of the spatial distribution of tins. Single and double tins both provided areas of shelter and protection and the thermal attraction capabilities for both tin sizes were equal (since double tins were two adjoining single tins). The higher number of slow-worms recorded under single tins may be a result of the distribution of tins across the site and the larger number of 'catchment areas' sampled. The ten single tins and five double tins both covered 2.5 m^2 of the site, but, single tins were more widely dispersed across the site (Figure 2.9).

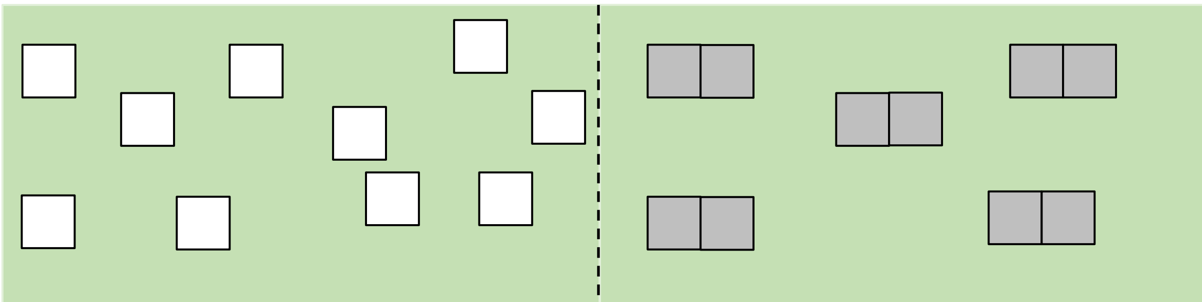


Figure 2.9: Distribution example of 10 single tins (left) and 5 double tins (right). Scale: one square signifies a 0.5 m x 0.5 m tin.

The smaller inter-refugia spacing between single tins allowed a higher number of potential slow-worm home ranges to be sampled. The low dispersal capacity of slow-worms and the wider distribution of single tins suggested that single tins are easier to locate within the environment (Schmidt et al. 2017).

2.6.2 Tin density comparison

Doubling the number of tins on site increased the number of slow-worms recorded, irrespective of tin size. The findings also highlighted that if tin density remained the same between survey years the number of slow-worms recorded remained the same. Overall, the results support the hypothesis that the more refugia used the higher the number of reptiles detected (Christian et al. 2016; Froglife 1999). In 2014, the 20 tins, covered 5 m^2 (0.075%) of the total site. In 2015, after the density was doubled, 10 m^2 (0.15%) of the site was covered in refugia. The doubling of the density resulted in a higher number of home ranges being sampled, increasing the number of slow-worms being detected per visit.

On average, 31 more slow-worms were recorded per visit in 2015. The biennial breeding cycle of slow-worms means that juvenile recruitment would only contribute a small proportion of the 31 additional slow-worms recorded (Platenberg 1999; Smith 1990). The increase in the number of slow-worms recorded is more likely to be a result of increasing the number of tins present on site. Consequently, the chance of detecting a slow-worm, if present, was increased.

2.6.2.1 Population assessment

Population assessments, based on the total number of adults recorded per visit, are used within commercial survey work to inform mitigation and avoidance actions after an initial reptile survey has been conducted. Population size estimates are used to make further evaluations relating to the capture efforts required for reptile mitigation: translocations.

For the purpose of creating this hypothetical scenario, attention is focused on the surveys conducted between May and September, as this represents the optimal timeframe for a typical commercial survey. Table 2.2 illustrates the amended adult and tin density boundaries for reptile translocations respective of the size of the site (Soakham Down: 0.665 ha and Earthworks: 0.335 ha).

Table 2.2: Minimum mitigation capture effort for slow-worm at Soakham Down and Earthworks (Population size = adult density / ha, Tin density = tin number / ha) (adapted from Herpetofauna Groups of Britain and Ireland 1998).

Original (1 ha)		**Soakham Down (0.665 ha)**		**Earthworks (0.335 ha)**		**Minimum number of suitable trapping days**
Population size	Tin density	Population size	Tin density	Population size	Tin density	
High (> 100)	100	High (>67)	67	High (>34)	34	90
Medium (> 50)	100	Medium (> 33)	67	Medium (>17)	17	70
Low (< 50)	50	Low (<33)	33	Low (<17)	17	60.

In 2014, 20 adult slow-worms (low population) were recorded at Soakham Down. After the number of tins on site doubled a peak of 47 adults were recorded, which highlighted a “medium” population was present on site. Doubling the tin density, increased the detectability of recording slow-worms, which in turn, increased the reliability of the capture effort required for translocations (Table 2.2).

In comparison, 25 and 29 adult slow-worms were recorded at Earthworks site during 2014 and 2015 respectively. The population of slow-worms on site remained constant within the "medium" category when refuge density was not altered. The minimum capture effort did not change between the survey years.

Froglife (1999) guidance suggests between 5 and 10 refugia should be used per hectare of site being surveyed. A total of 20 and 40 tins were used to survey the 0.665 ha site in 2014 and 2015, respectively. These densities are 4 times and 8 times greater than the minimum 5 tins recommended in the Froglife guidance. The findings have highlighted how variable population class assessments can be when tin density is increased. Fundamentally, using a greater number of tins on-site increases the likelihood of detecting a slow-worm if present. Furthermore, Froglife (1999) guidance must be updated and modified in light of these results to increase the certainty in detecting slow-worms, if present on-site.

2.6.3 Tin age comparison

Although old double tins were occupied by more slow-worms than new double tins, overall findings suggest that there was no statistically significant difference between the number of slow-worms recorded and tin age. Weathering of tins may change the thermal properties and the overall appeal as refugia. The ground beneath both aged tins was generally similar, and the older tins did not experience significant levels of weathering, which could have impacted the thermal attractiveness of the tins.

Differences in slow-worm numbers are likely to be seen between tins that have been placed on a site for a longer period (greater than one year). In most instances, the vegetative cover beneath tins placed on site for longer periods of time would begin to die off. Ultimately, this would result in changes in the refugia microclimate to become less suitable for slow-worms.

In general, there is sparse literature relating to the time taken for reptiles, more specifically slow-worms, to locate artificial refugia within their environment. Findings from this study have indicated that in 2014 100% of all tins were located by visit 3 and in 2015 90% of all tins were located by visit 3 (Appendix 5). This indicates that slow-worms find new refugia quickly. As previously mentioned, weathering of tins has the potential to change the thermal properties and appeal of the tins. Older tins have the potential to produce a more suitable microclimate beneath them which will aid with effective thermoregulation (Christian et al. 2016). Therefore, if the time frame between setting out the 2014 tins and new 2015 tins on the site was greater, a greater preference towards older tins may have been witnessed.

2.7 Conclusion

Slow-worm detectability was clearly impacted by tin layout. Tin size and tin density and distribution had the greatest influence on the number of slow-worms recorded. Using tins 0.25 m^2 in size and at density of 40 per hectare increases the reliability and validity of survey results. This emphasises the need for existing reptile survey guidance to be updated and modified, in light of the findings from this study.

Chapter 3: Slow-worm population monitoring: A study of local long-term monitoring in Kent.

3.1 Abstract

Population monitoring is an essential method for assessing the status of species, determining changes in biodiversity over time and develop our understanding of how management actions or practices affect species. Species-specific monitoring has been identified as a priority action to aid with the conservation of many Species of Principal Importance (SPI) in the UK, including the slow-worm. Habitat management is a key regime used to enhance and maintain areas of suitable reptile habitat. This study was undertaken to determine if and how slow-worm numbers have changed over time and identify whether population changes are comparable between sites within close proximity. Slow-worms were studied over a thirteen-year period, between 2005 and 2017, inclusive, at two sites within King's Wood, Challock known as Main site and Earthworks site. Habitat management in both areas was undertaken approximately over a five year cycle, however in recent years it was undertaken more regularly, every three years. Slow-worm numbers fluctuated differently between sites. Slow-worm numbers decreased by 41.9% at Main site and increased by 3.6% at the Earthworks site, over the thirteen-year period. Habitat change is likely contributing to the variation in slow-worm numbers at both sites. Findings from this study indicate that more frequent habitat management, i.e. annual habitat management, should be conducted at the Main site. Ultimately, these findings have highlighted how conservation action, in this case habitat management, should be determined on a site by site level however, management should also consider other species and the general biodiversity of the area when determining conservation and management actions.

3.2 Introduction

In the United Kingdom, 1,115 animal species have been assessed and assigned a conservation status (IUCN 2017b). Approximately 7.35% of these species are classified as threatened (Extinct, Critically Endangered, Endangered or Vulnerable) with *c.* 92.7% falling into the Near Threatened, Data Deficient and Least Concern categories, 59, 78 and 896 species respectively (IUCN 2017b). The Red Lists' primary goal is to gather species-specific information to assess status, threats and analyse trends to inform conservation (JNCC 2010).

Wildlife monitoring is a method used to influence conservation (Di Fonzo et al. 2015; Engeman et al. 2016). Population monitoring is primarily undertaken to assess or update the status of a threatened species, to determine changes in biodiversity of a given area over time, or to understand how management actions or practices affect species, especially 'biological indicators' (Witmer 2005).

Slow-worms are a cryptic, long-lived 'environmental indicator' species, present throughout the UK in varying abundance (Gleed-Owen et al. 2005). Although common and widespread in southern England, slow-worm occupancy generally decreases with latitude, with total absence of slow-worm in Northern Ireland and Isle of Man (Figure 3.1) (Inns 2009; Platenberg and Langton 1996; Wilkinson and Arnell 2013). Baker et al. (2004) highlighted that all regions in England have experienced declines in slow-worm numbers, with the greatest declines recorded in the Midlands.

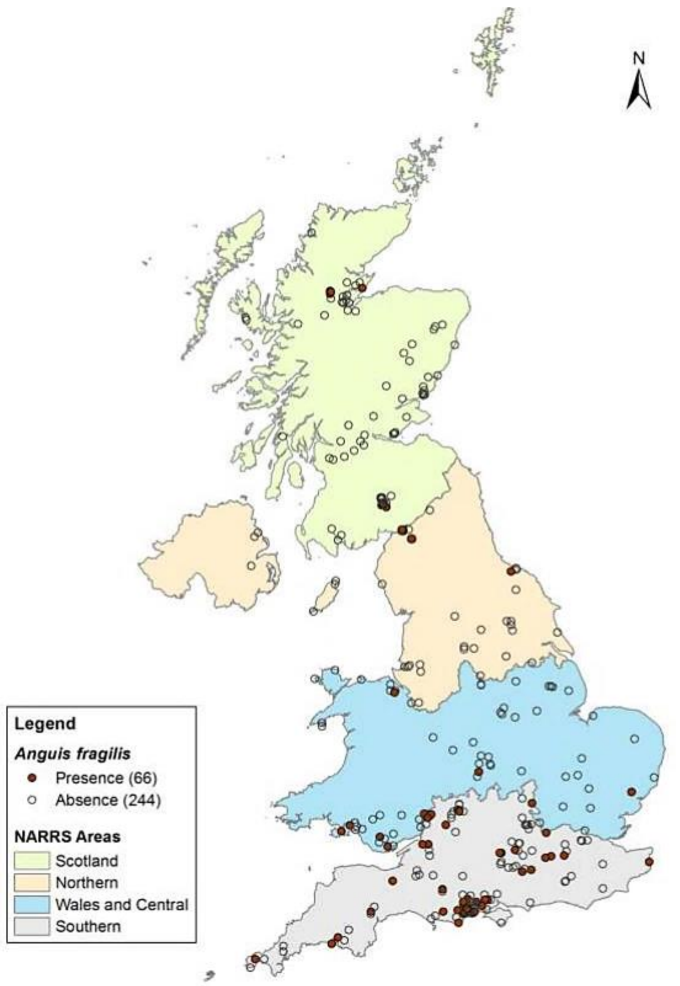


Figure 3.1: National Amphibian and Reptile Recording Scheme (NARRS) slow-worm occupancy map – highlights presence and absence of slow-worms within squares surveyed (Wilkinson and Arnell 2013).

Widespread habitat loss, especially relating to increased developmental pressure in rural and urban areas, is a major threat responsible for nationwide declines in slow-worm numbers (JNCC 2007; JNCC 2010). The slow-worm is a Species of Principal Importance (SPI), under Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006 (Natural England. 2014). SPIs are priority species that require conservation action due to major threats (JNCC 2016). Species-specific monitoring and surveying was identified as a priority action to aid the conservation of slow-worms, which involved the implementation of the National Amphibian and Reptile Recording Scheme (NARRS) to assess population levels and improve survey data

(JNCC 2007). Previously, reptiles were overlooked from a number of habitat management regimes (Edgar et al. 2010). In general, habitat management schemes primarily focus on main reptile habitat requirements; warmth, habitat connectivity and structural complexity, to aid with species conservation (Edgar et al. 2010).

NARRS, a volunteer-based programme established in 2007, aimed to assess species status and predict changes expected for populations in the future (Wilkinson and Arnell 2013). Generally, conservation strategies begin with generating an inventory, closely followed by monitoring and management (Engeman et al. 2016). Phase 1 of NARRS involved surveying randomly selected sites to generate a baseline inventory for reptile occupancy throughout the UK during the period of 2007 to 2012 (Wilkinson and Arnell 2013). NARRS Phase 2, a monitoring programme for reptiles, was introduced in 2013, where surveys are undertaken at a fixed location so population data can be obtained (Wilkinson and Arnell 2013). Surveying and monitoring known sites with slow-worm presence is vital for increasing knowledge of ecology and population status.

Conservation objectives for slow-worms in the UK are site-specific, due to substantial variations in slow-worm populations with different phenology, population size, habitat preferences and requirements and location (JNCC 2004). Increased monitoring and surveys should be undertaken to ensure the most appropriate conservation action is implemented.

This chapter sets out to analyse trends in slow-worm abundance over time within sites specifically managed for reptiles.

3.3 Methodology

King's Wood is an ancient woodland site located within Challock, Kent (approximate central OS grid reference: TR035492). Two sites, Main site (*c.* 1.42 ha) and Earthworks site (*c.* 0.335 ha) have been monitored over the last 13 years by University of Kent students and The Kent Reptile and Amphibian Group (KRAG) Figure 3.2). Materials and methods relating to the study species are given in Chapter 2.

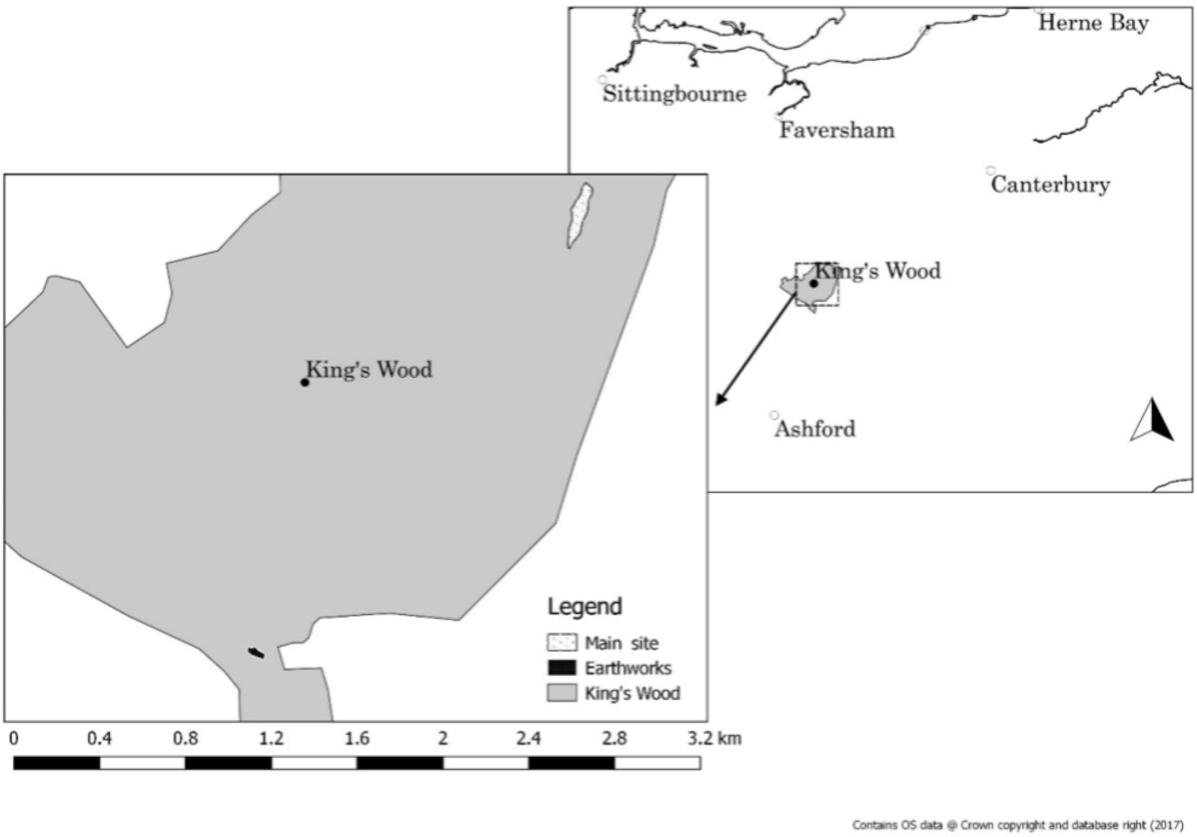


Figure 3.2: Map illustrates the geographical location of the study sites Main site and Earthworks, King's Wood, Challock.

3.3.1 Habitat characteristics and management

The Main site and Earthworks site have similar habitat structure and occupancy of slow-worms. Both sites are vegetated by tussocky calcareous grassland and dense scrub, including bramble (*Rubus fruticosus* agg.), with occasional scattered trees. Figure 3.3 and Figure 3.4 illustrate the vegetative characteristics of both sites showing examples of habitat prior and post clearance.

The Forestry Commission are responsible for the management of King's Wood (Forestry Commission 2016). Site management follows current habitat management guidance and good practice for reptiles (Edgar et al. 2010). Management involves creating brash piles and clearing vegetation to stop natural succession, to maintain the woodland clearing area. The main priority of habitat management is to generate continuous or connected mosaics of diverse habitat. Management of both sites usually occurs over a 5-year cycle, however, the mild and wet winters over recent year have resulted in bramble and bracken regenerating quicker. Survey records have highlighted that habitat clearance is occurring more regularly, with minor clearance work being undertaken by surveyors to prevent refugia being engulfed by areas of scrub. An exact inventory of habitat management for each site is unknown. Survey data sheets indicated incidences of habitat management undertaken during 2014 and 2017 at both sites with additional management occurring in 2011 at the Earthworks site.

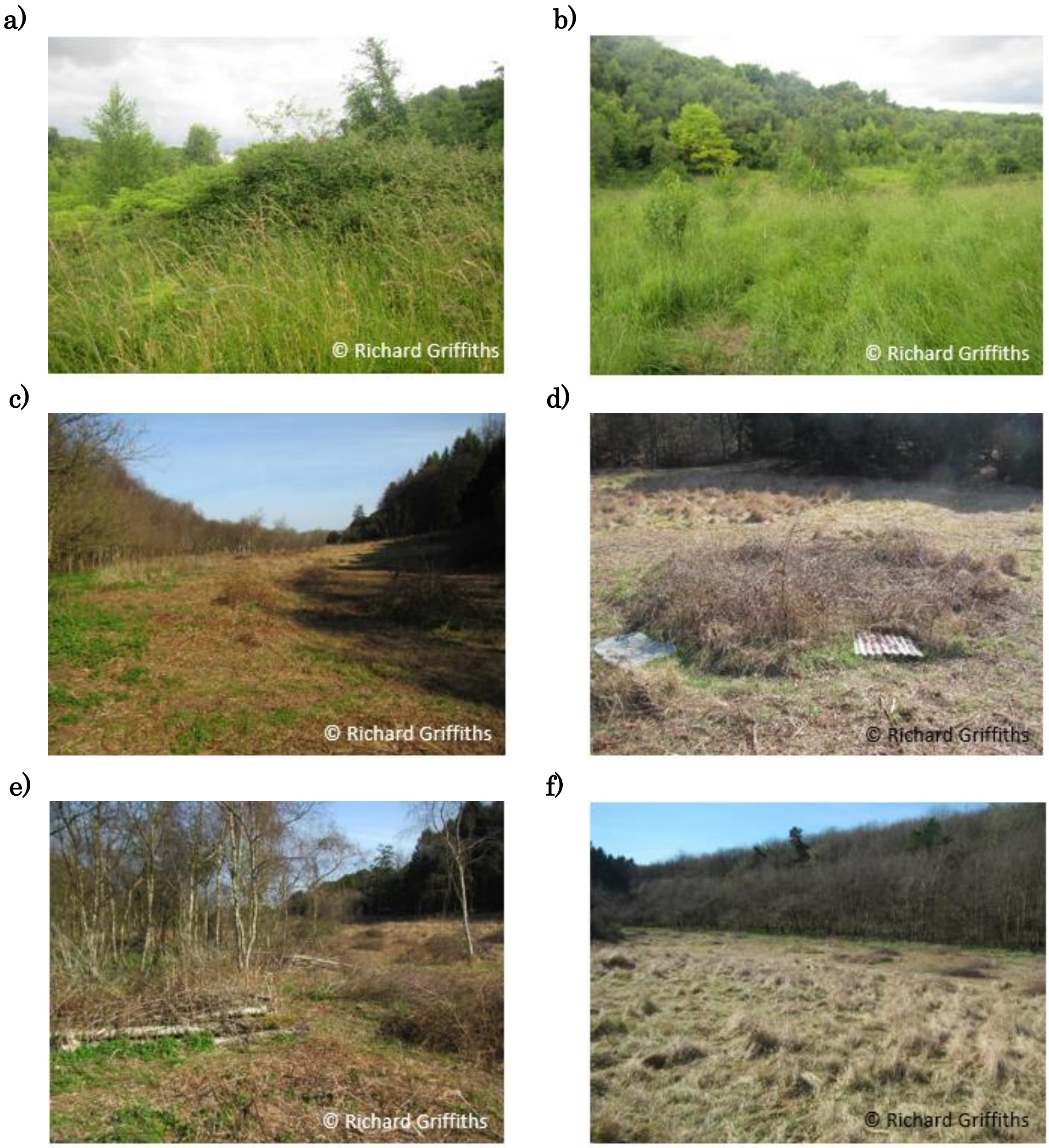


Figure 3.3: Main site habitat. **a)** Grassland and scrub (2016), **b)** Scattered trees and grassland (2016), **c)** Cleared habitat (2017), **d)** Regenerating scrub patch (2017), **e)** Felled trees and scrub patches (2017), **f)** Grassland (2017).

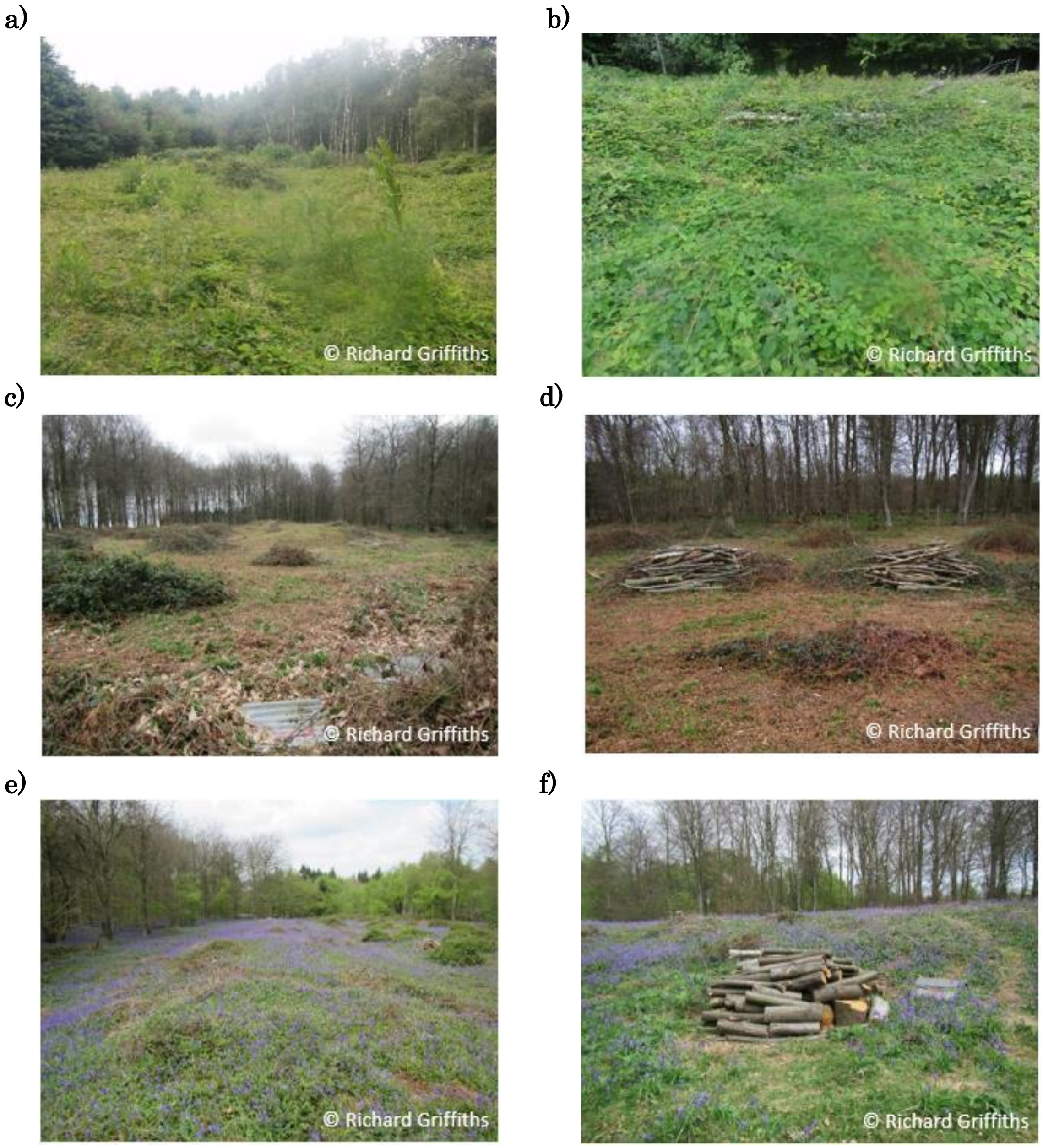


Figure 3.4: Earthworks site habitat: **a)** Grassland, scrub and brush piles (2014), **b)** A scrub covered log pile (2014), **c)** Grassland and scrub patches post-clearance (2017), **d)** Scrub patches and brash piles post-clearance (2017), **e)** Regenerating grassland (2017), **f)** Regenerating grassland with log pile (2017).

3.3.2 Experimental design

Survey visits were conducted every 1-4 weeks between the months of March and September from 2005 to 2017.

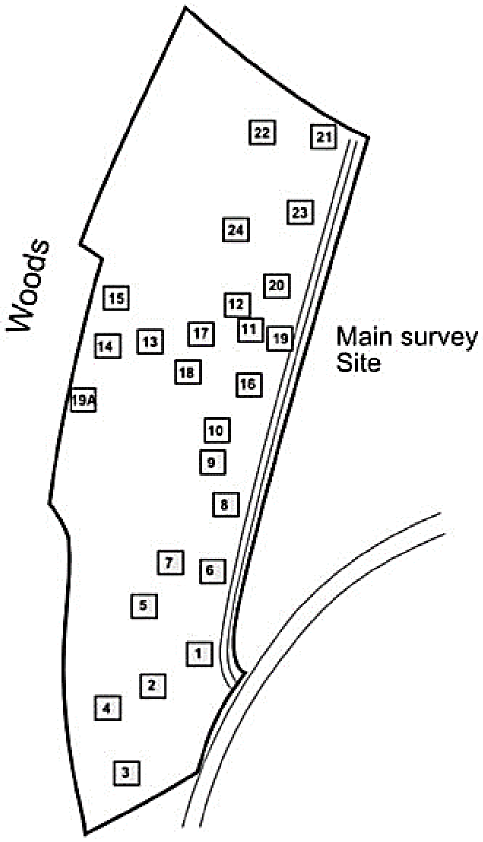
Artificial refugia, 0.25 m^2 sheets of lightweight corrugated iron sheeting (tins) and roofing felt sheeting (felts), were used to survey for slow-worms. The Main site and Earthworks site contain 48 (24 pairs of tins and felts) and 24 (12 tins and felts pairs) refugia respectively (Figure 3.5a,b). The number of refugia remained constant over the 13-year monitoring period and refugia were only replaced if damaged or degraded. Although there is public access to the sites, site disturbance was generally low and mainly occurred during survey visits where refugia were lifted to check for slow-worms underneath.

3.3.3 Data collection

Descriptions of the survey methods and parameters recorded are given in chapter 2. When slow-worms were present the following variables were recorded: refuge number and type (no slow-worms were found out in the open), the number of individuals, age class and sex (Appendix 2).

The survey period extends from May 2005 to September 2017. Survey years are denoted by the year in which surveys began. Each survey visit took between 0.5 hrs to 1.5 hrs to complete dependent on the number of slow-worms recorded. Surveys were generally conducted between the hours of 0900 hrs and 2030 hrs.

a)



b)

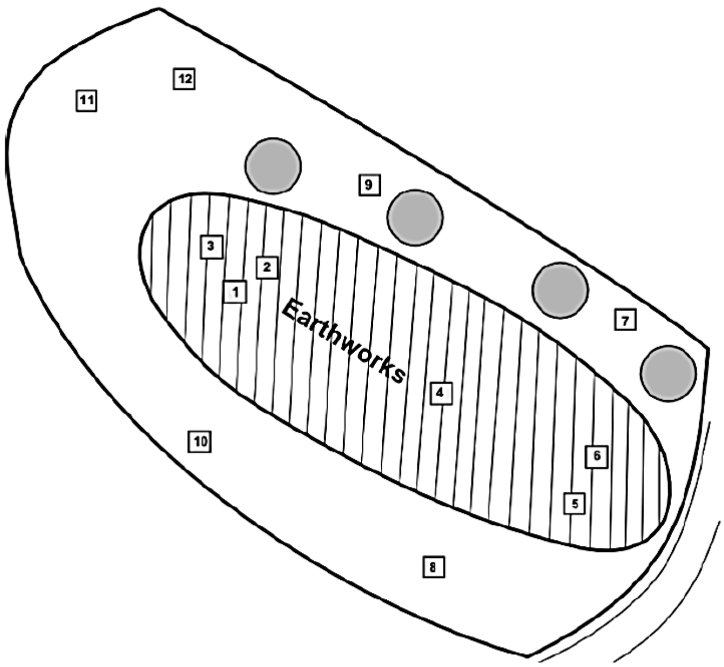


Figure 3.5: Map illustrating refugia distribution. **a)** Main site **b)** Earthworks site. The squares illustrate the location and number of the refugia pairs. The hatched area on the Earthworks site plan indicate the bronze-age mound and circles indicate log piles.

3.4 Data Analysis

Raw data sheets were organised and manipulated using Excel to generate the datasets for analysis. The total number of slow-worm captures per visit were used as the dependent variable for the statistical analysis. Date (week) was used as the replicate within each survey year because multiple survey visits were conducted within a year.

Assumption tests and statistical analysis were undertaken using R (Appendix 5).

In the results section, slow-worm patterns are highlighted within the text. The results of each statistical analysis, whether statistically significant or not, are included on the graphs included within the results section.

3.4.1 Population trend comparison

Simple linear regression analysis were performed for the Main site and Earthworks site datasets individually to study the relationships between slow-worm captures over time. To study the relationship between slow-worm captures over time between the sites, the regression slopes were compared.

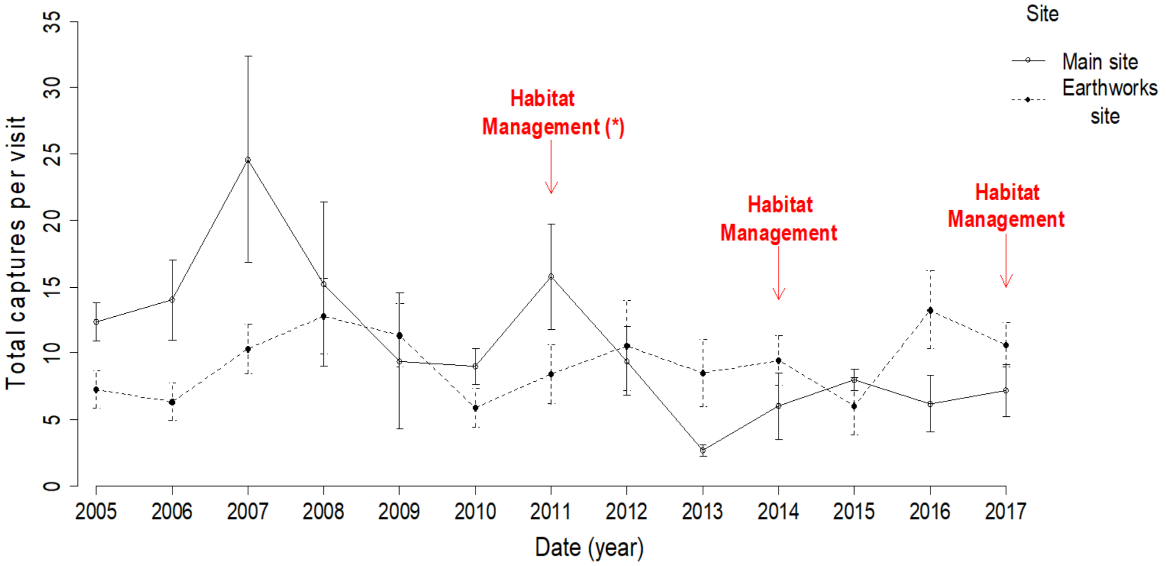
3.4.2 Age class comparison

Total slow-worm captures per visit were separated by age class: adult, male, female, sub-adult and juvenile.

To compare the proportion of slow-worms per age class between years, descriptive statistical analysis were undertaken to calculate the mean number of slow-worm captures per visit.

3.5 Results

Overall, 197 survey visits were conducted over the thirteen-year period across both survey sites (Main site: 63; Earthworks site: 134). On average, five surveys were conducted per year at the Main site, whereas approximately nine surveys were conducted per year at the Earthworks site (Figure 3.6). At the Main site total slow-worm numbers per visit fluctuated over time (Figure 3.6). Slow-worm numbers at the Earthworks site fluctuates repeatedly over time, however, the slow-worm numbers generally increase.



Survey visits per year

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Main site	3	5	5	5	5	5	4	5	6	5	4	5	6
Earthworks	12	6	13	14	9	10	10	9	12	11	10	8	10

Figure 3.6: Total slow-worm captures per visit – Main site and Earthworks site. Data grouped by week, data points illustrate total slow-worm captures per visit ± S.E. Red arrows indicate years where habitat management was undertaken on site. (*) – Habitat management only undertaken at the earthworks site. The table indicates the number of surveys conducted per year.

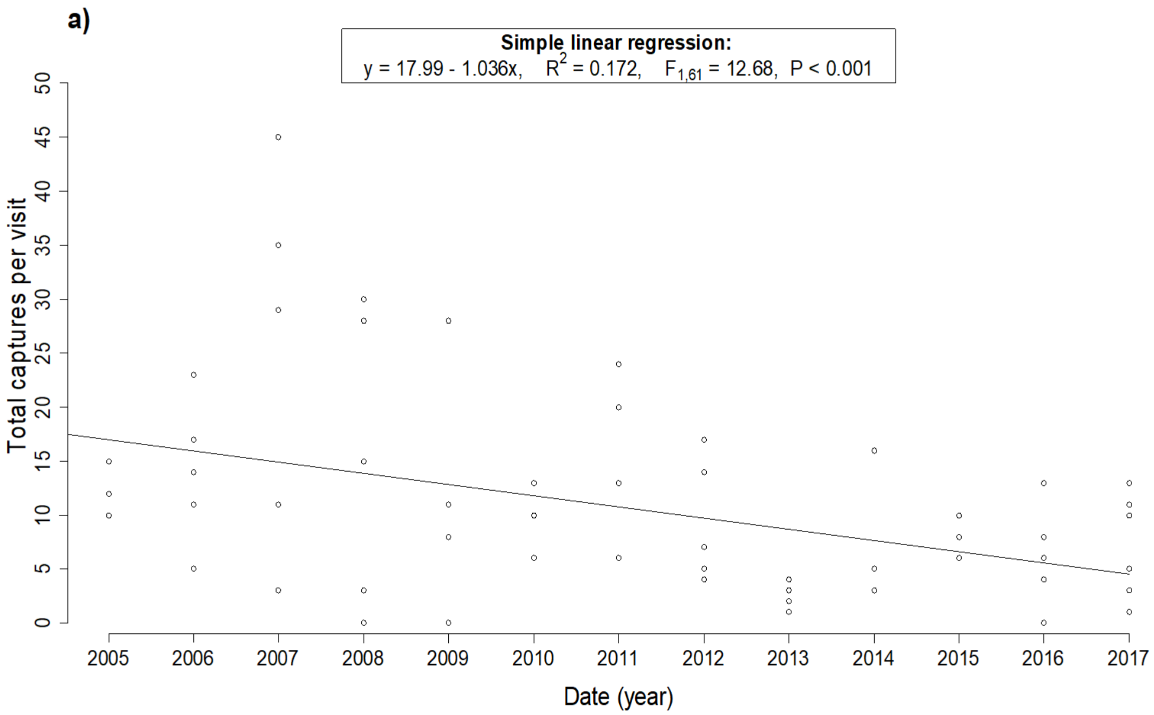
3.5.1 Population trend comparison

Slow-worm numbers declined by 41.89% at the Main site from 2005 to 2017 (Figure 3.7a). Approximately 17.2% of the variation in slow-worm numbers can be explained by the model containing only date.

The Main site regression indicates that slow-worm numbers will continue to decrease over time where slow-worm numbers are estimated to reach zero between the years 2021 and 2022 ($y = 17.99 - 1.036x$).

At the Earthworks site, there was little variation in slow-worm numbers over time, however slow-worm numbers increased by 3.6% over the thirteen year period (Figure 3.7b). Only 2.19% of the variation in slow-worm numbers can be explained by the model containing only date.

The Earthworks site regression indicates that slow-worm numbers will gradually increase over time ($y = 8.058 + 0.300x$) (Figure 3.7b). It is estimated that approximately 13 slow-worms could be recorded per visit by the year 2022, if slow-worm numbers continued to increase as indicated by the model.



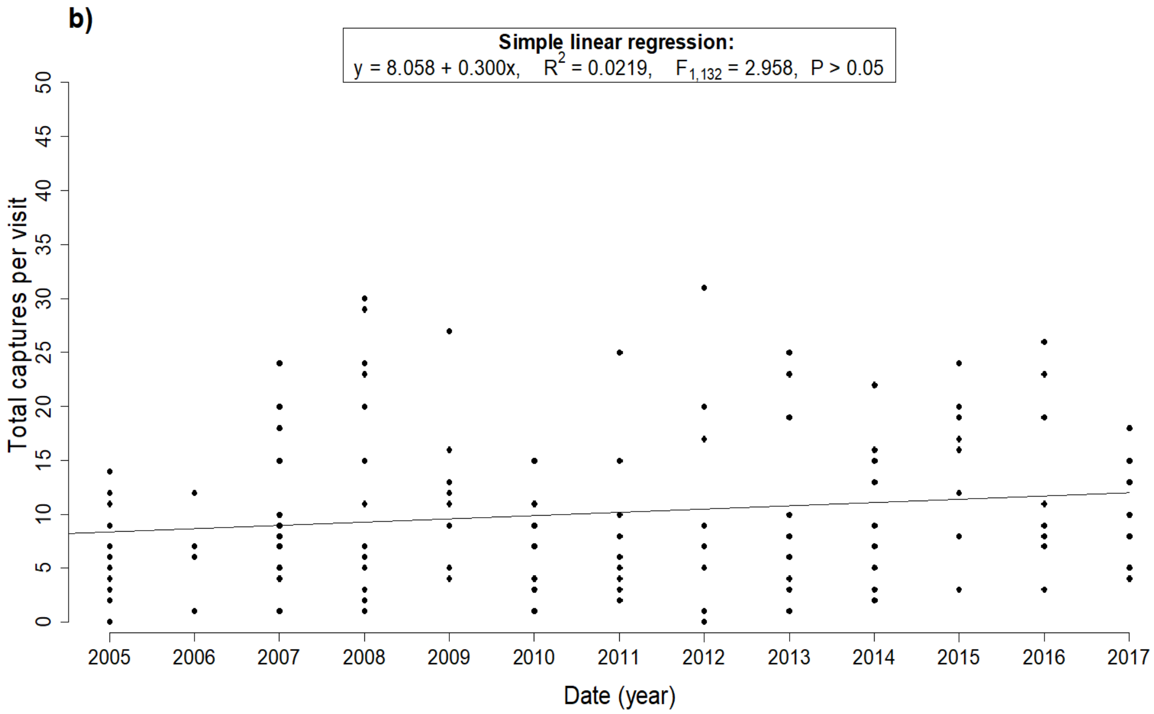


Figure 3.7: Total number of slow-worms captured per visit, during the study period, 2005 – 2017. **a)** Main site and **b)** Earthworks site. Regression lines fitted.

3.5.2 Age class comparison

A total of 2014 slow-worms were recorded over the thirteen-year period (Main site N = 658 and Earthworks site N = 1356). More adult slow-worms (adult, male and female) were recorded per visit than non-adult (sub-adult and juvenile) irrespective of site (Figure 3.8a,b). Female slow-worms were the highest recorded age class from 2005 to 2017, accounting for approximately 40.58% and 44.32% of all sightings at the Main site and Earthworks site respectively (Figure 3.8a,b). At the Main site, peak counts of female and sub-adult slow-worms per visit were observed during 2007. The highest number of male slow-worms recorded per visit occurred during 2011. Juvenile slow-worms were the least recorded of all the slow-worms. No juveniles were recorded during the period of 2013 to 2015.

During surveys at Earthworks site, adult slow-worms accounted for *c.* 78% of all sightings over the thirteen-year period. In 2007 and 2008, the highest numbers of female slow-worms were recorded. Peak counts of male and sub-adult slow-worms were witnessed in 2015. As at the Main site, juveniles were the least recorded age class (Figure 3.8b).

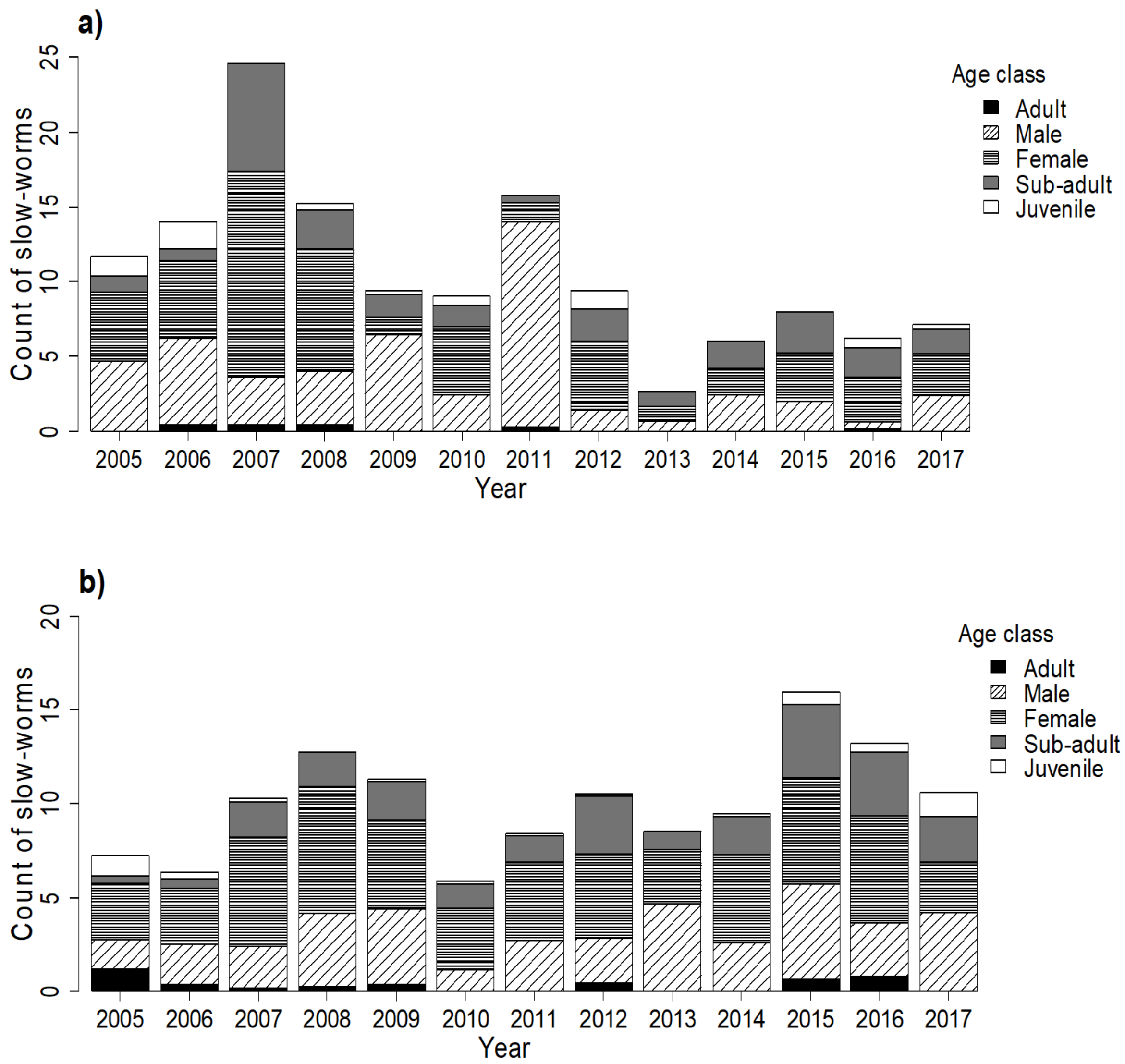


Figure 3.8: Average count of slow-worms per age class per visit. **a)** Main site, **b)** Earthworks site. Data grouped by age class.

3.6 Discussion

3.6.1 Population trend comparison

Slow-worm population trends fluctuated differently between sites, decreasing at the Main site and remaining stable at the Earthworks site. Slow-worm numbers were higher at the Main site during the early years of monitoring, but as time progressed numbers declined. The Main site (*c.* 1.42 ha) is four times larger than the Earthworks site (*c.* 0.335 ha). A greater number of slow-worms can be supported within a larger area of suitable reptile habitat (Froglife 1999). There is little difference between the overall number of slow-worms between sites, but as the Earthworks site is smaller in area, there is a higher population density of slow-worms present.

Although slow-worms can be detected at almost any time between March and September, climatic variables such as temperature, can influence the number of animals recorded per visit (Edgar et al. 2010; Sewell et al. 2012). Surveys were conducted during suitable conditions for reptile surveying, to increase the chance of detecting slow-worms (Froglife 1999). No surveys were conducted during periods of extreme weather. The Main site and Earthworks site exhibit similar vegetation characteristics, are within the same woodland and experience the same climatic pressures. Temperature and other climatic factors are therefore unlikely to have influenced the difference in slow-worm abundance.

On average, twice the number of surveys were conducted at the Earthworks site throughout the year compared to the Main site (Figure 3.6). Between three and four surveys are required to have 95% certainty that a species would be detected if present on a site (Sewell et al. 2012). A greater number of surveys are required to detect changes in population size, the power to detect is proportional with the number of surveys (Sewell et al. 2012). The results comply with NARRS Phase 2 requirements of at least four surveys conducted each year in suitable survey conditions, however, for a local scale comparison the difference in survey efforts could influence the population trends. The results support the requirement for conservation action to be tailored towards site-specific survey results and which have identified trends in slow-worm abundance.

Slow-worm counts increased post-habitat management at the Main site in 2014 and at the Earthworks site in 2011, however numbers began to decline in the following years. Habitat management can render basking reptiles more visible and easier to detect if present, shortly after the operation, resulting in higher survey counts immediately following management (Edgar et al. 2010). By using artificial refugia during reptile surveys, we may be exploiting and changing the behaviour, thermoregulation and breeding of slow-worms by potentially increasing site suitability and carrying capacity for this species. In turn, we are altering the habitat, which could negatively impact other species present within the site. These potential impacts therefore need to be taken into account when developing and implementing site-specific management, to ensure biodiversity as a whole is supported and conserved.

3.6.2 Age class comparison

More female slow-worms were recorded per visit compared to any other age class. In general, a slow-worm population consists of a higher number of adults compared to juveniles due to the longevity of the species (Beebee and Griffiths 2000). The biennial breeding cycle of female slow-worms could have influenced the fluctuations in juvenile numbers recorded throughout the study. Additionally, the higher number of female slow-worms recorded over the year is likely to be associated with the thermal preferences of the species. Refugia could provide a safe and sheltered environment for mating and incubation, therefore, sightings of adult slow-worms would be higher.

Previous studies have indicated slow-worm refuge preference at different life stages preference. Alfermann (2002), suggested that juveniles preferred utilising refugia more than adults. In contrast, Fish (2016) suggested that juveniles avoid using refugia where other slow-worms or reptiles of other species are present. Lower sightings of juvenile slow-worms could be associated with the higher proportion of refugia use by adult slow-worms. Juveniles therefore could be utilising aspects of denser habitat which provide suitable refuge, protect against predators and disturbance from other reptiles that were inaccessible to surveyors during survey visits.

Finally, fewer sightings of juvenile slow-worms is potentially associated with reduced reproduction of adult slow-worms (Ferreiro and Galán 2004). Fluctuating temperatures and weather, because of climate change, can increase the variability in the activity patterns of slow-worms and their prey. This has the potential to limit energy storage which would be used for reproduction. Reduced juvenile recruitment over the study period could be associated with food availability or climatic factors.

3.7 Conclusion

This study highlights that across the years slow-worm numbers have exhibited site-specific change. Slow-worm numbers at the Main site decreased across the thirteen-year period and the model indicates that numbers will continue to decrease over time. Slow-worm numbers at the Earthworks site remained stable over time with a very gradual increase. The findings indicate that slow-worm populations vary between sites, and therefore conservation should follow suit and be conducted on a site-specific level. Habitat management regimes, especially at the Main site could be undertaken annually to account for increased levels of regrowth of bramble, bracken and scrub within these areas. Decreasing the habitat management intervals will aid with maintaining the sites suitability through the wetter and warmer climate, and fundamentally maintain the sites overall suitability for reptiles. This study indicates that as a result of species-specific and site-specific monitoring conservation action should be focused at site-specific level. However, conservation and management actions should be implemented to benefit other biodiversity within the area not just slow-worms.

Chapter 4: General Discussion

This study set out to develop an understanding into how, if at all, artificial refugia characteristics impact slow-worm detectability, and analyse and compare long-term trends in slow-worm abundance between two sites. The effects of refugia characteristics on slow-worm detectability were assessed by the total number of slow-worms per visit, number of slow-worms per tin and the total number of slow-worm occupied refugia. The total number of slow-worms recorded per visit was used to assess the long-term changes in two local slow-worm populations over time.

Findings from the study indicate that slow-worm detectability is influenced by refugia size, refugia density and distribution, with more slow-worms recorded using single tins and when more – and more widely distributed – tins are used to survey a site. Refugia age had no effect on slow-worm numbers. In general, the population class assessments, based on the number of adult slow-worms recorded per visit, fluctuated when refugia density was increased. This indicates that more slow-worms are detected when a greater number of refugia are distributed across the site, resulting in a more realistic representation of population class within the site.

Slow-worm abundance fluctuated differently within the two sites that underwent similar habitat management regimes. Habitat management of both sites is likely to have maintained high numbers of slow-worms over time by ensuring the site remained suitable for reptiles. However, the lack of a management inventory, to compare to the long-term trends, meant that the full impact of habitat management, on slow-worm abundance could not be fully assessed.

Slow-worms will only be recorded during a survey if they are visible during the period of time in which the survey is conducted, therefore some individuals will go undetected (Kéry and Schmidt 2008; Morgan 2008; Sewell et al. 2012). Increasing the number of refugia used to survey the site increases the number of potential home ranges sampled and decreases the spacing between each refugia. Schmidt et al. (2017) stated that the best inter-refugia spacing for artificial refugia within a site was 28 m. During the investigation into how refugia characteristics impact slow-worm detectability, tin density on site was increased from 20 to 40 between years. The total area covered by all refugia present on site was 5 m^2 in 2014 and 10 m^2 in 2015. Increasing the number of tins between years therefore decreased the inter-refugia spacing by *c.*5 m. In theory, the inter-refugia spacing at the Main site and the Earthworks site for the long-term monitoring survey, were *c.*24 m and *c.*16 m respectively. Fundamentally, increasing the tin density on site allows for more home ranges to be sampled, although the catchment area size decreases as inter-refugia spacing decreases.

Reptile surveys are undertaken to gather specific information relating to presence / likely absence; population counts, densities and estimates. As part of this research, both studies set out to answer specific questions relating to slow-worms. The refugia characteristic study followed a similar methodology to a reptile presence / likely absence survey, to gather information on species persistence and population dynamics, whereas the long-term monitoring survey set out to gather information on population estimates over time. However, the main factor underpinning any reptile survey is the use of outdated guidance which lacks appropriate standards and sets out the advised methodology for how surveys should be completed.

Reptile detectability can be influenced by a number of factors, including geographical location, habitat characteristics, temperature, time of year, survey area and survey effort (Kéry 2002; Kéry et al. 2009; Sewell et al. 2012). This research has indicated that habitat characteristics, specifically relating to long-term management influence slow-worm detectability, and in addition to the factors listed above, refugia density and size also impact slow-worm detectability. Irrespective of the type of survey conducted, whether it be a presence / likely absence survey or a population estimate survey, the number of slow-worms recorded during each visit is influenced by survey effort, specifically associated with artificial refugia characteristics used to conduct the survey. Fundamentally, a higher number of refugia should be used to survey than the existing outdated unstandardized reptile survey guidance recommends. Use of a higher number of refugia will maximise the chance of detecting slow-worms if they are present within the area, and consequently, survey results are likely to be more reliable, valid and realistic to aid with:

- Assigning population class assessments to slow-worms during a presence / likely absence survey
- Informing mitigation i.e. translocation survey effort, based on more reliable population class assessments; and
- Informing species conservation action based on species conservation assessment deduced from long-term monitoring.

4.1 Limitations of this study

This analysis concentrated on a single species, single site and single refuge type. The lack of comparison between sites of differing sizes, habitat structures and locations, means that we cannot be certain that slow-worm numbers would react in a similar way when influenced by different refugia characteristics.

Tin and felt refugia were used to survey the comparison site. However, only tins were used at the Soakham Down site, which does not allow an assessment of slow-worm refuge preference at this site. Fish (2016) indicated that adult slow-worms do not show a significant preference towards artificial refugia material (felt and corrugated roof sheeting), however, juvenile slow-worms were more likely to be found under felts than other artificial refugia material. Previous research has also indicated that artificial refugia preferences by slow-worms can be site specific, where adult slow-worms have preferentially used roofing felt at one site but did not exhibit a refugia preference at other sites (Rijksen unpublished).

Keeping refugia locations constant over time can change the microclimate beneath, so that the substrate becomes unsuitable for reptiles. In this instance, if vegetation is killed off underneath the refugia, the microclimate may become too dry for reptiles, and therefore, individuals may go undetected and population assessments could be under-represented. However, keeping refugia locations constant over time allows for effective long-term monitoring to be conducted and allows for comparability between years.

4.2 Future research

The effects of tin density, size and distribution should be analysed further. Without further research into the effects of refugia characteristics on slow-worm detectability, standardising reptile guidance would be difficult. Future actions should involve: determining when increasing the number of refugia used to survey reaches a detection limit and investigating how refugia size and density affect slow-worm detectability at sites of different sizes, locations, habitat characteristics and reptile assemblages.

Conservation action should be directed towards a more site-specific, species-specific approach. Habitat management regimes could be undertaken more regularly, on an annual basis, to aid with maintaining site suitability through wetter and warmer climatic conditions.

Finally, continually undertaking species-specific monitoring of sites will further aid with the conservation of species, especially, to increase our understanding of how climate change and other contributing factors are influencing slow-worm activity patterns and population abundance.

It is fundamental to ensure that future reptile survey guidance is supported by an evidence base of relevant research, whereby the detectability issues surrounding surveys are taken into account and minimised.

4.3 Conclusion

This research provides evidence to support the argument to review and improve current outdated reptile guidelines, considering the effects that refugia size, density and distribution have on slow-worm numbers. Inevitably, more reliable and accurate survey methods would increase the validity of survey results, which would consequently increase certainty when making mitigation decisions.

Guidance should be generated from evidence-based conservation rather than historic and current anecdote and expert opinion. This would ensure the most appropriate conservation actions are undertaken which have the best outcomes for biodiversity. If reptile survey guidance is not updated and amended based on up-to-date evdence, slow-worm and potentially other reptile species will continue to be under-represented by survey results obtained using methds set out in current guidance, which do not take into account methods of increasing species detectability. Furthermore, this could result in ineffective conservation management strategies being implemented, and further declines in slow-worm populations and other biodiversity in the future, on both local and national scales.

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Appendix 1: Soakham Down survey recording sheet.

SLOW-WORM

Site:	Soakham Down	Date:	
Air temp:		Rain:	
Wind speed:		Wind direction:	
Cloud cover:		Ground conditions:	
Start time:		Stop time:	

	Single				
Refuge	Male	Female	(Adult)	Juvenile	Subadult
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
Totals					
Grand total					

	Double				
Refuge	Male	Female	(Adult)	Juvenile	Subadult
1					
2					
3					
4					
5					
Totals					
Grand total					

SLOW-WORMS IN THE OPEN					
LOCATION/BEHAVIOUR	Male	Female	(Adult)	Juvenile	Subadult

Appendix 2: Earthworks survey recording sheet

SLOW-WORM

Site:	Earthworks	Date:	
Air temp:		Rain:	
Wind speed:		Wind direction:	
Cloud cover:		Ground conditions:	
Start time:		Stop time:	

	Tin				
Refuge	Male	Female	(Adult)	Juvenile	Subadult
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
Totals					
Grand total					

	Felt				
Refuge	Male	Female	(Adult)	Juvenile	Subadult
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
Totals					
Grand total					

SLOW-WORMS IN THE OPEN					
LOCATION/BEHAVIOUR	Male	Female	(Adult)	Juvenile	Subadult

Appendix 3: Soakham Down data for the total number of slow-worm captures throughout the study. (The number of slow-worm captures for double tins is the combined total of tins A & B, that form a double tin.)

		2014	2015
Visit No.	Tin type	No. of captures	No. of captures
1	Single	7	32
	Double	3	21
2	Single	18	23
	Double	5	15
3	Single	5	44
	Double	11	31
4	Single	6	31
	Double	12	31
5	Single	15	15
	Double	7	6
6	Single	7	28
	Double	0	15
7	Single	5	28
	Double	2	16
8	Single	17	40
	Double	4	30
9	Single	13	16
	Double	4	20
10	Single	14	24
	Double	2	23
11	Single	12	
	Double	13	
12	Single	13	
	Double	7	

Appendix 4: Soakham Down assumption testing and data transformations.

Independence of observations

The data relating to the dependent variable, number of slow-worm occupied 'single' tins for the age comparison analysis complies with the assumption of independence. Each observation is independent to one another, as the occurrence of slow-worms under refugia during an individual survey visit does not change the probability of detecting slow-worms at another occurrence.

Homoscedasticity (Homogeneity of variances)

A Levene's test was used to test for homogeneity of variance across groups. The dataset relating to single tin occupancy for the age comparison analysis failed to comply with the homoscedasticity assumption, therefore to comply with this assumption a data transformation was required.

Normally distributed dependent variable

A Shapiro-Wilk test was undertaken to test for normality within datasets, normality was assumed when P-values were greater than 0.05.

The dataset relating to the number of slow-worm occupied 'single' tins for the age comparison analysis had a P-value less than 0.05. Therefore, these data were therefore transformed,

Data transformations

A data transformation was undertaken for the 'single' occupied tins for the age comparison analysis.

A reflected square root transformation adjusted on the negatively skewed data to follow a normal distribution whilst having homoscedasticity (Levene's Test: F = 0.215, P = 0.648, Shapiro-Wilk: P = 0.189).

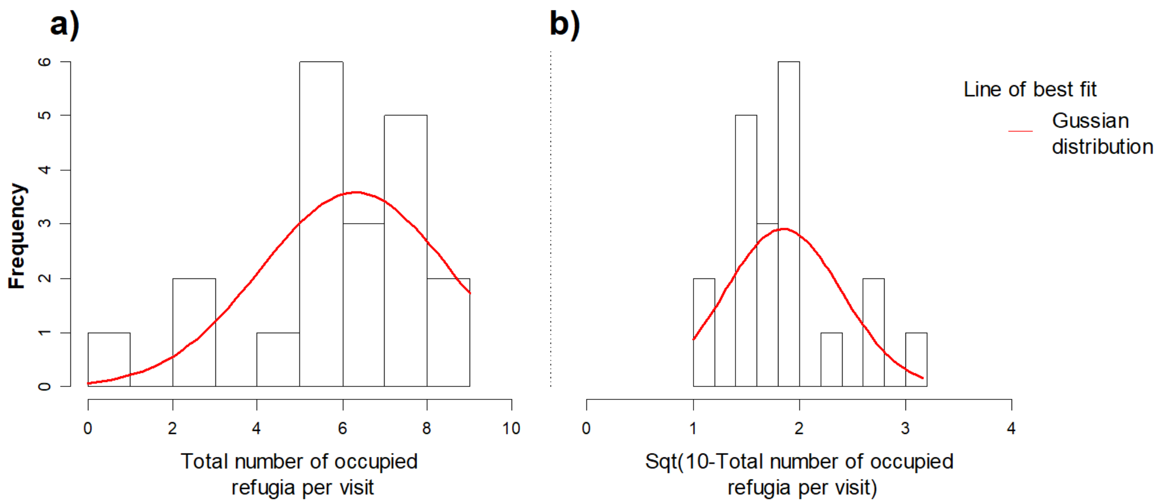

Figure 4.1: Histogram of total number of slow-worm occupied single refgia per visit: **a)** Original data (P < 0.05), **b)** Reflected square root transformation (P > 0.05).

Appendix 5: Number of previously unoccupied tins found by slow-worms per visit.

Visit number	**Number of previously unoccupied tins found by slow-worms**	
	2014	*2015*
1	6	25
2	8	7
3	2	4
4	2	1
5	2	1
6	0	0
7	0	0
8	0	0
9	0	0
10	0	1
11	0	
12	0	