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Post-reintroduction monitoring: artificial refugia provides insight into threatened reptile assemblages

A technical report prepared from an MRes Wildlife Conservation project, Marwell Wildlife & University of Southampton

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Abstract

Reintroductions are a powerful tool used in the conservation of threatened species and to enhance the stability of an ecosystem. This method, however useful, remains flawed in its application with poorly designed pre-and post-monitoring protocols. These protocols rarely consider the community structure as a factor and post-monitoring surveys are often limited in scope and duration. This study used monitoring data from surveys based on artificial refugia to understand reptile assemblages at Eelmoor Marsh SSSI, post-reintroduction of Sand Lizards (*Lacerta agilis*). Chi-square and Fisher's Exact analyses found intra-interspecific interactions influenced refugia use and thus, detectability during surveys. Results also revealed life stage preferences for different material types of refugia. Yielding recommendations for the most appropriate material for optimal observations of reptile surveys. Refugia survey data enable studies of the population dynamics and extension risk of cryptic reptile species. Thus, having the ability to study their extinction rates, of which we know very little. This study calls attention to the importance of monitoring a species after reintroduction to see the effects on pre-existing community assemblages and to monitor population dynamics.

Keywords: Artificial refugia · Reintroductions · Community Assembly · Monitoring protocols · Extinction rates · Reptiles · Conservation management

Target Journal: Biological Conservation

1. **Introduction:**

The rapid loss of global biodiversity is largely due to anthropogenic climate change (Martínez-Freiría *et al.*, 2013). Changes in climate leads to an increase in environmental changes such as, shifts in vegetation density and diversity along with water access, which affects territorial ecosystems (Araújo *et al*., 2006; Martínez-Freiría *et al.*, 2013). This alteration produces changes in activity patterns and shifts in animal species abundance and distribution (Araújo *et al.*, 2006; Ripple *et al.*, 2017). Some species, for example, Elk (*Cervus elaphus*), can adapt well to changes in the environment, as do most large migrating herbivores due to large distribution ranges. Elk's distribution ranges from 2,500 to 10,000 acres, therefore, can migrate along with their habitat as it changes (Bennett and Tang, 2006; Böhm *et al.*, 2013; Meiri and Chapple, 2016). However, other species, such as Gaboon adder (*Bitis gabonica*), having low distribution capabilities of only four acres, cannot adapt well to changes in the environments and are at high risk of extinction (Edgar *et al.*, 2010; Gibbon *et al.*, 2000; Linn *et al.*, 2006; Meiri and Chapple, 2016; Tingley *et al.*, 2016).

The International Union of Conservation of Nature, IUCN, highlights the importance of studying species with high extinction risk (Böhm *et al.*, 2013), and in particular, the life histories of reptiles and the environmental stressors they face, to better predict extinction rates (Gibbon *et al.*, 2000; Tingley *et al.*, 2016). Reptiles can live in a variety of habitat types and typically have small home ranges, making them particularly vulnerable to extinction and also difficult to monitor, so gaining estimates of population size is difficult. By learning more about their environmental stressors, we can better predict where they can be found, and thus, advance monitoring protocols of this species (Brown *et al.*, 2011; Carvajal-Cogollo and Urbina-Cardona, 2015; Edgar *et al.*, 2010; Grillet et al., 2010; Hodges and Seabrook, 2016; Tingley *et al.*, 2016, 2016).

Reptiles fill a variety of functional niches within the ecosystem. Some serve as top predators, such as alligators, and control herbivore abundance, while others serve as a vital food resource (Böhm *et al.*, 2013). Reptiles can provide key ecosystem services by decreasing the spread of disease and providing pest control by consuming rodents. Additionally, through prey consumption reptiles can also act as important seed dispersers (Biaggini and Corti, 2015; Brown *et al.*, 2011). Therefore, reptiles' ecosystem services support ecosystem stability and provide direct benefit to humans and it is thus vital to address our lack of knowledge on this essential taxonomic group. Within terrestrial vertebrates, reptiles are the most species-rich group (Tingley *et al.*, 2016). The IUCN Red List of Threatened Species has near-complete extinction assessment coverage of birds and mammals and 83% of amphibians, but only 45% when it comes to reptilian species. This is due to insufficient data on population trends (Tingley *et al.*, 2016). There are three broad themes when it comes to the lack of knowledge on these creatures: 1) global patterns and processes of extension risk; 2) taxonomic and regional knowledge gaps; 3) conservation interventions, such as reintroductions protocols, specifically a lack of pre-and post-reintroduction monitoring (Gibbon *et al.*, 2000), the focus of this study.

The use of refugia to monitor and study reptiles has shown to not only help with finding individuals and aid with population estimates but also improves their habitats (Glorioso and Waddle, 2014; Grillet *et al.*, 2010; Hodges and Seabrook, 2016). Natural refugia, such as rabbit burrows or hollowed-out logs, provides shelter for reproduction, protection from predation and hunting grounds for reptiles (Grillet *et al.*, 2010). As habitat degradation and fragmentation continues to increase, natural refugia decreases, resulting in a decrease of reptile population size and diversity (Grillet *et al.*, 2010).

Reptiles have also been shown to thrive in man-made/artificial refugia (Zappalorti and Reinert, 1994). Artificial refugia can enhance reptile management plans to aid in population growth through reductions in predation and increase in fecundity (Grillet *et al.*, 2010; Hodges and Seabrook, 2016). Reptiles use the refugia as a resource, making refugia an optimal tool for observational studies. Artificial refugia have also provided valuable insights into inter-and intraspecific interactions, such as competition for refugia space use (Glorioso and Waddle, 2014; Hodges and Seabrook, 2016). In amphibians, competition and material type influence trap use, and therefore species presence in monitoring studies (Glorioso and Waddle, 2014). Life history stages may also affect refugia use (Hodges and Seabrook, 2016). The Common European viper, *Vipera berus*, uses felt refugia in preference to tin as juveniles but prefers tin to felt as adults (Hodges and Seabrook, 2016). It is suggested that this is due to the application of felts closer to the ground given the nature of the material (Hodges and Seabrook, 2016). Thus, the individual using them would get warmth from direct contact with the felt. Due to juveniles smaller size, they need to regulate their temperature differently than adults (Hodges and Seabrook, 2016). Therefore, it makes sense for juveniles to be more drawn to a material that would make them warmer faster. Thus, for monitoring to provide a valuable estimate of population size, the preferences of different life-history stages need to be considered when designing monitoring protocols, using a variety of refugia materials to maximize survey success. This could be particularly pertinent following a reintroduction, to determine the success of the project (Glorioso and Waddle, 2014; Grillet *et al.*, 2010; Hodges and Seabrook, 2016).

Artificial refugia, therefore, provides a valuable tool for reptile population monitoring but often the methodology is not fine-tuned enough to use it to its fullest potential. Reptile surveys may involve one type of refugia being distributed at a site (Brown *et al.*, 2011) for a limited amount of time, and surveyed infrequently (Edgar *et al.*, 2010). In the United Kingdom, artificial refugia are most commonly used by ecological consultants as part of mitigation planning due to building developments (GOV.UK 2015). This use of refugia has marginalized the opportunity for reptilian population estimates. As a remote survey technology, similar to camera traps which are focused towards warm-blooded animals, artificial refugia provides a valuable opportunity to be used to maximize conservation of this at-risk taxonomic group. In particular, there is an opportunity to improve upon the monitoring of the reintroduction conservation method by using artificial refugia. By inspecting the biotic interactions in the area around the refugia, for example, other animals using the refugia; we can improve the placement and choice of material for the refugia. This will allow optimal selection based on the positive and negative interaction with the other populations (Glorioso and Waddle, 2014).

This study investigated the interactions of reptilian species found in Eelmoor Marsh in the United Kingdom with the use of artificial refugia. These species include Adders (*Vipera berus*), Common Lizards (*Zootoca vivipara*), Grass Snakes (*Natrix natrix*), Slow Worms (*Anguis fragilis*), and reintroduced Sand Lizards (*Lacerta agilis*). According to the Amphibian and Reptile Conservation Trust (ARC 2020), all of the species listed were native to Eelmoor; sand lizards were extinct in the area until they were reintroduced by Marwell Wildlife in 2016 (Gardner 2020). This study analyzed the post-reintroduction of sand lizards using artificial refugia. Due to the lack of knowledge on life histories of sand lizards and other reptile species on site (IUCN, 2009; Edgar *et al.*, 2010; Meiri and Chapple, 2016), this study, took a community ecology approach and focused on measuring the use of the artificial refugia. Adders are known predators of the other four species, thus could present negative interspecific interactions along with other lizard species competing for the same food source (ARC 2020). In different life stages, adult lizards have been known to eat juveniles, and large adults and subadults could compete for room on the refugia (Edgar *et al.*, 2010; Hodges and Seabrook, 2016). This research aimed to investigate post-monitoring artificial refugia protocols for conservation reintroductions of reptilian species, specifically by:

A) Examining the presence and absence of species found on artificial refugia to gain insight on interspecific interactions.
B) Investigating reptile life stages' refugia material preference.

2. **Methodology:**

2.1 Study Area: Eelmoor Marsh Site of Specific Scientific Interaction (SSSI)

Eelmoor Marsh SSSI, North Hampshire, UK, is owned by Qinetiq and therefore not open to the general public, making it an ideal site for the release of sand lizards (QinteiQ 2020). The study area was a total 40,000m^2, with a mown strip within the area that was 2400m^2 (Figure B1). The area was oriented in line with the southeast aspect of Pyestock Hill (51.275N, 0.802W). The mown strip was the release site for the reintroduced sand lizards by Marwell Wildlife. They were released in September 2017, 2018, and 2019. All refugia use data was collected by Rachel Gardner during her PhD study from 2016 to 2019, which included pre-reintroduction surveys in 2016-2017 and post-reintroduction surveys following releases in September 2017 and 2018.

Figure B1: Eelmoor Marsh Dry Heath survey area. The original, 200 x200m, and extended limits. The extent of the grid was limited by dense vegetation. The lower mown strip in the center of the grid, ~2 m, is where the sand lizards were released. (Satellite imagery georeferenced and annotated from Google™ Earth image in ArcMap).

2.2 Artificial Refugia:

Gardner (2020) used four different types of materials, felt, slate, tile, and tin. Materials were placed in an array of four in a compass direction, north, south, east and west, every 20m in the area outside the lower mown strip, release site (Figure B2). Within the release site, the arrays were placed every 10m, given the interest in sand lizards dispersal abilities, to maximize the chances of observing the lizards. Sand lizards can disperse anywhere between 10m to 100m from the release site (Edgar *et al.*, 2010). Therefore, a more intense grid was deployed, placing an array every 10m rather than 20m, this covered 2400m^2 of the 40,000m^2 area.

Material size was 500 x 250mm and arrays were placed randomly, given a 20m distance. The arrays were spread throughout a grid covering 40,000m^2. Within the larger area, some arrays were not able to be placed every 20m due to inaccessible dense vegetation or the grid fell out of the Dry Heath range and completely off Eelmoor Marsh SSSI.

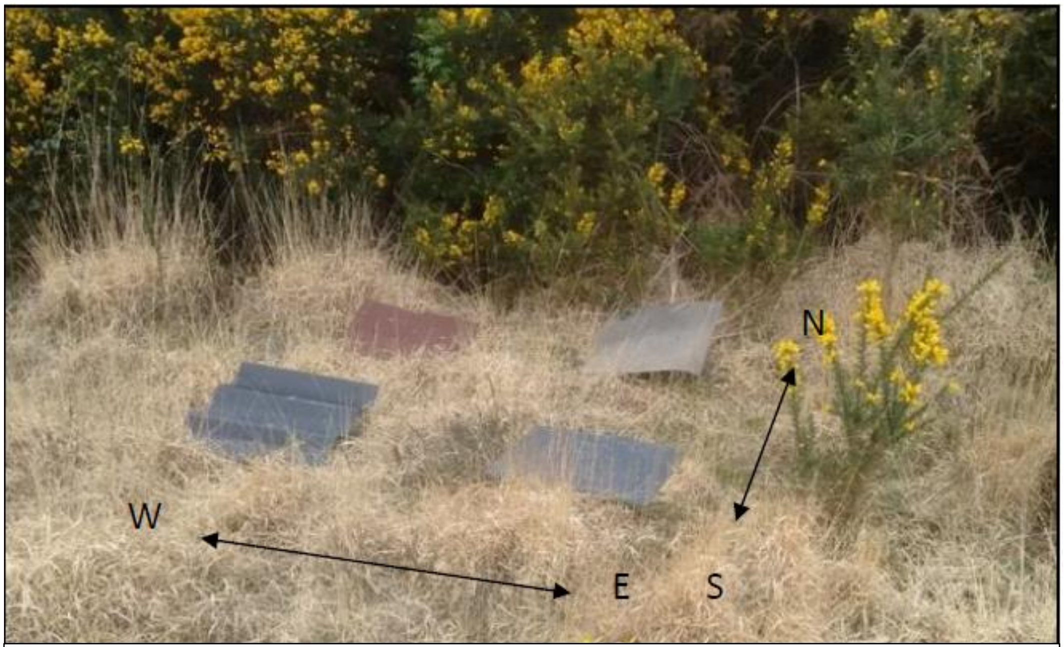

Figure B2: Above is an example of a refugia array showing clockwise from top left: tile, felt, slate and tin, oriented along north-south and east-west bearings.

2.3 Reptile survey data used in the study:

Post-release surveys were conducted at the dry heather site between September 19, 2017, and November 17, 2017; March 22, 2018, to November 11, 2018; February 26, 2019, to August 29, 2019. Surveys ran three times a week after post-release in September to the end of the season in November in both 2017 and 2018. In the months of mid-March to early September, surveys were conducted one or two times a week. There was a reduction in the number of surveys in the summer of 2018 due to a heatwave (Gardner 2020), making observations of reptiles on refugia decrease dramatically and surveys unproductive. Surveys totalled at 117, amounting to 614 hours and 50 minutes surveying time.

Within the data collected, life stages were identified based on size: adults, subadults, juveniles, adult/subadults, subadult/adult, juvenile/subadult, yearlings, hatchlings or juvenile (tiny). Since this was a strictly observational study only, Gardner had to use her judgment of size. For example, if the individual was larger or had the marking of an adult, she listed adult. If the individual was mostly medium in size, it fell under subadult. For purposes of this project, the life stages were narrowed down into three different categories based on size. Adults were large (A, SA/A), subadults were medium in size (SA, Juv/SA, A/SA), and the juveniles were small (Yearling, Hatchling, Juv, Juv (tiny); (Hodges and Seabrook, 2016, Gardner, 2020).

2.4 Statistical Analyses:

A) Examining the presence and absence of species found on artificial refugia to gain insight on interspecific interactions.

Due to having two independent categorical variables, species count of two different species, a Chi-square model was chosen to analyze the differences between observed versus expected frequencies in the count data for presence and absence of species found at artificial refugia (Zappalorti and Reihert, 1994). A Fisher Exact test was implemented, opposed to a Chi-square test, if any of the expected values were below five. On each survey, individual sightings were documented. So, if only one individual was seen on refugia, this resulted in one sighting for the location on that survey. There was only one occurrence of six individuals being sighted at the same location at the same time. All five species were compared to each other. Therefore, a 2 x 2 Chi-Square table was used to compare the presence and absence of paired species. There were 10 possible pair-wise combinations of these comparisons. Analyses were carried out using R.

B) Investigating reptile life stages' refugia material preference.

Chi-Square analysis was performed to examine the independent categorical variables, count of life stage present as type of refugia. Chi-square analysis will show the differences between the observed and expected frequencies of life stage preference to material type; if any of the expected values were below five, a Fisher Exact was used to test life stage against material type (Hodges and Seabrook, 2016). Each species was examined separately from each other. Once the species were filtered down, they were categorized by life stage (adults, subadult, and juvenile) and material type (felt, slate, tile, and tin). This produced a 3 x 4 table to which the Chi-square analyses or a Fisher Exact test was performed.

If the Chi-square or Fisher Exact produced a significant result (α p= 0.05), the life stage proportion on each material type was tested using a Kruskal-Wallis test (Dytham, 1999). Within Excel, the count of life stage present at refugia was transformed to percentages to find the proportion of life stage at refugia. This ran through R programming using the Kruskal-Wallis test. Kruskal-Wallis test was used over an ANOVA because of there are no assumptions of the homogeneity of the variance nor are the variables normally distributed (Dytham, 1999).

3. Results:

A) Examining the presence and absence of species found on artificial refugia to gain insight on interspecific interactions

Table 1 presents the results of all 10 presence and absence combinations of the five reptile species found at the artificial refugia. Fisher Exact and Chi-square analyses were used. Each test is represented in a mosaic plot.

Table 1: The interspecific analyses effects. Fisher's Exact test when expected values were lower than five; and Chi-square test with the result for X^2. The positive, negative, or neutral column represents the type of interaction. * represents results alpha p-value of 0.05.

Species interactions	**Comparison Test**	**Degrees of Freedom**	**P-value**	**Positive, Negative, or Neutral**
Adder and Common lizard	Fisher's Exact	1.00	0.0003*	Negative
Adder and Grass snake	Fisher's exact	1.00	1.00	Neutral
Adder and Sand lizard	Fisher's exact	1.00	1.00	Neutral
Adder and Slow worm	Fisher's exact	1.00	0.1368	Negative
Common lizard and Grass snake	Fisher's exact	1.00	1.00	Neutral
Common lizard and Sand lizard	Fisher's exact	1.00	0.0005*	Positive
Common lizard and Slow worm	Chi-square $X^2 = 21.43$	1.00	3.679e-06*	Neutral
Grass snake and Sand lizard	Fisher's exact	1.00	0.0876	Neutral
Grass snake and Slow worm	Fisher's exact	1.00	0.6382	Neutral
Sand lizard and Slow worm	Fisher's exact	1.00	5.25e-05*	Neutral

<u>Species interaction 1 - Adders and Common lizards</u>: There was a significant difference between the observed and expected frequencies that adders were found together or apart (Table 1). The interaction between the species is shown in a mosaic plot of the residuals (figure B3). In figure B3 the blue colour indicates a significant difference in the observed frequencies being higher than the expected. In other words, the species were found to be together more frequently than expected, indicating a negative interaction between these two species.

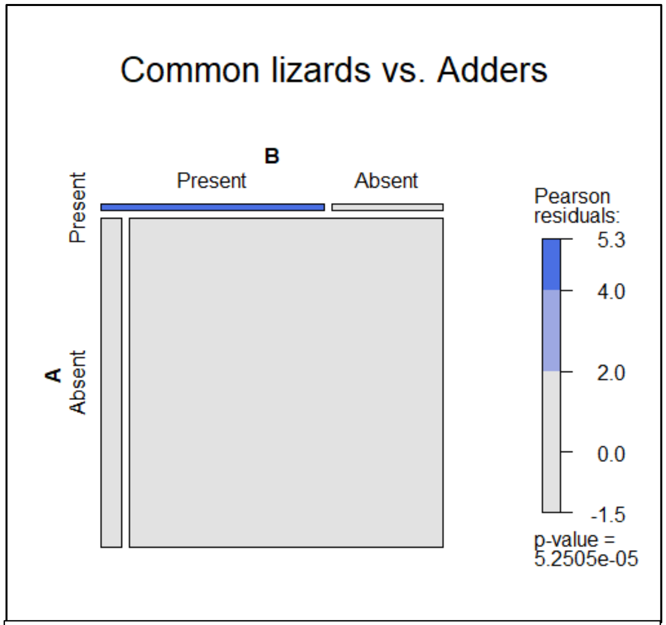

Figure B3: Mosaic plots of observed and expected frequencies of presence and absence analyses of common lizards (A) and adders (B).

Mosaic plots: A mosaic plot is a representation of the residuals. When the residuals are high, the colouration is blue, which mean the observed frequencies are significantly higher than the expected. When the residuals are low, the colouration is red, which means the observed frequencies are significantly lower than expected. The size of the boxes represents the number of observed individuals.

<u>Species interaction 2 - Adders and Grass snakes</u>: There was not a significant difference between observed and expected frequencies of when adders and grass snakes were found together (Table 1). Figure B4 shows that these species were never found together and were almost always absent from the same area. The low sample size makes it hard to determine whether interspecific effects are neutral or negative.

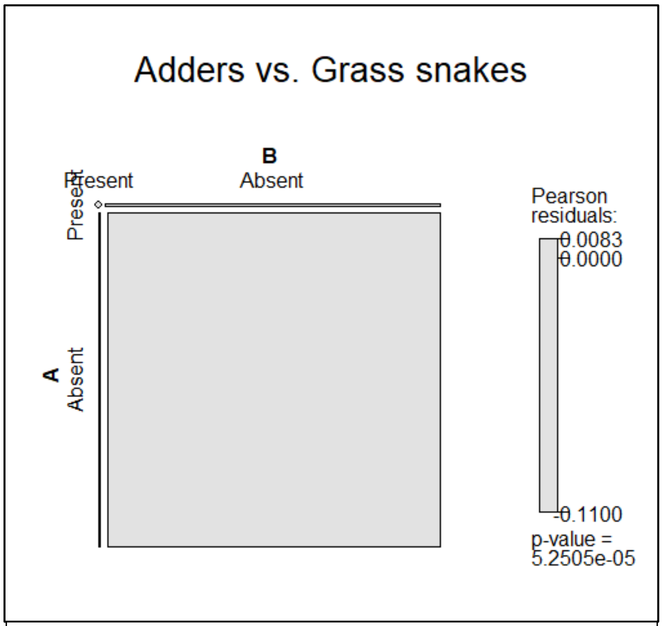

Figure B4: Mosaic plots of observed and expected frequencies of presence and absence analyses of adders (A) and grass snakes (B).

Species interaction 3 - Adders and Sand lizards: There was not a significant difference between observed and expected frequencies of when adders and sand lizards were found together (Table 1). Figure B5 shows that these species were never found together and were almost always absent from the same area. The low sample size makes it hard to determine whether interspecific effects are neutral or negative.

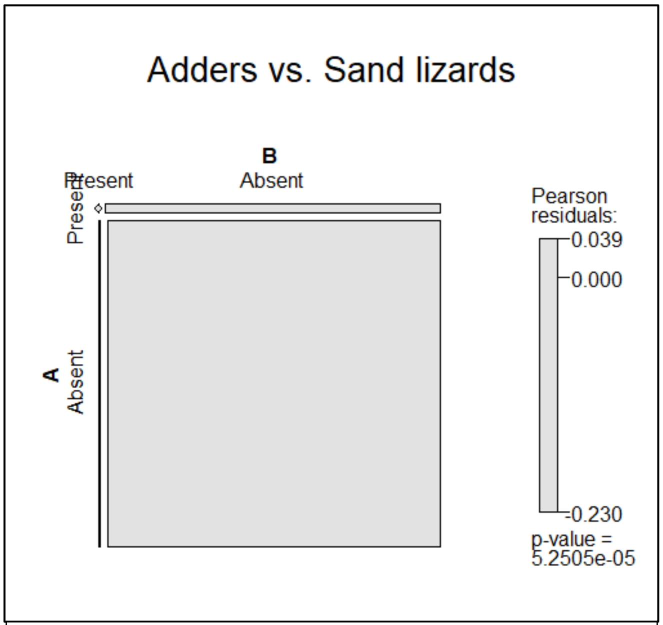

Figure B5: Mosaic plots of the observed and expected frequencies of presence and absence analyses of adders (A) and sand lizards (B).

Species interaction 4 - Adders and Slow worms: There was not a significant difference between observed and expected frequencies of when adders and slow worms were found together (Table 1). Figure B6 shows that these species were found together and slow worms were mostly found in the absence of adders. The low sample size makes it hard to determine whether interspecific effects are neutral or negative.

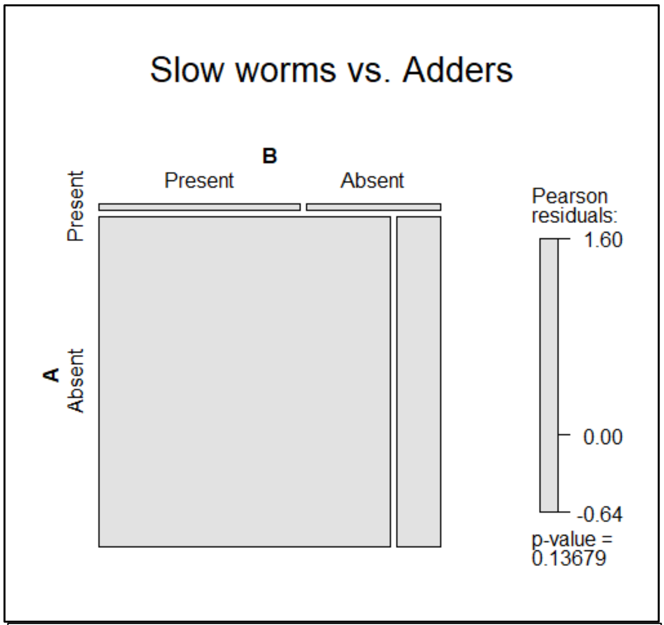

Figure B6: Mosaic plots of the observed and expected frequencies of presence and absence analyses of slow worm (A) and adders (B).

<u>Species interaction 5 – Common lizards and Grass snakes</u>: There was not a significant difference between observed and expected frequencies of when common lizards and grass snakes were found together (Table 1). Figure B7 shows that these species were never found together and mostly absent from the same location. The low sample size makes it hard to determine whether interspecific effects are neutral or negative.

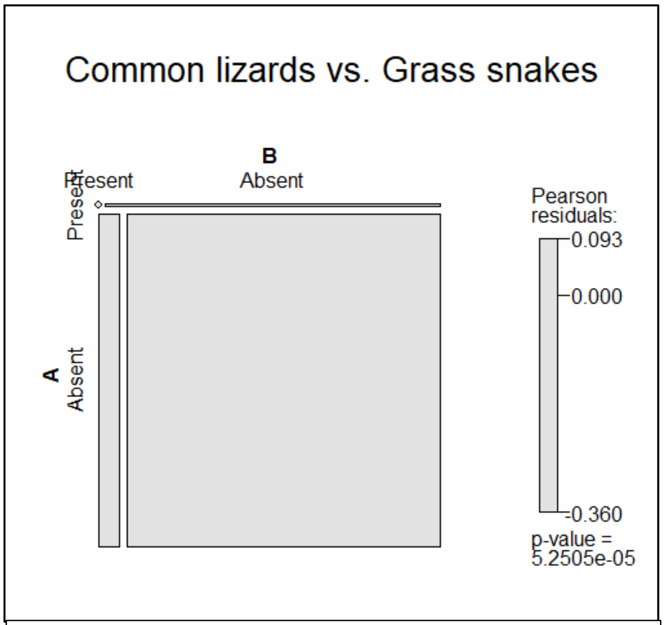

Figure B7: Mosaic plots of observed and expected frequencies of presence and absence analyses of common lizards (A) and grass snakes (B).

Species interaction 6 – Common lizards and Sand lizards: This interaction resulted in a significant difference between observed and expected frequencies of when both species were absent or present under different refugia (table 1). Figure B8 shows both species being present together is higher than expected. This alludes to a positive interspecific interaction between these two species.

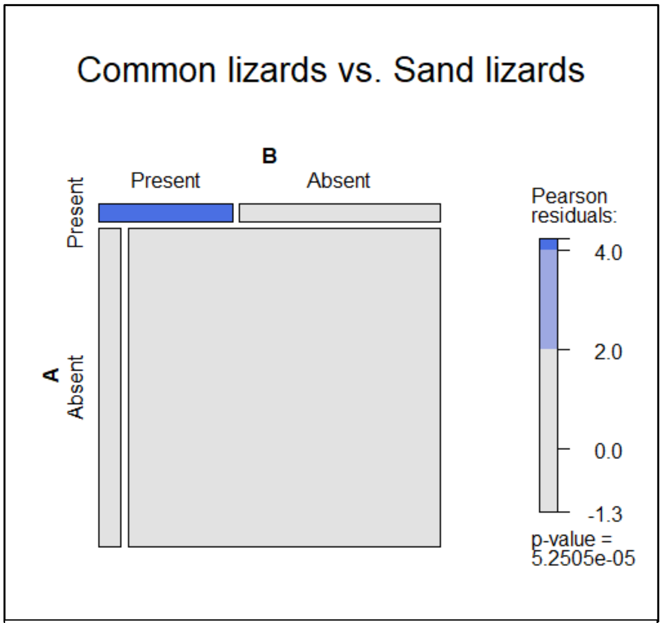

Figure B8: Mosaic plots od the observed and expected frequencies of presence and absence analyses of common lizards (A) and sand lizards (B).

Species interaction 7 - Common lizards and Slow worms: There was a significant difference between observed and expected frequencies of the presence and absence of slow worms and common lizards (Table 1). Figure B9 indicates that slow worms and common lizards were observed together frequently, the observed frequencies coincided with the expected. It was observed less than expected for both species to be absent from the same location. Slow worms were observed without common lizards more than expected. This indicates that it is odd to find slow worms in the absence of common lizards, signalling a neutral interspecies interaction

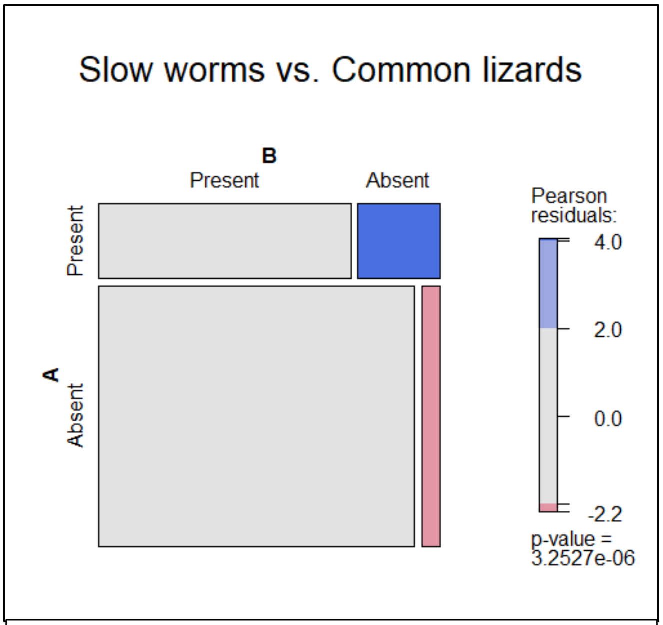

Figure B9: Mosaic plots of observed and expected frequencies of presence and absence analyses of slow worm (A) and common lizards (B).

Species interaction 8 - Grass snakes and Sand lizards: There was no significant difference between observed and expected frequencies of grass snakes and sand lizard's presence and absence (Table 1). These two species were most often not seen together and were only present at the same time once in a three-year study (Figure B10).

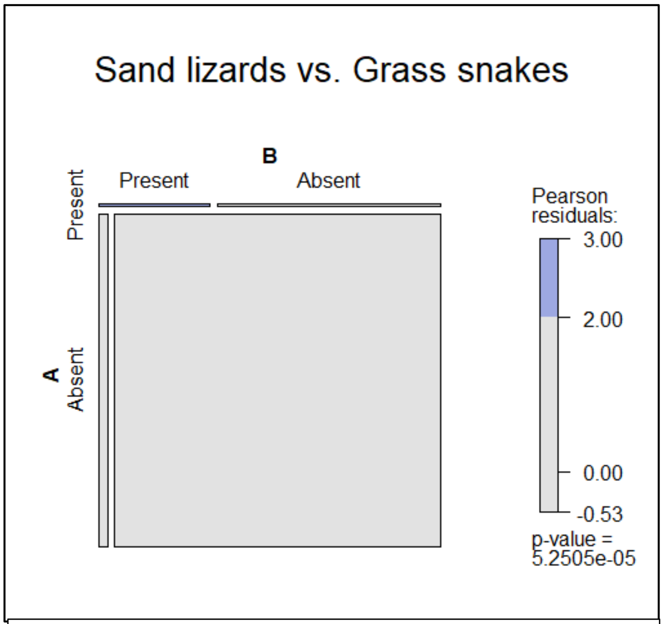

Figure B10: Mosaic plots of observed and expected frequencies of presence and absence analyses of sand lizards (A) and grass snakes (B).

Species interaction 9 - Grass snakes and Slow worms: There was no significant difference between observed and expected frequencies of grass snakes and slow worms' presence and absence (Table 1). Slow worms and grass snakes were at times found together; however slow worms were mostly found without grass snake (Figure B10). The low sample size makes it hard to determine whether interspecific effects are neutral or negative.

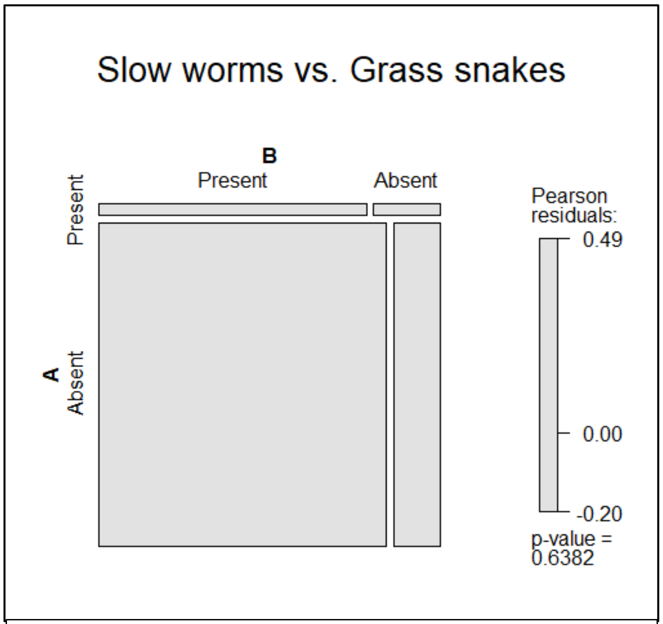

Figure B11: Mosaic plots of observed and expected frequencies of presence and absence analyses of slow worm(A) and grass snakes (B).

Species interaction 10 - Sand lizards and Slow worms: There was a significant difference between observed and expected frequencies of the presence and absence of sand lizards and slow worms (Table 1). Figure B12 indicates that most often sand lizards were seen in the absence of slow worms, observed frequencies concur with the expected. Slow worms and sand lizards both being present at the same time, that was as expected. However, it was higher than expected for slow worms to be observed without sand lizards. This alludes to a neutral interspecific interaction.

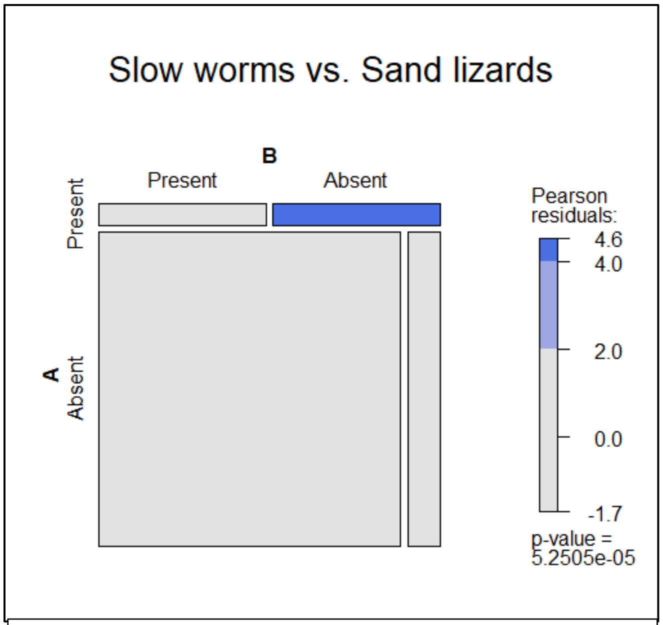

Figure B12: Mosaic plots of observed and expected frequencies of presence and absence analyses of slow worm(A) and sand lizards (B).

B) Investigating reptile life stages' refugia material preference:

Comparison of refugia preference by different species at different life-history stages is shown in Table 2. The sample sizes for each species life stage were relatively low due to the natural challenges of observing such cryptic species in the field.

There was a significant difference between observed and expected frequencies of different refugia material type by different life-history stages of two of the five species investigated in this study.

Table 2: Statical analyses results for each species life stages. * represents results alpha p-value of 0.05 and therefore a significant effect.

Species	**Comparison test used**	**Degree of Freedom**	**P-value**
Adder	Fisher exact	6	0.415
Common lizard	Chi-square, $X^2 = 4.87$	6	0.560
Grass snake	Fisher exact	6	0.287
Sand lizard	Fisher exact	6	0.011*
Slow worm	Chi-square, $X^2 = 75.75$	6	2.695e-14*

Refugia preference by adder life stages:

Statistical analysis found no significant differences between the observed and expected frequencies of the adder life stages and material type, see Table 2. The differences can be visualized in Figure B13.

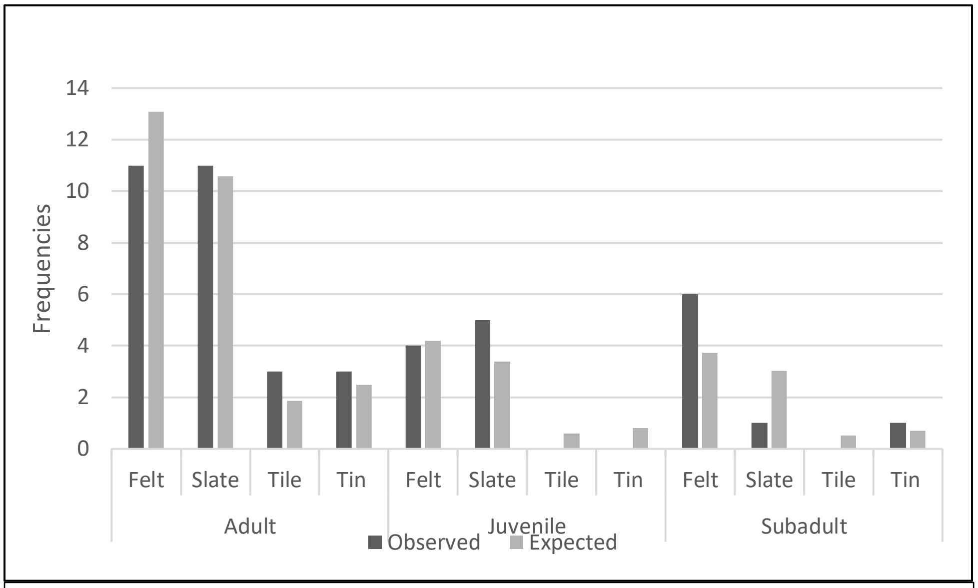

Figure B13: Relationship between adder age groups and use of refugia material type. Representing the differences between the observed and expected frequencies.

Refugia preference by common lizard life stages:

Statistical analysis found no significant differences between the observed and expected frequencies of the common lizard life stages and material type, see Table 2. The differences can be visualized in Figure B14.

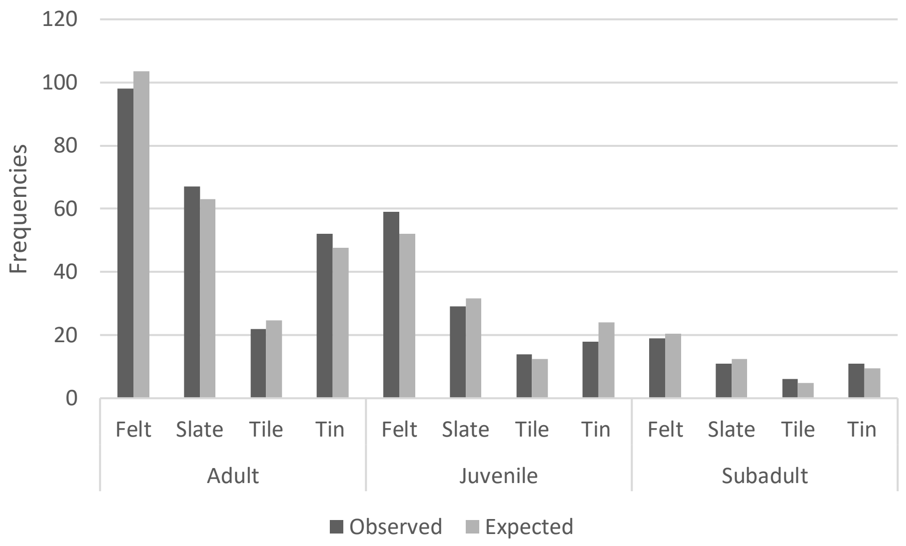

Figure B14: Relationship between common lizard age groups and use of refugia material type. Representing the differences between the observed and expected frequencies.

Refugia preference by grass snake life stages:

Statistical analysis found no significant differences between the observed and expected frequencies of the grass snake life stages and material type, see Table 2. The grass snake life stage material preferences are depicted in Figure B15.

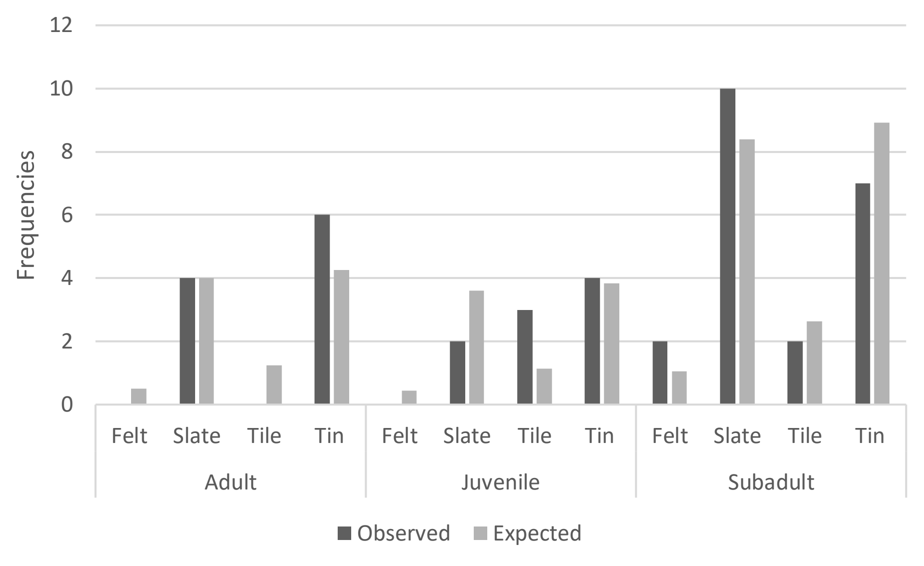

Figure B15: Relationship between grass snake age groups and use of refugia material type. Representing the differences between the observed and expected frequencies.

Refugia preference by sand lizard life stages:

Statistical analysis found significant differences between the observed and expected frequencies of the sand lizards life stages and material type, see Table 2. The sand lizard life stage material preferences are depicted in Figure B16.

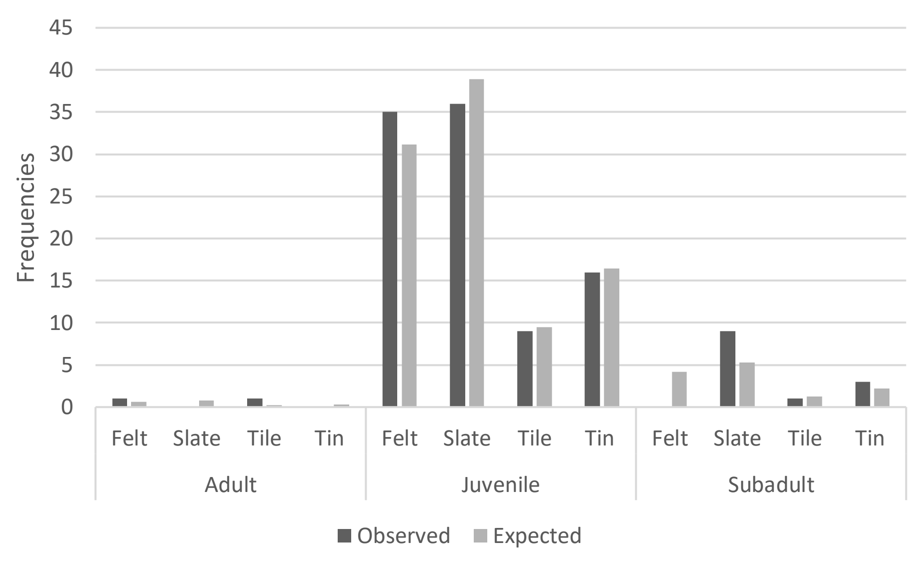

Figure B16: Relationship between sand lizard age groups and use of refugia material type. Representing the differences between the observed and expected frequencies.

Due to the Fisher Exact test coming back with significant results, a Kruskal-Wallis test was performed on the proportion of life stage at material; however, no significance was found (Kruskal-Wallis X2= 7.33, df = 8, P = 0.501) between use of each material type by the life history stages. In Figure B17, the high and low residuals are depicted. Here we can see that subadults were observed less than expected on felt (red colour) and juveniles were the most abundant life stage, table presenting the percentage of life stage found on material can be found in Appendix B. This abundance was due to the release of sand lizard juveniles during this study. Due to the skewness of the life stage data towards juveniles, it is difficult to determine intraspecific interaction occurrence as well as preference.

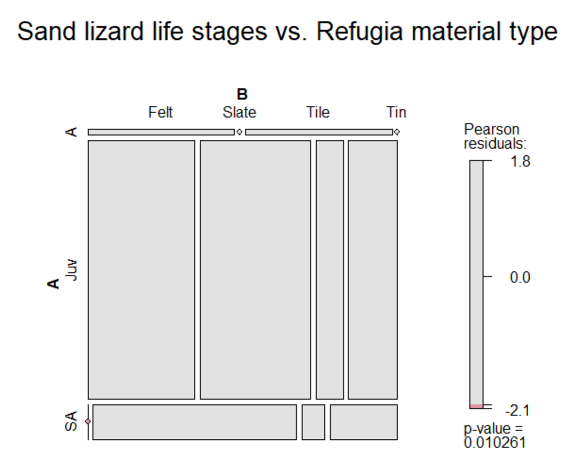

Figure B17: Mosaic plot illustrating the low and high residuals of the observed and expected frequencies of sand lizard life stage on material type. Life stage (A), material type (B)

Refugia preference by slow worm's life stages:

Statistical analysis found significant differences between the observed and expected frequencies of the slow worm life stages and material type, see Table 2. The slow worm life stage material preferences are depicted in Figure B18.

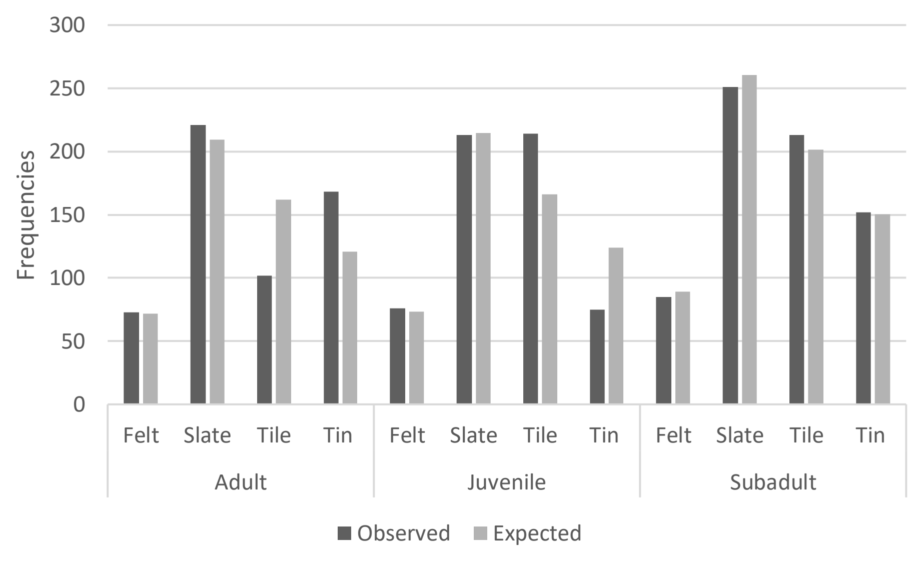

Figure B18: Relationship between slow worm age groups and use of refugia material type. Representing the differences between the observed and expected frequencies.

Due to the Chi-square analysis resulting in significant differences, a Kruskal-Wallis test was performed on the proportion of life stage at material; however, no effect was found (Kruskal-Wallis X^2= 11, df = 11, P = 0.443). In Figure B19, the high and low residuals are depicted. Here adults were observed less than expected on tile (red colour) and more than expected on tin (blue colour). Juveniles were observed more than expected on tile and less than expected on tin. This alludes to life stage preference of material or negative intraspecific interactions.

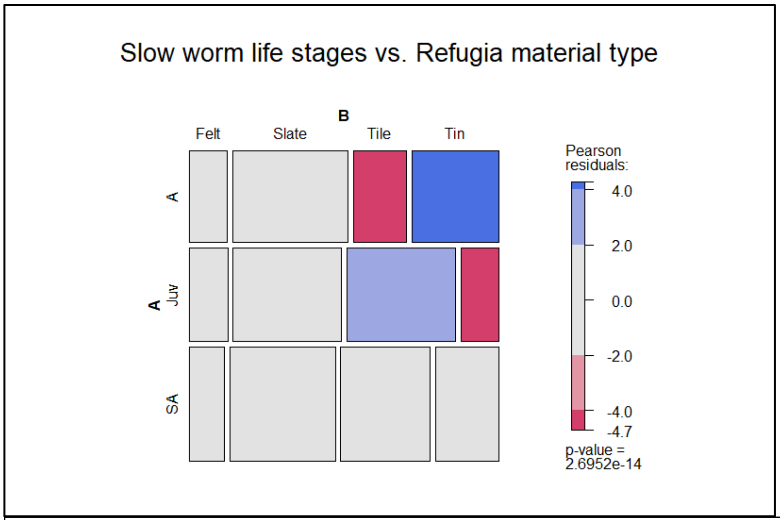

Figure B19: Mosaic plot illustrating the low and high residuals of the observed and expected frequencies of slow worm life stage on material type. Life stage (A), material type (B)

4. **Discussion**:

This study aimed to improve post-reintroduction monitoring by fine-tuning use of artificial refugia and incorporating evidence of intra-interspecific interaction between species. Highlighting the importance of community ecology within the reintroduction process.

4.1 Aims A and B:

A) Examining the presence and absence of species found on artificial refugia to gain insight on interspecific interactions:

Adders and common lizards were observed apart more often than together. This was expected because adders prey on other snakes and lizards, as well as small mammals and birds (Edgar *et al.*, 2010, ARC). However, some adders were observed with common lizards, which could have been due to material preferences and environmental conditions at the time of those observations. Since reptiles are cold-blooded they need to regulate their body temperature; the temperature of the day, humidity, or canopy cover could influence which material is used based on the conducting ability of that material (Owen, 1989; Brown *et al.*, 2011; Hodges and Seabrook, 2016). However, seeing these two species together could be an observation of adders hunting common lizards, as common lizards have been observed in the adder diet (Edgar *et al.*, 2010). The absence of common lizard in the presence of adders may suggest avoidance of areas with higher adder abundance. Possible avoidance was also observed in the adder-slow worm comparison and the grass snake-slow worm comparison. This alludes to a negative interspecific interaction through probable predation between the adders and the other lizard species.

The comparison of common lizards and sand lizard distribution revealed another interspecific interaction as they were observed together more often than expected. Overlapping ecological niches between common lizards and sand lizards may have caused competition avoidance and limited observation together at one site. However, the positive interspecific interaction of coexistence observed at Eelmoor indicates species redundancy at this site (Kokkoris *et al.*, 1999; Laughlin *et al.*, 2012; Tilman, 2004). The site itself is low in canopy cover, so plenty of sun exposure for basking and is fairly homogenous when it comes to vegetation density, temperature and humidity. Preliminary analyses were performed on environmental factors around refugia, these results are located in Appendix A. Because reptiles are quite selective when it comes to habitat, this habitat structure seems to be optimal for these two species (Biaggini and Corti, 2015; Rotem *et al.*, 2016). Information about habitat features of release sites should therefore be considered as part of a reintroduction programme.

The comparison of common lizards and slow worm's had similar findings to the sand lizard/slow worm comparison. Common lizards are expected to be seen without slow worms as their ecological niches do not overlap, common lizards are terrestrial and slow worms are fossorial, therefore they use the habitat differently. However, observed frequencies were higher than expected for both slow worms and common lizards to be absent. This pattern was the same for sand lizards and slow worms, yet this observation could be due to small sample size. The observed pattern between sand lizards and slow worms could imply that they can share habitat without too much conflict, again suggesting that they do use habitat differently, which coincides with their life histories, sand lizards are terrestrial and slow worms are fossorial. Clear neutral interspecific interactions are occurring at artificial refugia sites with these species. Therefore, it appears the sand lizards are not having a negative effect on the surrounding reptile communities.

B) Investigating reptile life stages' refugia material preference:

Different preferences for refugia type were statistically proven in sand lizards and slow worms. However, it should be noted that from observed counts only, life stage count differed between all five species on use of material. For example, in adders, juveniles were observed most on the slate, common lizards' juveniles were found most on felt, grass snake juveniles seen most on tin, sand lizard juveniles were observed most on slate, while slow worm juveniles were found most on tile (see Tables 8 – 17 in Appendix B). This indicates that species differ in preference of materials and also preferences change as the species age. Life stage population percentages showed that adults were the most pronounced life stage in adder and common lizards' populations; subadults were most abundant in grass snakes and slow worms' populations, and juveniles were the highest found life stage for sand lizards' population (see Tables 8-17 in Appendix B).

In slow worms, juveniles are found on tile significantly more than adults and adults were found on tin significantly more than juveniles. Slow worms juveniles may be using tile over tin due to preference, tile is lighter than tin (Gardner 2020), therefore juveniles would be able to lift refugia easier to get on top for basking (Hodges and Seabrook, 2016). Tile could be a faster conductor of heat, so juveniles might be prioritizing tile over other materials. Juveniles could also be partitioning from adults that frequenting used the tin. The partitioning could be due to material size not allowing room for both life stages, as adults and subadults are bigger than juveniles. Juveniles could be avoiding adults, out of fear of being prey upon, as adults lizards have been known to prey upon juveniles (Edgar *et al.*, 2010; Ljungström *et al.*, 2016; Olsson and Shine, 1997). However, the fact that adults are seen where juveniles are not and vice-versa may support the hypothesis that intraspecific competition influences material use. This negative intraspecific interaction could be because different life stages use habitat differently. Adults search for nesting sites or mates, while juveniles mostly concern themselves with prey.

4.2 Pre- and post- reintroduction improvements:

With the use of artificial refugia, we can monitor an area before a reintroduction to help find what other reptile species and age groups are already present and provide insight on species richness, diversity, and ecological stability of the area under consideration (Boulangeat *et al.*, 2012; Loreau, 2001; Mason *et al.*, 2005). In particular artificial refugia can provide an understanding of intra-interspecific interactions that might affect population growth of the species in the area, thus providing data useful for examining extinction risks (Böhm *et al.*, 2013; Meiri and Chapple, 2016; Tingley *et al.*, 2016). The ability to identify species of ecological equivalence that share functional traits helps determine species redundancy in the area, and therefore ecosystem stability (Mouchet *et al.*, 2010; Roche and Campagne, 2017), providing a clear understanding of where intervention is needed to stabilize the extinction risk and also the ecosystem.

Artificial refugia can enhance reptile surveys and especially post-release monitoring in reptile reintroductions (Glorioso and Waddle, 2014; Grillet *et al.*, 2010; Hodges and Seabrook, 2016), yielding vital information on population dynamics (Hodges and Seabrook, 2016). Refining survey techniques, by testing and selecting refugia material, can improve detection rates and allow for analysis of species distribution by life stages. The use of artificial refugia will yield more in-depth insight into the community assemblies in the study area. By considering the existing community ecology, researchers can see how the newly reintroduced species is interacting with the species around them and thus ascertain if the reintroduced species is having a negative or a positive effect on the community. Understanding how the community assemblages are effected, conservationists have a better chance to ascertain the stability of the ecosystem these species live in.

4.3 Future Studies:

This project found that artificial refugia can be used to study life stages of reptiles and also to investigate interactions between reptile species. Artificial refugia can be used in future studies to investigate the population dynamics of cryptic species and thus provide data for extinction rate models. To enhance observations, this study started identifying life stage preference to material type, however further studies are needed to statistically diagnose preference by incorporating thermo ecology (Hodges and Seabrook, 2016). If artificial refugia are continued to be used, then future studies need to evaluate the long term effects that man-made refugia has on the environment (Glorioso and Waddle, 2014; Hodges and Seabrook, 2016).

Behavioural studies following this project could further investigate intra-interspecific interactions, such as hunting behaviour, which would confirm negative effects of one species on another. In understanding the interactions between species, reintroductions can be enhanced. In choosing a location with low negative interspecific interaction, release sites can be better selected for optimal reproduction success. Therefore, it is encouraged for action plans to include investigations on the community structure of species pre-and post-reintroductions. Learning about community structure can provide clues about the functional diversity within the area and therefore the stability of the ecosystem (Gitay *et al.*, 1996; Naeem, 1998; Nash *et al.*, 2016; Petchey *et al.*, 2007). By incorporating community ecology in reintroduction programmes, better analysis of how a reintroduction will affect the stability of the ecosystem can be made (Vellend, 2010), therefore enhancing the diagnosis of a successful reintroduction.

Conclusion:

Species reintroduction programmes are a great conservation method to combat the increase in extinction rates, however, this method is still in need of improvement. This project took a novel approach in using artificial refugia survey data to diagnose refugia material preference by life stage x species and investigating intra-interspecific interaction amongst species found. It is strongly recommended that multiple types of refugia should be employed when conducting a herpetofauna survey as different species and life stages prefer different materials. This study provides population dynamics data to be used in the future by providing observation counts of individuals ages of each species. Artificial refugia have shown to provide insight on the community ecology of species residing in the environment by finding evidence of inter-intraspecific interactions. In learning which species compete and which species can coexist, a more thorough investigation on the functional diversity and species redundancy can be made within the environment. Reintroduction programmes can use the information on community structure to enhance diagnosis of reintroduction success. By incorporate effects on community structure, reintroduction success can be measured in ecosystem stability along with species richness and population dynamics. As reintroductions grow in success, extinction rates will start to reverse and biodiversity can increase.

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Appendices

Appendix A: Preliminary analysis of Environmental Factors

Methodology:

Environmental factors were categorized as: hourly relative temperature, hourly relative humidity, vertical cover, ground cover and canopy cover. All of these factors were calculated from the raw data provided by Gardner (2020). Hourly relative temperature and hourly relative humidity were chosen over relative humidity and air temperature due to the lack of consistency in data (Brown et al., 2011; Carvajal-Cogollo and Urbina-Cardona, 2015; Rutten et al., 2015). Both relative humidity and temperature were calculated by a Kestrel 3000. The vertical cover was calculated by first using the Dumbenmire Cover Chart from the north, east, south, and west measurements that Garnder (2020) collected in both summer and spring months, therefore taking the average from the appropriate time of the year at which the individual was sighted. The summer months included the months of June to October, the spring months included the months of November to May, December and January were left out due to the hibernation cycles of these species (Edgar et al., 2010). Gardner (2020) had also collected ground cover readings of both times of the year. To obtain particular measures, bare ground was subtracted from the total ground cover. The canopy cover was calculated from averaging the canopy cover reading from the north, east, south, and west directions and multiplying it by 1.04 (Forestry Suppliers), this calculation came with the raw data provided (Gardner, 2020, Lemon, 1956).

Statical Analysis:

A Poisson generalized linear model (GLM) within R programming was used to study the environmental factors around the artificial refugia where species were found (Brown et al., 2011; Rutten et al., 2015). Each species was compared separately, therefore five GLM's were run. For each species, the relative temperature, relative humidity, canopy cover, vertical density, and ground cover were chosen as the explanatory variable and were compared to life stages present at refugia as the response variable. Due to the fact that this data was count data, a poisson GLM was used. A negative binomial and a quasi-poisson were also run to check for overdispersion on the data.

Results:

A generalized linear poisson model was used to compare individual sighting at refugia. No significant effects of environmental factors on species sightings, most likely due to sampling size. The findings are represented in Table 3-7.

Table 3: Poisson GLM results for adder life stages against all environmental factors

	Adder			
Coefficients	Estimate	Std. Err.	z value	P
Intercept	14.183205	7.642707	1.856	0.0635
Rel Temp ˚C	-0.004323	0.028734	-0.15	0.8804
Rel Hum %	-0.001324	0.008642	-0.153	0.8782
Canopy Cover %	-0.113237	0.21575	-0.525	0.5997
Vertical Cover %	0.004368	0.005829	0.749	0.4536
Ground Cover %	-0.140593	0.074988	-1.875	0.0608
AIC	162.01			

Table 4: Poisson GLM results for common lizard life stages against all environmental factors

	Common Lizard			
Coefficients	Estimate	Std. Err.	z value	P
Intercept	1.3091445	0.8415828	1.556	0.12
Rel Temp ˚C	0.0128869	0.0102892	1.252	0.21
Rel Hum %	-0.0007462	0.0026377	-0.283	0.777
Canopy Cover %	-0.0065472	0.006514	-1.005	0.315
Vertical Cover %	-0.0003719	0.0019039	-0.195	0.845
Ground Cover %	-0.010476	0.0019039	-1.303	0.193
AIC	1138.4			

Table 5: Poisson GLM results for grass snake life stages against all environmental factors

	Grass snakes			
Coefficients	Estimate	Std. Err.	z value	P
Intercept	-1.820976	4.0466291	-0.45	0.653
Rel Temp ˚C	0.0264654	0.0279033	0.948	0.343
Rel Hum %	0.0043385	0.0106153	0.409	0.683
Canopy Cover %	-0.006779	0.0124421	-0.545	0.586
Vertical Cover %	0.0005805	0.005661	0.103	0.918
Ground Cover %	0.0193592	0.0386991	0.5	0.617
AIC	141.38			

Table 6: Poisson GLM results for sand lizard life stages against all environmental factors

	Sand Lizard			
Coefficients	Estimate	Std. Err.	z value	P
Intercept	0.8965362	0.5822041	1.54	0.124
Rel Temp ˚C	-0.0042257	0.0172467	-0.245	0.806
Rel Hum %	-0.0002316	0.0040831	-0.057	0.955
Canopy Cover %	0.0043358	0.0157144	0.276	0.783
Vertical Cover %	0.001045	0.0039583	0.264	0.792
Ground Cover %	-0.001437	0.0055026	-0.261	0.794
AIC	354.49			

Table 7: Poisson GLM results for slow worms life stages against all environmental factors

	Slow worms			
Coefficients	Estimate	Std. Err.	z value	P
Intercept	8.15E-01	2.60E-01	3.138	0.0017
Rel Temp ˚C	3.67E-03	4.39E-03	0.835	0.4038
Rel Hum %	-1.16E-03	1.14E-03	-1.022	0.3068
Canopy Cover %	-2.40E-04	1.51E-03	-0.159	0.8736
Vertical Cover %	2.78E-05	8.26E-04	0.034	0.9731
Ground Cover %	-7.60E-04	2.41E-03	-0.316	0.752
AIC	5414.4			

Appendix B: Tables from Investigating reptile life stages' refugia material preference:

All following tables were created from the artificial refugia survey count data.

Adder population tables -

Table 8: Adder life stage percentage found on material type.

Life Stage	Felt	Slate	Tile	Tin
Adult	39.29 %	39.29 %	10.71%	10.71 %
Juvenile	44.44 %	55.56 %	0.00 %	0.00 %
Subadult	75. 00 %	12.50 %	0.00 %	12.50 %

Table 9: Per cent of life stage found in total for adders.

Life Stage	Total per cent found
Adult	62 %
Juvenile	20 %
Subadult	18 %

Common Lizard population tables -

Table 10: Common lizards life stage percentage found on material type.

Life Stage	Felt	Slate	Tile	Tin
Adult	41.00 %	28.03 %	9.21 %	21.76 %
Juvenile	49.17 %	24.17 %	11.67 %	15.00 %
Subadult	40.43 %	23.40 %	12.77 %	23.40 %

Table 11: Per cent of life stage found in total for common lizards.

Life Stage	Total per cent found
Adult	59 %
Juvenile	30 %
Subadult	12 %

Grass snake population tables -

Table 12: Grass snake life stage percentage found on material type.

Life stages	Felt	Slate	Tile	Tin
Adult	0.00 %	40.00 %	0.00 %	60.00 %
Juvenile	0.00 %	22.22 %	33.33 %	44.44 %
Subadult	9.52 %	47.62 %	9.52 %	33.33 %

Table 13: Per cent of life stage found in total for grass snakes.

Life Stage	Total per cent found
Adult	25 %
Juvenile	23 %
Subadult	53 %

Sand lizard population tables -

Table 14: Sand lizard life stage percentage found on material type

Life Stage	Felt	Slate	Tile	Tin
Adult	50.00 %	0.00 %	50.0 %	0.00 %
Juvenile	36.46 %	37.50 %	9.38 %	16.67 %
Subadult	0.00 %	69.23 %	7.69 %	23.08 %

Table 15: Per cent of life stage found in total for sand lizards.

Life Stage	Total per cent found
Adult	2 %
Juvenile	86 %
Subadult	12 %

Slow worm population tables -

Table 16: Slow worm life stage percentage found on material type

Life Stage	Felt	Slate	Tile	Tin
Adult	12.94 %	39.18 %	18.09 %	29.79 %
Juvenile	13.15 %	36.85 %	37.02 %	12.98 %
Subadult	12.13 %	35.81 %	30.39 %	21.68 %

Table 17: Per cent of life stage found in total for slow worms.

Life Stage	Total per cent found
Adult	31 %
Juvenile	31 %
Subadult	38 %