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Habitat Selection of a Non-Native Snake: Implications for Future Management of *Zamenis longissimus* in Colwyn Bay, North Wales

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Abstract

Invasion biology is the study of non-native species so to better understand the impacts that they have on their new environment. Non-native species have the potential to become invasive, causing severe negative ecological and economic impacts. Snakes in particular have shown themselves to have high invasive potential due to their life-history traits, and behaviours: high fecundity, secretive lifestyles, and generalist diets. To effectively control invasive species and mitigate their impact requires the implementation of wildlife management schemes with sound ecological understanding of the focal species is required. One such aspect of ecology which applies to invasion biology is habitat selection. Habitat selection underlies the survival and population distribution of a species. If an individual is to choose their habitat incorrectly they may find themselves without the necessary resources for survival. Snakes, as ectotherms, have intrinsic ties to their environment as they are thermally constrained, requiring specific temperature thresholds for physiological functions. Often, snakes select their habitat based upon its thermal quality, yet, prey availability has also been shown in some snake species to affect population distribution. The Aesculapian snake (*Z. longissimus*) is a large colubrid snake found throughout Europe, yet a population of *Z. longissimus* exists in the Welsh Mountain Zoo (WMZ), North Wales, at a much higher northern latitude than is found in its native European range. We investigated the WMZ *Z. longissimus* population to establish key habitat features, including prey availability (small mammals), to be able to predict population densities of the snake in the future. We used novel marking methods in capture-mark-recapture studies to assess the snake and small mammal population and calculated macro-habitat characteristics coverage across the grounds of the WMZ. We calculated snake encounter rates across the grounds of the WMZ from search effort and snake captures. By use of multiple regression analysis we identified significant relationships between specific habitat characteristics to generate a multiple regression equation predicting snake encounter rates. In contrast to other snake habitat selection studies, we found that the best predicting habitat feature for *Z. longissimus* is prey abundance. This can be explained by the fact that the climate in the WMZ is continuously cooler than the climate in the snake native range; therefore, as the snake cannot reach optimal temperatures as seen in its native range, the snakes select their habitat based on other important habitat characteristics. Alternatively, it may be that the snakes are reliant on other methods of thermoregulation i.e. use of anthropogenic sites, to overcome the cooler climate. Future studies should implement radio-telemetry to further clarify *Z. longissimus* habitat selection at a micro-scale, allowing greater insight into the impact that the non-native snake has in North Wales.

Introduction

Invasion biology is the combined application of ecology and conservation biology to monitor and better understand the impacts of non-native organisms; that is, organisms who have established themselves outside of their native range (Blackburn *et al*., 2011). Though there has been some critique of invasion biology (Richardson and Ricciardi, 2013), the increasing number of biological invasions (Bellard *et* al., 2017) in conjunction with the fact that non-native species have the potential to become invasive, causing severe negative ecological impacts (Luque *et al.,* 2014) i.e. outcompeting native species in their new habitat (Willson, 2017), warrants its existence. Long term ecological studies are highly valuable, offering insights into species relationships and biodiversity change (Lindenmayer et al., 2012). Likewise, long term ecological studies observing non-native species would also be of huge benefit (Simberloff, 2003) for several reasons; the first being that the transition from non-native species to invasive species often occurs after a lag time, wherein the non-native species remains localised for an extended period of time before the population expands dramatically and causes irreversible damage (Crookes *et al.*, 1999). And secondly, the way in which an organism acts in its new environment may be very different to how it behaves in its native range (Fisher *et al.*, 2019). Therefore, historical ecological data for the non-native species in its native range cannot always be applied to the introduced species in its new environment. In recent years, the impact of non-native and invasive species has been debated (Vitule *et* al., 2012), with some suggesting that their presence boosts biodiversity (Sagoff, 2005) and aids conservation (Schlaepfer *et al*., 2011; Schlaepfer, 2018). However, invasive species are generally regarded by ecologists as one of the major driving forces of biodiversity loss and the extinction of native species globally (Simberloff, 2005; Bellard *et al*., 2016). Since the publication of "100 of the World's Worst Invasive Alien Species" (Lowe *et al*., 2000), invasion biology has gained some attention (Richardson *et al.*, 2011); invasion biologists have expanded on previous invasive species lists (Hirsch *et al*., 2016; Nentwig *et al*., 2018), and published literature on invasive species of the same taxa (Kumschick *et al.*, 2016). For example, Weber (2017) produced a compendium of 500 invasive plant species, discussing their impacts i.e. native species displacement, alteration of soil composition and vegetation structure, and severe agricultural disruption. Similarly, Downs and Hart (2020), compiled and discussed the impact of several invasive bird species.

Snakes in particular have shown themselves to have high invasive potential, and more recently the awareness of invasive herpetofauna and their impacts has increased (Kraus, 2018). Pitt *et al*. (2005) describe successful invasive reptiles to have high fecundity, a generalist diet, and cryptic behaviours. In addition to this, Bomford *et al*. (2008) found that the best predictor of invasive reptile establishment was the similarity between the species' native climate, and the climate of their new environment. Typically, invasive species that have a greater impact on their new environment receive greater attention from invasion biologists (Guerin *et al.*, 2017). This is certainly the case in the context of herpetofaunal invasive species. Though there are other examples of invasive snakes (Cabrera-Pérez*et al*., 2012, Montes *et al.*, 2020, Worthington-Hill *et al*., 2012),

two species of snake are most well- represented in the literature: the brown tree snake (*Boiga irregularis*) on the isle of Guam, and the Burmese python (*Python bivittatus*), in the Everglades. Both of these snakes have had huge economic, and environmental impacts in their new environments. Burmese pythons have been directly linked to the decline of mammal species in the Everglades (McLeery *et al*., 2015; Sovie *et al*., 2016) as well as introducing novel parasites into the ecosystem (Miller *et al*., 2020); whilst the brown tree snake has single-handedly driven native bird species to extinction, and generated huge economic losses and health concerns (Engeman *et al*., 2018). While preventative action to stop herpetofaunal invasions would be the ideal (Kraus *et al*., 2018) – indeed invasion biologists have made attempts to predict the invasive potential of non-native species (Goodwin *et al.*, 1999; Howeth *et al.* 2016), and are now suggesting measures to prevent further introductions (Kraus *et al*., 2015) – this has proven a challenging task due to the fact that invaders are largely introduced via human pathways (Hulme *et al.*, 2008; Hulme, 2009), such as cargo-hitchhikers (Kraus *et al*., 2018), and in more recent years, as a result of the exotic pet trade (Stringham and Lockwood, 2018). Invasion biology, with respect to herpetofauna, has significant knowledge gaps. Kraus (2015), author of the most comprehensive overview on herpetofaunal invasions to date, evidenced this, finding that only 31 of the 780 herpetofaunal invasions reported had substantial data explaining the invasive impact of that species. This, in addition to a lack of snake ecology data in general (Böhm *et al.*, 2013; Mullin and Seigel, 2011), again highlights the need for long-term, comprehensive studies into the ecology of invasive species. Unfortunately, snakes are cryptic by nature and often occur in low densities (Boback *et al.*,2020), so collecting such data may prove to be time consuming and require high effort or rely on expensive technology, such as radio-telemetry devices (Weatherhead and Blouin-Demers, 2004).

It is evident, given the breadth of invasive species and their impacts, that effective management schemes are essential to the conservation and protection of native species from non-native introductions (Simberloff, 2010). One key element of wildlife management is habitat selection (Morris, 2003). Habitat selection, or habitat ecology, underlies population distribution and population expansion, and is fundamental to the survival of an individual or species. If an individual selects inadequate habitat without the necessary resources for survival they will die (Sibly and Hone, 2002). Many habitat selection studies have been carried out, largely with consideration to the conservation of the focal species, so to advise wildlife management schemes (MacDonald *et al*., 2018; Robson *et al*., 2018; Romano *et al*., 2017). Ectotherms in particular, are significantly restricted by their habitat due to their reliance on specific temperature thresholds (Huey, 1982) for physiological functions (Stevenson *et al*., 1985). As an example, Pringle *et al*. (2003) demonstrated the restrictive nature of certain habitat features on the broad-headed snake (*Hoplocephalus bungaroides*) which affected thermoregulatory opportunities. The intrinsic relationship between habitat selection patterns by ectothermic species and thermoregulation has been evidenced many times (George *et al*. 2015; Halliday and Blouin-Demers 2016; Howze *et al*., 2019; Kapfer *et al*. 2010; Row and Blouin-Demer 2006; Sperry and Weatherhead 2009). However, more recently, ecologists have investigated alternative habitat features as drivers for habitat selection patterns in ectothermic species, such as preyabundance. Prey abundance has been

shown to influence spatial patterns of many different species (Apolloni *et al.*, 2018; Filla *et al.*, 2017; Waggitt *et al*., 2018), and has also been observed in certain snake species (Baxley and Qualls, 2009; King and Duvall, 1990; Glaudas and Rodriguez-Robles, 2011; Himes *et al.*, 2006; Wittenberg, 2012), potentially highlighting an under-studied area of habitat ecology.

Whilst the detrimental impact of invasive species is clear, which species have invasive potential is not, and further research is needed to identify and prevent non-native species from reaching the status of invasive. One such example of a non-native species with invasive potential is the Aesculapian snake (*Zamenis longissimus*) in Colwyn Bay, North Wales. *Z. longissimus* are large colubrid snakes found across mainland Europe. The snakes grow to 1.8 metres at the northern limits of their native range, but exceed this in southern populations, reaching 2.25 metres (Edgar and Bird, 2006). They are low-energy, and exhibit cryptic and secretive behaviour (Lelièvre *et al.*, 2010a). *Z. longissimus* is semi-arboreal, and has a generalist diet consisting of small rodents, birds, reptiles (Capula and Luiselli, 2002), and bats (Théou, 2016). As of 2020, there are three isolated populations of *Z.* longissimus in the UK: the Colwyn Bay population (Edgar and Bird, 2006), the Regents Park population, and, most recently discovered, the Bridgend population (Clemens and Allaine, 2020). The *Z. longissimus* population in North Wales has existed in the grounds of the Welsh Mountain Zoo (WMZ) since the late 1960s, where a single gravid female is thought to have escaped captivity and given rise to the now established population. We investigated habitat selection in the Colwyn Bay *Z.longissimus* population, in an attempt to identify habitat characteristics, including prey abundance, which could be used to predict the presence of the snake, and provide a foundation for future management plans, and to assess the species' invasive potential.

Methods

To be able to establish habitat selection patterns for *Z. longissimus* in North Wales, we performed a capture-mark-recapture study in the grounds of the WMZ, Colwyn Bay, to estimate snake encounter rates. We marked adult snakes using PIT tags, and marked juvenile snakes using a novel marking method devised for this study.

Macro-habitat characteristics, such as canopy cover, were recorded across the grounds of the WMZ. In order to assess food availability as a habitat characteristic we performed an additional capture- mark-recapture study on the small mammal population. We performed a multiple regression analysis using robust standard errors to determine which habitat characteristics could best be used to predictthe presence of *Z. longissimus* in the grounds of the WMZ.

Welsh Mountain Zoo Study Site

The WMZ is situated in the hills above Colwyn Bay town covering 37 acres of mixed grassland and deciduous forest (Figure 1.). The zoo houses 140 different species of exotic animals and plants (Welsh Mountain Zoo website – https://www.welshmountainzoo.org/about/about-us). There are man-made structures within the grounds of the zoo offering a range of micro-climates. Due to the high numbers of animals living on site, food availability is higher in the grounds of the zoo than in the surrounding areas in the form of animal feed. Additionally, waste products e.g. faecal matter, used bedding, or expired food, are piled on site; hereafter referred to as the 'manure pile'.
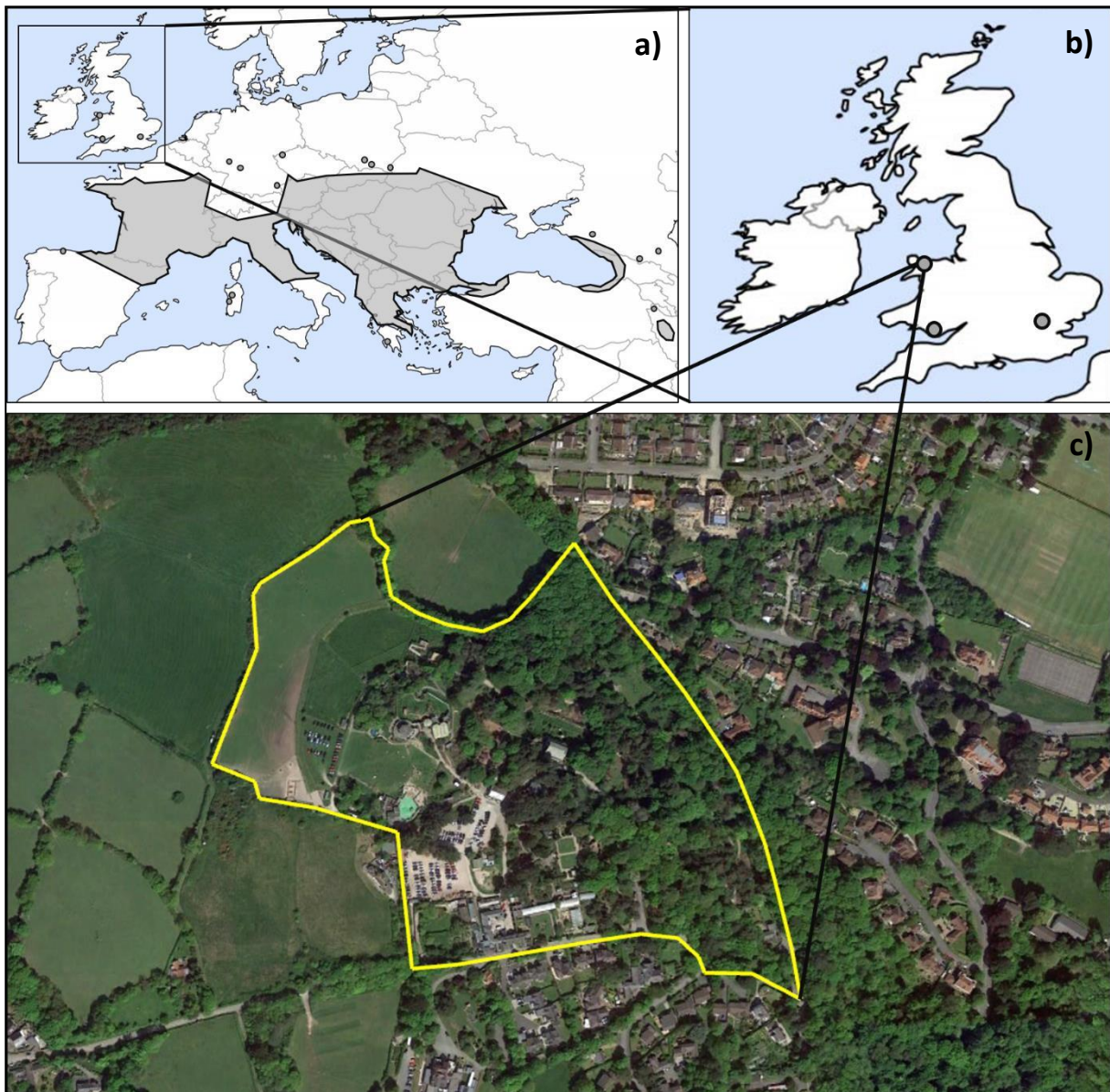

Figure 1. (a) shows the current distribution of *Z. longissimus* across the UK and central Europe (Musilová *et al.*, 2007). Isolated populations are shown by grey dots. (b) shows the three isolated populations in the UK. (c) shows the outline of the Welsh Mountain Zoo, in Colwyn Bay.

The zoo is host to two setts of European badgers (*Meles meles*). Furthermore, from the zoo staff, we received anecdotal reports of stoats (*Mustela erminea*) in the zoo grounds. Ravens (*Corvus corax*), and buzzards (*Buteo buteo*) have also been seen in the zoo grounds. Throughout the 2019 summer there was a considerable amount of groundwork undertaken by the WMZ in the form of aggressive grounds management and landscaping. As such, the environment changed over the course of the study period i.e. reduced canopy cover and turn over, or complete removal, of top-soil.

Snake Mark Recapture

Snake Survey

To establish the encounter rate of *Z. longissimus* in the grounds of the zoo, we performed refugia surveys as part of a capture-mark-recapture study. Roofing felt was used as refugia due to its thermal conductivity (Engelstoft and Ovaska, 2000), exploiting the snakes dependency on the environment to maintain their internal body temperature. The felt was cut into 50 centimetre by 50 centimetre squares (Sewell *et al.*, 2013), and numbered with white paint. A message alerting the public to avoid tampering with the refugia was also painted on the refugia. The zoo grounds and neighbouring areas were surveyed to identify the best refugia locations with regard to *Z. longissimus* habitat suitability; forests, due to prior literature identifying this environment as a key habitat for *Z. longissimus* (Edgar and Bird, 2006), and east-facingbanks and habitat edges, as they receive the most solar radiation, were favoured. The refugia were surveyed daily (Čeirāns and Nikolajeva, 2016) from April until November in 2018 and 2019, to coincide with the snakes' active period (Edgar and Bird, 2006). During each of the surveys, every mat was checked once and reset in the same position. Rest days were taken roughly every two weeks after intense surveying to 'refresh' the refugia, allowing the snakes to use the mats without disturbance to prevent trap shyness . Additionally, in adverse weather conditions, such as heavy rain, surveys were not performed. *Z. longissimus* rely on refugia for thermoregulation. Should the refugia become too hot or too cold its thermal quality reduces and is no longer useful for thermoregulation; this may lead to refugia avoidance by the snakes. To overcome this, the surveys were performed at different times of the day to coincide with optimal refugia temperature, decreasing refugia avoidance and increasing the likelihood of a snake capture. Whilst surveying, the route taken through the zoo was recorded using a GPS watch (Garmin). During the snakes' hibernation period, through late autumn to early spring, perished mats were replaced. In addition to refugia surveys, we performed a thorough search through the onsite manure pile: a large mound consisting of animal feed and waste. We postulated that the manure pile would be a suitable oviposition and hibernation site for the snakes due to its warm climate compared to that of the zoo (Löwenborg *et al.* 2010; Löwenborg *et al.* 2012). In October 2019 we excavated the manure pile to search for *Z. longissimus* individuals, and *Z. longissimus* eggs.

Snake Capture and Marking

Upon snake capture the capture location was recorded using a handheld GPS (Garmin GPSMAP 64s). New snakes were marked and processed onsite on the same day in a vet room in the zoo grounds. The work surfaces were cleaned with anti-bacterial spray and wipes before and after the snakes were processed. During processing the snakes were observed for signs of stress i.e. hissing, lethargy, and striking. Snakes exhibiting these behaviours were placed in a covered box until they had calmed.

Individuals new to the project were classified broadly by life-stage. Juveniles were identified by the appearance of their yellow collar, chequered body pattern (Edgar and Bird, 2006) and small size (<50cm), whilst adults were identified by their olive-green body colour (Major *et al.*, 2020) and large size (>90cm); sub-adults displayed intermediate characteristics. Photographs of the snake's head, body and tail were taken. The snakes were scanned using a photocopier to provide a clear image of their underside (Figure 2.).

Figure 2. (a) showing the underside of a juvenile *Z. longissimus* revealing the chequered pattern on the chin. (b) shows the monocoloured underside of an adult *Z. longissimus*.

As the pattern on the chin of juveniles is unique to each individual, these images were kept for future reference to identify recaptured snakes. This method of identification is only reliable whilst the snake is young, as during development individuals lose their chin pattern (Major *et al.*, 2020).

Snakes were marked using one of two methods: visible implant elastomer (VIE) for juvenile snakes weighing <40 grams, and passive integrated transponder (PIT) tagging (Animal ID 1.4x6mm) for sub-adult and adult snakes weighing >40 grams. PIT tagging could not be used to mark juvenile snakes due to their small size. VIE has been used to mark a huge variety of animals including amphibians (Bainbridge *et al.*, 2015; Nauwelaerts *et al.* 2000; Tapley *et al.*, 2017), and certain reptile species such as skinks (Penney *et al.*, 2001), and turtles (Anderson *et al.*, 2015). VIE, however, has never been used in wild snakes. As such, this study describes the first case of wild snakes being marked using VIE (as published by Major *et al.*, 2020). We developed the use of VIE for this study given the difficulty of marking and reliably identifying small snakes (Winne *et al.*, 2006). VIE is an inert, fluorescent polymer which can be injected between the layers of an animal's skin, and can be used reliably for marking small animals, without any damaging effect. A mark scheme was generated using Salamarker (Figure 3.), creating unique combinations of fluorescent red and yellow marks in different positions along the flanks of the snake, with respect to the snake's ventral scales, which could be used to identify the individual. Two VIE marks were used providing over 250 identities.
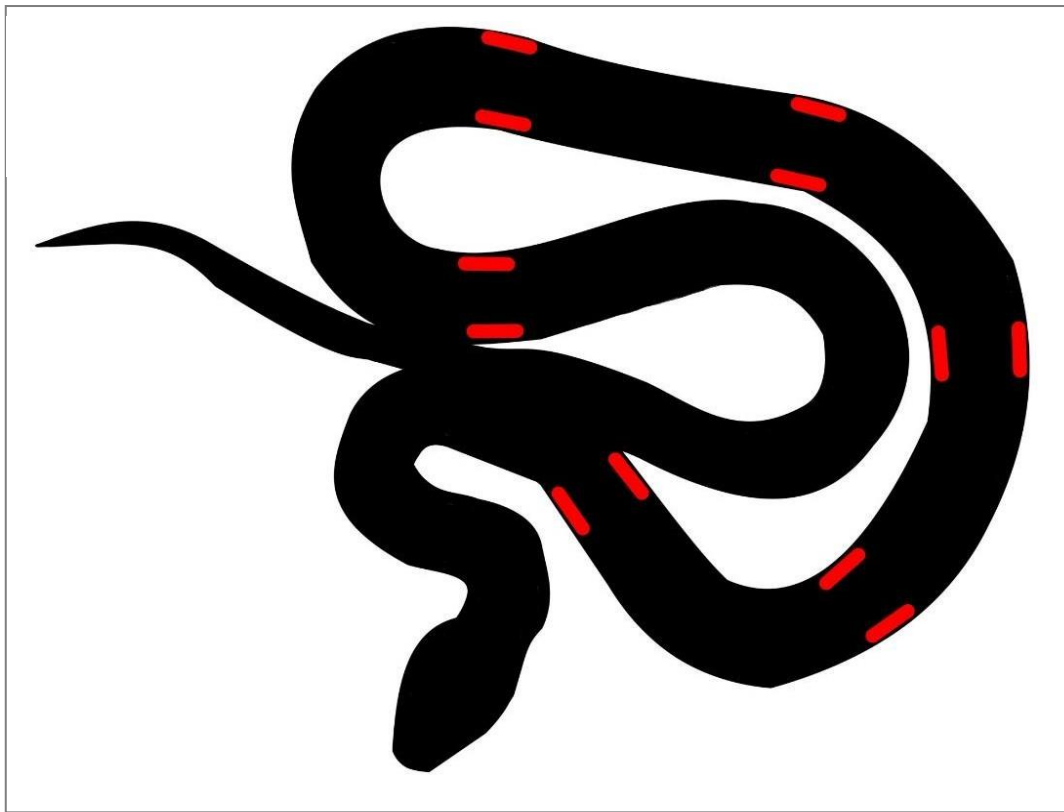
Figure 3. Mark locations generated in Salamarker (Major *et al*., 2020)

Red and yellow polymers were selected as they stood out most against the snakes' scales (Figure 4.). The ventral scales were counted to find the mark location. The polymer was then injected between the scale rows at said location using a 29-gauge needle, directed anteriorly beneath the skin. The needle was slowly withdrawn during the injection to create a stripe of VIE polymer beneath the skin. Once made, the marks were

checked for visibility using a UV-B torch. In some instances, the injection site was sealed using super glue to ensure that the elastomer did not bleed from the injection site. The marking process took 10 minutes, including the time taken to count the ventral scales to find the mark position.
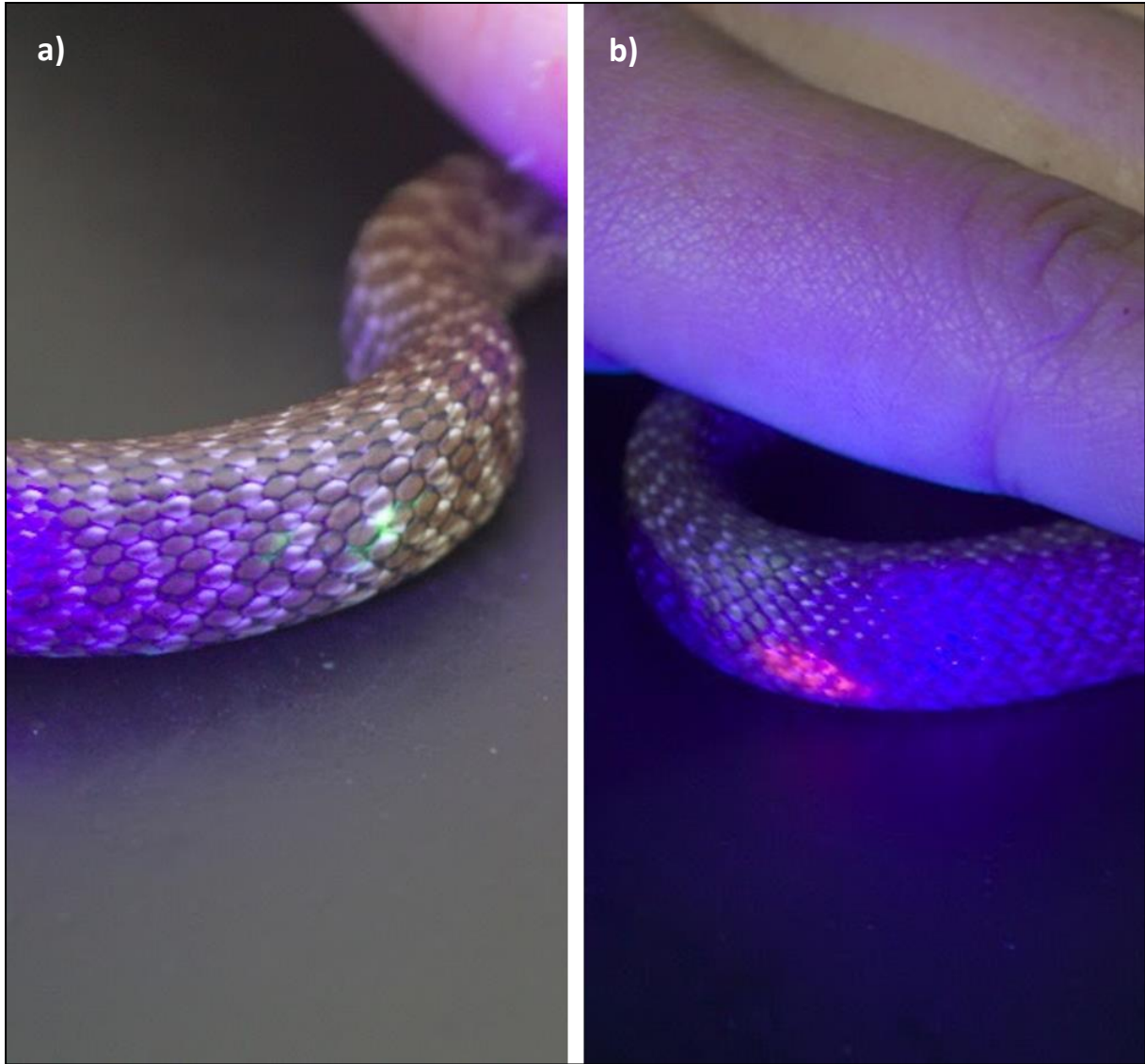

Figure 4. Examples of VIE marking: a) yellow elastomer under mark applied to juvenile *Z. longissimus* under UV light b) shows the red elastomer used for marking under UV light.

PIT tagging was performed in sub-adult and adult snakes due to difficulty detecting VIE marks on large snakes and increased reliability over VIE as an identification method (Elbin and Burger, 1994; Gibbons and Andrews, 2004). Prior to application, the tag was checked for a signal response using a PIT tag reader device. A pre-loaded needle was used to inject the PIT tag into the subcutaneous tissue of the snakes. The needle was directed anteriorly, and the injection made laterally between the scale rows in the final third of the snake but before the vent, to avoid damage to vital organs (Keck, 1994). Upon insertion, the tag was checked for a signal response using a PIT tag reader. Following the application of the PIT tag the entry wound of the needle

was sealed using super glue. After processing, the snakes were checked for signs of injury e.g. blood seeping from the injection site of the VIE and PIT tags, and observed for stress-related behaviours e.g. hissing, striking, lethargy. Individuals expressing such behaviours were held in a covered box to allow the individual to recuperate. PIT-tagging took significantly less time than the VIE marking method (less than one minute) due to the PIT tagging process being far less complex. Processed snakes were transported to their place of capture and released.

Recaptured snakes were identified by their elastomer mark or PIT tag ID and then released. The marks of recaptured individuals were examined for visibility and mark quality i.e. had the mark moved position. The location of the capture site was recorded using the handheld GPS.

Mammal Mark-Recapture

Mammal trapping took place in 2019, in the second year of the study. During the first year of the study, we caught five snakes who had consumed prey items. In two of these instances the snakes regurgitated their prey during processing (the snakes were closely monitored for signs of injury, and allowed to fully recover i.e. no longer showing signs of stress in the form of striking or lethargy, before they were released). The prey items were confirmed to be wood mice (*Apodemus sylvaticus*) by onsite zoo staff, by the characteristic large ears, long tail, and light brown fur. In addition to these observations, the small mammal species existing in and around the grounds of the WMZ were identified through a Cofnod data report to determine which trap types would be required for the study. For example, common shrews (*Sorax araneus*) do not survive for very long when trapped, and so the presence of the common shrew at the WMZ would require a trap which allows for their escape (Longworth trap with shrew hole). The Cofnod report returned several sightings of wood mice within the grounds of the zoo and the surrounding areas, and one sighting of a brown rat (*Rattus norvegicus*) in the Colwyn Bay town. Anecdotal reports from the WMZ staff suggested that there was a small likelihood of trapping a bank vole. From this, it was reasoned that wood mice were the main prey items of *Z. longissimus*. As the mark recapture study therefore primarily focused on small mammals, and there was no evidence of shrews existing within the grounds of the zoo or surrounding areas , standard Longworth Traps (Flowerdew *et al.*, 2004) were used in this study. Additionally, the Longworth trap has space to provide significant amounts of bedding and food, which is important for the survival of trapped animals in temperate climates (Flowerdew *et al.*, 2004)

As a note, during the third year of the snake capture-mark-recapture study (following the completion of this piece of work), researchers found a deceased snake outside of the grounds of the WMZ which had consumed a nest of rat pups, suggesting that they also consume other rodents species.

Pilot Study

A pilot study was conducted in Treborth Botanical Gardens, Bangor, Gwynedd prior to the mammal trapping at the WMZ to assess the effectiveness of the methodology and provide the research team with experience of capturing and handling small mammals.

Trap Setting

To ensure that the zoo was evenly surveyed the trap locations were established prior to the traps being set in the WMZ. To determine the trap locations, a trap map was generated. A birds-eye-view screenshot was taken of the WMZ using Google Earth, and a grid overlaid onto the screenshot (figure 5.). The grid was sized to use as many of the available traps so that they covered the grounds of the zoo uniformly, and so that each zoo square used in the statistical analysis had roughly the same number of traps within it. The trap locations were made at the regular intersections of the lines of the grid, and were set approximately 40 meters apart.. Whilst being set, trap locations were adjusted to avoid inaccessible areas, areas where traps would be susceptible to human interference, and areas with high exposure to the elements. Traps were relocated as close to the original trap location as possible. In this project we used 47 Longworth Traps. Initially, 48 traps were to be used, however, one trap was removed from the project due to logistical problems, and research effort constraints.

All traps were washed thoroughly with Vircon solution before being set in the WMZ to avoid cross contamination of wildlife diseases from Treborth Botanical Gardens. Numbered flags were set alongside the traps to ensure that no traps were missed during set up and collection. Between May and October of 2019, five trapping sessions were undertaken. Each session consisted of three nights of trapping over four days, wherein the traps were baited and set out on the evenings of the first, second, and third days; and collected in on the mornings of days two, three, and four. Between each session, a ten-day rest period was allowed. The traps were baited each evening with two slices of apple, two squares of chocolate, 10 grams of oats, 10 grams of raisins, and a fistful of straw provided for bedding. We added 10 grams of meal worms to the trap in the unlikely event of a shrew being caught.
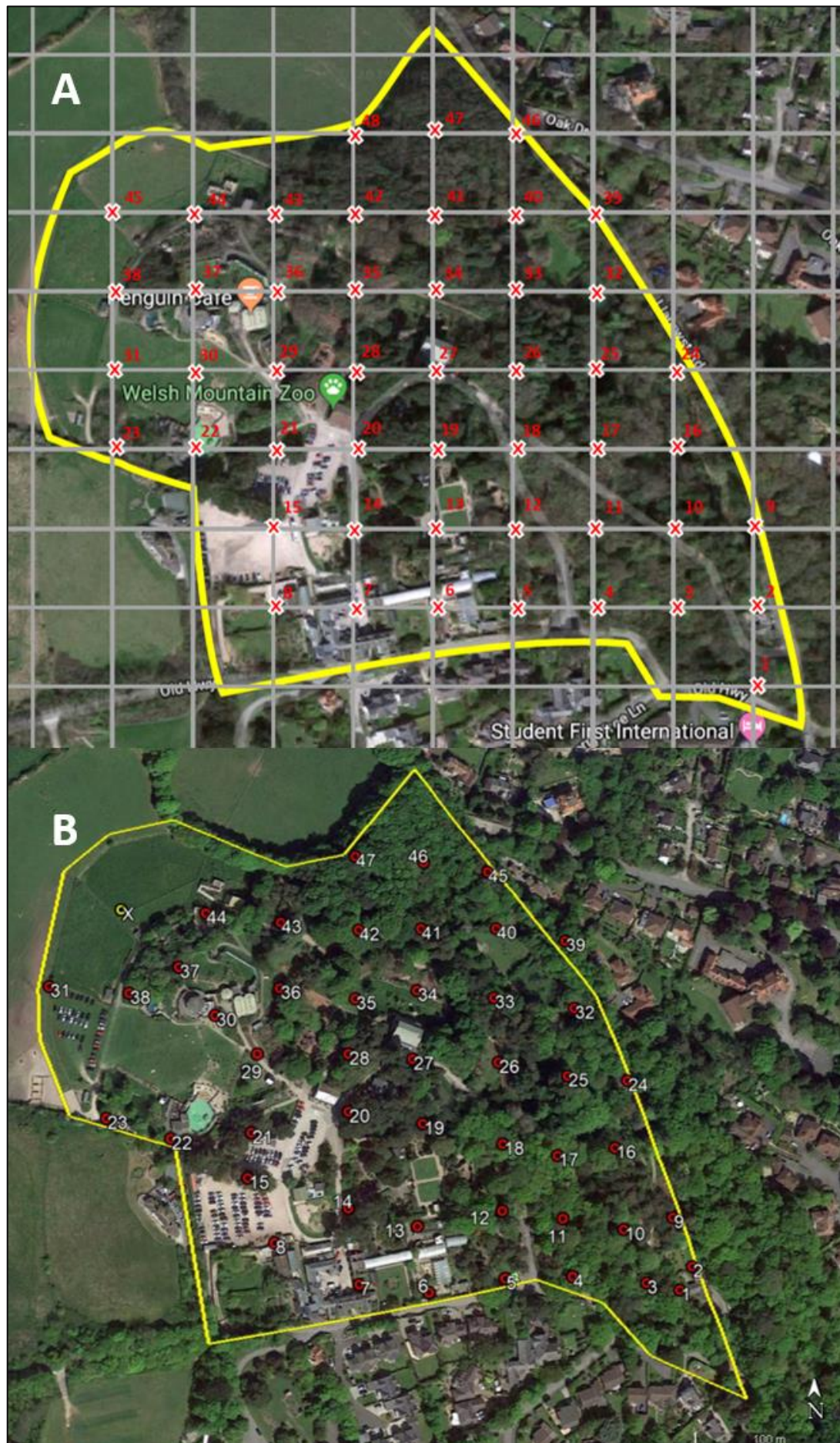
Figure 5. A) Showing the grid overlay used to identify initial trap locations in the WMZ such that the grounds of the zoo are adequately covered, and B) showing the actual locations of the traps in the WMZ. The yellow marker 'X', seen in B), shows the trap which was removed from the study.

Casing Design

We experienced high levels of trap disturbance caused by badger interference in the first trapping session. The traps were often dismantled and the bait consumed, compromising the trap's function (Figure 6.). As such, this trapping event was disregarded, as it did not provide an accurate representation of the small mammal population within the zoo. To overcome this issue for future trapping sessions a novel protective casing was developed.

Figure 6. An image showing a Longworth trap dismantled and its contents emptied, in the foreground, and its pink marker flag showing where the trap was originally placed, in the background.

The trap casing was designed to prevent badgers from dismantling the trap and carrying the trap away.The casing consisted of three components: a single length of PVC piping and two iron stakes. The PVC pipes used in the final trap design measured 66 centimetres long by 15 centimetres across, with a one millimetre edge thickness. Iron stakes were used to fix the traps to the ground through two pairs of holes made 15 centimetres away from either end of the pipe. A series of trap casings were designed using different lengths of piping. The cases were tested in pilot studies. A single trap was baited (the shaft door left open so not to sacrifice a small mammal to the badgers) and placed inside the casing, which was set next to an active badger den. Three traps with casings were setfor one evening, and the interactions between the badgers and the traps recorded using wildlife camera traps. Trap designs (a) and (b) showed that the badgers could reach into the shorter casings and interfere with the trap; as such the length of the casing was increased as seen in design (c) (Figure 7.). In addition to the casings providing protection from badgers, we also believe that the casings mitigate two controlling factors for trap location: direct sunlight and rain fall. Whilst in many studies the traps will be placed under the cover of hedges and bushes, the protective casing would allow the traps to be placed elsewhere in the field, in more exposed locations, increasing the potential study areas for mammal surveys.
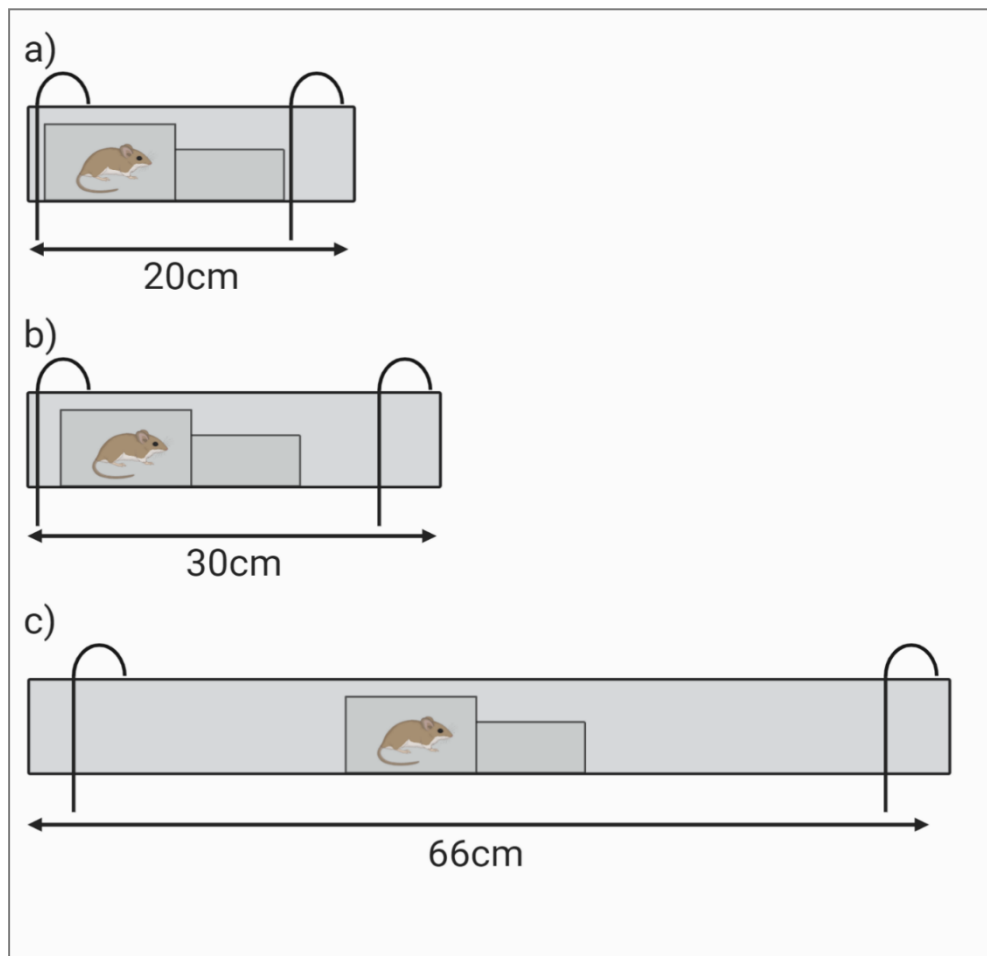

Figure 7. The evolution of the protective casing designs for the Longworth traps. a) and b) show the first and second trap designs respectively. c) shows the final trap design used in the study.

Trapping

The traps were re-set in the same locations with the protective casings between August and October 2019. The casings were slightly angled along slopes to assist with water drainage from the trap. To reduce the likelihood of heat-related deaths, the casings were set in shaded areas. The traps were set in the late evening and collected in the early morning for three consecutive nights every ten days. Due to adverse weather conditions, however, a week was given between the penultimate and final trapping occasions of the fifth trapping session. The traps were collected in the same order as they were set.

Traps that were not triggered were collected in and the time of collection recorded. Triggered traps were placed in a large bucket and opened, providing the research team with a confined space to work with any trapped animals. Trapped mice were caught and processed.

Mammal Processing

During processing, the mice were observed for signs of stress e.g. lethargy, or injury. Should the individual show signs of stress then the animal was given time to rest in a covered container. Every captured small mammal was marked for future identification. All small mammals were marked using ear notches, which were made with a biopsy tool (Bonaparte *et al*., 2013). The notches were made high in the ear to avoid blood vessels near to the head. Due to the high volume of wood mice captures ear notch combinations alone were not feasible for identifying all of the captured mice. As such, we combined ear notching with the use of VIE. This, to our knowledge, is the first instance where VIE has been used to mark small rodents. VIE was injected subcutaneously into the flank of the base of the mouse's tail using a hypodermic needle (Figure 8.). This method prevented damage to the blood vessel supplying the mouse's tail. Red and yellow VIE was mixed to create orange due to low supplies of red VIE. Yellow and orange VIE was used to mark the mice. Each mark was checked for visibility using an ultraviolet torch. Yellow VIE represented "100", and orange represented "200". For example, an individual whose ear notch denoted "10" (a notch on the inside of the left ear, and a central notch in the right ear) and whose tail mark denoted "100" (a yellow dot of elastomer in the base of the tail), would have the identity "110". The animal was released immediately after processing at the location of its capture. The total time spent marking depended on whether the mouse was due to receive one mark (ear notch), or two marks (ear notch and VIE). In both cases the marking method was very quick; the ear notching taking less than one minute, and the ear notching and VIE marking taking less than two minutes. To apply the VIE mark took less than one minute.

Figure 8. Elastomer marks applied to *Apodemus sylvaticus* tails, in addition to ear notching, for identifying small mammals. a) and b) show red and yellow elastomer marks, respectively, underUV light.

Statistical Analysis

In an attempt to explain the environmental features which drive habitat selection patterns in *Z. longissimus*, a multiple regression analysis (MRA) was performed. MRA identifies which habitat features contribute significantly to the population distribution pattern of *Z. longissimus*. MRA also determines the weight of each habitat feature i.e. which habitat feature is the best predictor of *Z. longissimus*.

A birds-eye-view of the zoo and surrounding areas where snake captures were recorded were divided into 70 metre by 70 metre squares using a grid overlay in Google Earth. The square size was selected based upon the

need to have mouse and snake capture data for each square. Equally, we did not want the squares to be so large that habitat selection inferences could not be made. We recorded the different habitat types within the zoo and the surrounding areas (Table 1.) To avoid issues of multicollinearity between independent variables during our analysis (i.e. difficulty separating canopy cover from forest habitat), each habitat characteristic was recorded with or without canopy cover. We considered canopy cover to be an important habitat characteristic for *Z. longissimus* based upon the results of other studies (Kurek *et al*.,2017; Kurek *et al*., 2018). To calculate the percentage cover of the habitat types per square, the squares were divided into 100 component squares, each representing one percent of the total coverage. The best represented habitat type was recorded for each component square to generate a complete picture of the percentage cover of each habitat type.

Unfortunately, due to the small reptile and mammal datasets, the preferred option of obtaining population density estimates could not be used. Initially, we performed mark recapture studies to provide population density data which could be analysed using the Spatially Explicit Capture Recapture (SECR) package within R. However, with a small data set such as ours, the SECR package could not resolve the population densities to a fine, and useful, scale. Therefore, for this project we chose to use snake encounter rates and individual mouse captures instead of population density values. The small datasets collected in this study make it difficult to perform capture-mark-recapture analyses, hence we used statistical methods which explain the data, but may be less accurate.

Snake encounter rate per square was calculated by dividing the total number of snakes caught per square by the search effort per square. Snake encounter rates were used for the analysis as this allowed more snake captures to be included in the data set. Due to the small amount of capture data collected over the course of the two survey years, removing recaptures from the analysis to calculate snake encounter estimates would significantly reduce the number of data points that could be used. Using capture and recapture data, whilst slightly inflating the encounter estimates, provides better information regarding the snakes habitat usage. Snake encounter estimates per square was calculated by dividing the total number of individual snakes caught per square, by the search effort per square. We considered search effort to be the number of times a squarewas visited by the team. To calculate this we mapped 30 randomly selected survey tracks onto the map of zoo and counted each time the survey track passed into a square. The total number of visits per square over the 30 surveys was calculated to give the search effort per square.

Table 1. Summary of the different habitat characteristics measured in the zoo.

Habitat Characteristic	Explanation
Number of individual mice caught	The total number of individual mice caught by each trap in a square across all trapping sessions.
Number of refugia in square	The total number of refugia in each square.
Habitat Edge - With Canopy	The percentage of ground covered in each square by habitat edge with canopy cover. Habitat edge is defined as the area where two different habitats.
Habitat Edge - Without Canopy	The percentage of ground covered in each square by habitat edge without canopy cover.
Forest Floor - With Canopy	The percentage of ground covered in each square by forest floor with canopy cover. Forest floor is defined as any ground covered by detritus.
Forest Floor - Without Canopy	The percentage of ground covered in each square by forest floor without canopy cover
Grassland - With Canopy	The percentage of ground covered in each square by grassland with canopy cover. Grassland is defined as any field, meadow, flower beds, or lawn
Grassland - Without Canopy	The percentage of ground covered in each square by grassland without canopy cover
Building - With Canopy	The percentage of ground covered in each square by buildings with canopy cover
Building - Without Canopy	The percentage of ground covered in each square by buildings without canopy cover
Other Ground - With Canopy	The percentage of ground covered in each square by obscure ground types, rarely observed in the zoo e.g. wood chipping and areas of extended concrete, with canopy cover
Other Ground - Without Canopy	The percentage of ground covered in each square by other ground types without canopy cover

Multiple regression analysis was used to generate models which could predict the habitat types and habitat characteristics which could predict high densities of *Z. longissimus*. The independent variables were tested for multicollinearity - high correlation between two independent variable - which showed high associations between Forest Floor With Canopy and Grassland Without Canopy (VIF > 1000). The data was analysed whilst excluding each of the independent variables; in both instances the violation of multicollinearity was

resolved (VIF < 2). Statistical analysis was continued with Forest Floor With Canopy as an independent variable. The remaining variables were tested to ensure that none of the assumptions of multiple regression analysis were violated (Osborne and Waters, 2002; Williams *et al*., 2013). Testing showed that the data violated the assumption of heteroscedasticity. Toovercome this, robust standard errors were used in the final analysis (Hayes, 2007).

Results

Snake Survey Results

Over the course of two summers we completed 198 refugia surveys. 108 individuals were caught 235 times between the months of April and October of 2018 and 2019. We averaged 1.18 (*s.d* ± 3.08) recaptures over the course of the survey season. Of the snakes caught, 23 were adults, 13 were subadults, and 72 juvenile. In the 2018 season, 59 individuals were captured 93 times (46 refugia captures, 47 opportunistic captures). In 2019 capture rates decreased, but recapture rates increased: 39 individuals captured 134 times (95 refugia captures, 39 opportunistic captures).

The VIE marking method used on the juvenile snakes in this study proved to be highly successful. The marks of recaptured juvenile snakes remained largely intact and in their original position; only one of the 14 marks applied to recaptured snakes had moved position along the flank of the snake inbetween captures. This is likely due to the application of excessive elastomer upon initial mark application. Recaptured juvenile snakes showed growth and increased in body mass (full results published in Major *et al*., 2020). These results demonstrate the effectiveness of VIE as a method of marking small snakes.

Mammal Trapping Results

Through August to October we completed four trapping sessions consisting of 12 trapping nights. 243 individuals were caught in 380 capture instances. Three species of small mammal were caught: 240 wood mice (*Apodemus sylvaticus*), two bank voles (*Myodes glareolus*), and one house mouse (*Mus musculus*). Both capture and recapture rates increased with each trapping session. We observed a mean recapture rate of wood mice of 0.55 (*s.d.* ±0.89) over all trapping sessions.

The use of a protective case for the Longworth traps also showed high success, preventing the badgers from accessing the Longworth traps. Prior to the use of the protective casing we observed a 65% trap disturbance rate; after the implementation of the protective casing, trap disturbance rates declined dramatically, with only one trap being disturbed throughout the rest of the study (Table 2.)

Table 2. Trap disturbance rates prior to and after implementation of the protective casings.

Trapping Session	Protective Casing Used	No. of Traps Set	No. of Traps Disturbed	% of Traps Disturbed
1	No	188	124	65.96
2	Yes	141	0	0.00
3	Yes	141	0	0.00
4	Yes	141	1	0.71
5	Yes	141	0	0.00

Chi-Square analysis showed a significant relationship between the use of protective casings and a decrease in trap disturbance, $X^2(df = 1, N = 754) = 438.4$, $p < 0.01$.

<u>Habitat Characteristic Calculations</u>

We recorded twelve different habitat characteristics for this study (Table 3**.**). Of the 12 habitat characteristics recorded, 10 represented the coverage of a specific habitat type per square, and two recorded the count value of a habitat feature: Number Of Refugia and Individual Mice Captures. Forest Floor With Canopy, Grassland Without Canopy, and Habitat Edge With Canopy covered the largest proportion of the zoo (76.76%). Forest Floor With Canopy was the largest single habitat type found across the zoo grounds (34.47%). We placed an average of 1.5 refugia in each square at the zoo. The highest number of refugia recorded in one square was eight. Refugia was placed in 20 of the 38 squares included in the statistical analysis.

Snake captures were confined to the grounds of the zoo. Although we did survey areas outside of the zoo, overall search effort was far higher in the WMZ. Of the 32 squares surveyed, 23 had snake encounter rate values. Snake encounter rate values per square were low. In all instances snake encounter rates were less than 1.0; at its highest, snake encounter rate was calculated to be 0.4 snakes per survey effort per square. However, this is to be expected given the cryptic behaviour of the snakes and typical low densities of snake populations.

Table 3. Summary of the percentage coverage of each habitat type per square

Habitat Characteristic	Habitat Characteristic abbreviations	Percentage Covered per Square
Habitat Edge – With Canopy (%)	HABCAN	18.89
Habitat Edger – Without Canopy (%)	HABNOCAN	8.87
Forest Floor – With Canopy (%)	FORCAN	34.47
Forest Floor - Without Canopy (%)	FORNOCAN	0.29
Grassland - With Canopy (%)	GRASSCAN	3.16
Grassland - Without Canopy (%)	GRASSNOCAN	23.39
Building - With Canopy (%)	BUICAN	0.58
Building - No Canopy (%)	BUINOCAN	6.63
Other Ground - With Canopy (%)	OTHCAN	0.58
Other Ground - No Canopy (%)	OTHNOCAN	3.14
Number of Refugia	NOREF	n/a
Number of Individual Mouse Captures	INDMIC	n/a

Mouse capture rates varied between squares: the highest number of mice caught in one square being 24, and the lowest four. On average 7.4 mice were captured per square. Per square, an average of 1.2 traps were set. The total number of individual mice captured per square, increased with the number of traps set per square. Due to limited resources, 12 of the squares in the zoo which were surveyed for snakes were not surveyed for mice.

Stepwise, forwards, and backwards multiple regression analysis was performed to determine which habitat characteristics were the most significant predictors of snake encounter rate. All three tests returned the same model, showing the highest levels of significance between three independent variables: Individual Mice

Captures (INDMIC) ($p < 0.001$), Number of Refugia (NOREF) ($p = 0.01$), and Forest Floor NoCanopy (FORNOCAN) ($p = 0.018$) and the dependent variable, Snake Encounter Rate (SNER). Multiple regression with use of robust errors produced a significant regression model ($F(3,34) = 6.95$, $p < 0.001$) to predict snake population density based on habitat characteristics with the following equation:

$$\text{SNER} = -0.0172 + 0.0058\text{INDMIC} + 0.0127\text{NOREF} + 0.0229\text{FORNOCAN}$$

where Forest Floor No Canopy is reported as habitat type ground coverage, and Individual Mice Captures and No of Refugia as count data per square. The equation shows all of the independent variables contribute positively to Snake encounter rate per square. Of these, Forest Floor No Canopy is the strongest predictor. This model is considered a suitable fit ($R^2 = 0.75$). Of the three independent variables included in the final model,Individual Mice Captures was the only statistically significant predictor variable ($p = 0.001$); Number of Refugia ($p = 0.181$), Forest Floor No Canopy ($p = 0.4578$). Whilst the latter independent variables could have been removed from the analysis due to their statistical insignificance, the models generated without these variables did not have as strong goodness-of-fit value.

Discussion

Habitat Selection

In this study we used combined data from a two-year mark-release-recapture study to elucidate habitat selection of *Zamenis longissimus* in the WMZ, Colwyn Bay. We expected *Z.* longissimus to use forest habitats as it typically does within its native range, for protection from predators as well as for foraging. Additionally, as Colwyn Bay is further north latitudinally than the native habitat of *Z. longissimus* we expected the snakes to use habitat which had higher thermal quality in order for the snakes to achieve their optimal body temperature i.e. areas with high amounts of habitat edge, as this habitat type offers foraging and basking opportunities, as well as quick access to shelter. By use of multiple regression analysis, we resolved macro-scale habitat characteristics which can be used to predict the presence of *Z. longissimus*. We found that *Z. longissimus* responds positively to prey abundance, and so believe that population densities of *Z. longissimus* can be predicted by high prey abundance. *Z. longissimus* does respond to forest habitat, however, not to the presence of increased amounts of habitat edge. This is highly unexpected as our results are in direct contrast with current literature reporting on habitat selection in colubrid snakes. This is the first report of *Z. longissimus* selecting habitat based upon prey abundance.

The driving factors behind habitat selection in colubrid snakes has been investigated in many species. Certainly, the reliance of snakes on their environmental temperature for important physiological processes, such as movement and digestion, is well understood (Stevenson *et al*., 1985). Webb and Shine (1998) further

developed the relationship between snakes and their environmental temperature by showing that large-scale movements and retreat-site usage could be accurately predicted based upon the habitat's thermal quality. This prioritisation of environmental temperature during habitat selection by colubrid snakes has been well demonstrated in milk snakes (Row and Blouin-Demer 2006), bull snakes (Kapfer *et al.* 2010), garter snakes (Halliday and Blouin-Demers 2016), and ratsnakes (Sperry and Weatherhead 2009; Howze *et al.*, 2019; George *et al.* 2015).

Whilst literature which directly assesses the impact of prey abundance on habitat selection in colubrid snakes is sparse, there is evidence supporting both habitat selection based upon thermal quality, and habitat selection based upon prey abundance. Halliday and Blouin-Demers (2019) assessed habitat selection patterns in the common garter snake (*Thamnophis sirtalis*) showing that garter snakes do notselect their habitat based upon prey abundance, but rather vegetation structure, suggesting that garter snake's prioritise the thermal quality of their habitat. In contrast, Baxley and Qualls (2009) demonstrated that the black pine snake (*Pituophis melanoleucus logingi*) utilised available habitat which had higher prey abundance. This was also seen in the Louisiana pine snake (*Pituophis ruthveni*) (Himes *et al.*, 2006), and has been suggested by Miller *et al.* (2012) in the Florida pine snake (*Pituophis melanoleucus mugitus*). It is worth noting, however, that the snakes were frequently observed using prey burrows for shelter, suggesting that it is not simply a case of increased prey abundance which affects habitat selection, but also habitat characteristics which are associated with the prey item. This was touched upon by Baxley and Qualls (2009), who noted that the ideal macro-habitat for the black pine snake was very similar to that of its prey.

A number of studies focusing on other species of snake outside of the colubrid family, have set out to establish a more direct relationship between snake habitat selection and prey abundance. Typically, these studies have focused on ambush predators. Such studies have shown the importance of prey abundance to snakes during habitat selection and use. King and Duvall (1990) found that Prairie rattlesnakes (*Crotalus viridis*) migrate in an attempt to find areas of higher prey abundance. Similarly, timber rattlesnakes (*Crotalus horridus*) (Wittenberg, 2012) and speckled rattlesnakes (*Crotalus mitchellii*) (Glaudas and Rodriguez-Robles, 2011) also select habitat with greater prey abundance. Additionally, experimental studies using supplementary feeding evidenced that snakes who receive additional food items altered their habitat use compared to those snakes who did not. Wasko and Sasa (2012) performed an experimental study to assess the impact of supplementary feeding on the foraging behaviour of a fer-de-lance (*Bothrops asper*) population. They demonstrated that the snakes which received additional food altered their spatial activity and foraging behaviour, further characterising the response of snakes to prey abundance, and the complex nature of this relationship.

Due to the above-mentioned reliance of colubrid snakes on their environmental temperature, we should now ask, why does *Z. longissimus* seemingly prioritise prey abundance over the thermal quality of the habitat in a

thermally challenging environment? There are several answers to this question, the most obvious being that the habitat of the WMZ is thermally suitable for *Z. longissimus*, despite being much cooler. This is certainly feasible as energetic studies performed by Lelièvre *et al*. (2010a) have shown that *Z. longissimus* in western France actively select cooler habitat (20∘C - 25∘C), such as forest rather than open fields (20∘C - 40∘C), maintaining a body temperature between 21∘C and 25∘C – lower than that of other rat snakes (Blouin-Demers and Weatherhead, 2001). This is in accordance with a low energy lifestyle. *Z. longissimus* was able to, and better performed, certain physiological processes, such as digestion, at lower temperatures (18∘C - 25∘C); higher temperatures (30∘C) were shown to be unfavourable, compromising the snake's ability to digest their prey (Lelièvre *et al*., 2010a). In its native range *Z. longissimus* was shown to thermoconform, matching its body temperature to that of the environment when in closed shelter (Lelièvre *et al*., 2011). Additionally, higher numbers of individuals in the isolated population in southeast Poland, studied by Kurek *et al* (2018), were found in habitats with average annual temperatures between 5.9∘C and 6.3∘C. It is rational then, to posit that the thermal quality of North Wales is suitable for *Z. longissimus*. Whilst it could be that the snakes reach their optimal body temperature (21∘C – 25∘C) in North Wales, this is unlikely as in 2019 temperatures in North Wales reached a maximum of 20∘C (Young and Galvin, 2020). This suggests that the snakes cannot reach body temperatures similar to *Z. longissimus* populations in their native range, rather that the snakes reach body temperatures that allow the snakes to perform necessary physiological functions i.e. digestion, regardless of the cooler climate. This may suit *Z. longissimus* as their low thermal requirements are related to a cryptic and secretive lifestyle (Lelièvre *et al*., 2011). Without the pressures of thermal quality and maintenance of high body temperature, thermoregulation no longer becomes the priority and *Z. longissimus* can therefore use its environment in ways to serve other functions, such as foraging. Despite suggestions that *Z. longissimus* is a slow-moving ambush predator (Lelièvre *et al*., 2010a), observations in our study suggest otherwise; rather that *Z. longissimus* is an active forager. Snakes in our study regurgitated consumed prey items during processing; sometimes regurgitating several mice of different age classes, leading us to believe that *Z. longissimus* is a nest raider. The ability of Z. *longissimus* to actively forage further suggests that the snakes can function effectively in cooler climates and do not need to prioritise thermal quality of the habitat.

A second explanation for this result is that we did not see thermoregulatory behaviour by the snakes during this study. Of the 108 snakes caught, we observed three individuals perform thermoregulatory behaviour i.e. basking. For a slow-moving snake such as *Z. longissimus*, basking behaviour poses predatory risks: the individual must expose themselves to receive solar energy, increasing the likelihood of predation (Herr *et al*., 2020). Typically snakes use habitat edges to thermoregulate, as these areas offer open space to receive solar energy, as well as providing quick access to shelter (Bauder *et al*., 2018; Sutton *et al*., 2017). This has been seen in *Z. longissimus* (Kovar *et al*., 2016a), however, in our study we did not observe such habitat use. The WMZ is home to European Badgers (*Meles meles*) and common buzzards (*Buteo buteo*), in addition to the zoo animals, all of which have the potential to kill *Z. longissimus*. And indeed, during our study period we encountered snakes which had been killed by badgers, an unknown bird species, and emus. Furthermore, the

zoo experience high footfall from human visitors during the summer months, coinciding with the snakes' active period. This highlights the risk of exposure to the snakes. As such it would be beneficial for the snakes to thermoregulate in other ways, such as thigmothermy (Belliure and Carrascal, 2002), or in sheltered habitat, such as buildings. During the study, five snakes were caught within buildings. Furthermore, anecdotal reports from zoo staff suggest that the snakes use the buildings regularly, although for what purpose is unknown. The use of anthropogenic sites by *Z. longissimus* has been documented in several populations in its native range (Edgar and Bird, 2006; Lelièvre *et al.*, 2010b). Kovar *et al.* (2016a) demonstrated the dependence of *Z. longissimus* on man-made structures at the northern limits of its native range. The snakes frequently used buildings for shelter and thermoregulation; one snake exclusively used man-made structures throughout the study. Additionally, *Z. longissimus* was found to use anthropogenic sites for overwintering (Kovar *et al.*, 2016b). Given *Z. longissimus*' inclination to use anthropogenic sites in its native range, it is not unreasonable to assume that they also use anthropogenic sites in a potentially thermally challenging environment for thermoregulation, shelter, or overwintering. The use of anthropogenic sites would allow the snakes to thermoregulate safely and then perform other tasks, such as foraging.

It is important to note that this study suffers two limitations. The first limitation being that to catch *Z. longissimus* we had to use artificial refugia. Although the refugia was placed in locations thought to already be used by snakes i.e. habitat edges and east-facing banks, there is still potential bias for snakes occurring in that area as a result of the improved thermal conditions offered by the refugia (Zappalorti and Reinert, 1994). Additionally, the refugia was not evenly distributed across the zoo, and so refugia 'hot spots' were created in areas of assumed increased snake presence, creating further sampling bias. However, due to the highly cryptic nature of the snakes, use of, and the selective placement of the refugia, was necessary to obtain adequate data for analysis. This limitation also extends to the locations of the Longworth traps, as they could not be precisely distributed across the grounds of the zoo. This limitation could be resolved by the use of population density analyses such as the SECR statistical package described previously.

The second limitation of this study is the reliance of secondary data to make habitat selection inferences, as a result of the survey method. Due to the high thermal conductivity of the artificial refugia, the environmental temperature under the refugia was often considerably higher than that of the natural environmental temperature in the WMZ. As such, in addition to the fact that the majority of the snakes were caught under refugia, the environmental temperature of the snakes capture location was not included in the analysis as it likely does not accurately represent the natural environmental temperatures. Therefore, any inferences made in the discussion regarding *Z. longissimus* habitat selection, are based upon secondary thermal data from other studies.

Population Expansion

The thermal quality of a habitat significantly influences the distribution of ectotherms, and therefore snakes (Harvey and Weatherhead, 2010), due to the dependence on specific temperature thresholds for physiological processes (Stevenson *et al*. 1985). Given that *Z. longissimus* is considered an active snake with a large home range (Naulleau and Bonnet, 1995), we were surprised to find in our study that the snakes were mostly confined to the grounds of the zoo, with the exception of rare capture occasions in nearby urban areas of Colwyn Bay, particularly as *Z. longissimus* has shown to be comfortable in the Colwyn Bay climate. We believe that lack of adequate nesting sites is the factor currently limiting the range of *Z. longissimus* in North Wales. In our study we discovered one clutch of snake eggs buried in the peripheries of the manure pile in the WMZ. We think these to be *Z. longissimus* eggs given the absence of other snake species in the area, and believe that *Z. longissimus* relies on manure piles and other anthropogenic sites for egg laying. Whilst this is the only egg sample collected in this study, this finding does conform with other literature. Grass snakes (*Natrix natrix*) have been found to overcome thermal constraints by use of artificial oviposition sites such as manure piles (Löwenborg *et al*. 2010; Löwenborg *et al*. 2012). Eggs laid in manure piles had a higher hatching success rate than eggs laid in naturally occurring oviposition sites. More recently, Struijk *et al*. (2019) showed that introduced Russian rat snakes (*Elaphe schrenckii*) in the Netherlands used compost heaps to tackle the challenging environmental temperatures. As the manure pile seems to be a viable oviposition site for *Z. longissimus* it is possible that the snake will remain tied to the grounds of the zoo, due to the absence of similar man-made sites elsewhere in Colwyn Bay.

Control of *Z. longissimus* in Colwyn Bay

Although this study did not assess the impact of *Z. longissimus* on the native fauna and flora of Colwyn Bay, we have provided an insight into the behaviour of the snake outside of its native environment, and have made suggestions about how the snake uses its environment to survive in a thermally challenging setting. This study has shown that the snakes respond to prey abundance aboveall else and may rely on anthropogenic sites for thermoregulation and reproduction. Our results can aid the development of wildlife management plans for future monitoring and assessment of *Z. longissimus* in the WMZ.

Longworth Trap Disturbance

The protective cases for Longworth traps designed in this study show a novel and highly effective method of preventing Longworth trap disturbance by badger populations. The trap casings not only allow the Longworth traps to be placed in more exposed locations, as the traps are protected from direct sunlight and rain, but also affords the traps better protection from larger animals who may interfere with the traps. This will allow for more robust and accurate small mammal surveys in future projects.

<u>Conclusion</u>

This study has provided a novel insight into macro-scale habitat selection patterns of *Z. longissimus* outside of its native range, showing that prey abundance is the main driving factor behind population distribution behaviours of the snake. Our results highlight the importance of including prey abundance as a habitat feature when characterising habitat selection in colubrid snakes. Though this is an interesting find with possibly wide ecological implications, this study comes with a caveat. This being that the majority of the *Z. longissimus* population studied in this project were juvenile snakes who were caught under refugia. As the refugia were strategically placed with respect to favourable habitat characteristics for *Z. longissimus*, it may be that the snakes appeared at the observed densities in the surveyed areas of the zoo as a result of refugia placement bias. At a macro-scale teasing apart the relationship between the snakes and the use of refugia is challenging without micro-habitat data.

Future studies should incorporate radio telemetry to characterise micro-habitat usage of *Z. longissimus,* in addition to collecting first-hand thermal data of the snakes' environment, to attempt to fully understand how the snakes use the environment and to properly clarify the impact of a non-native snake in Colwyn Bay.

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