Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Ecology

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Sammanfattning

Rörelsemönster och habitatanvändning hos ormar är ett område där relativt lite forskning har gjorts trots tecken på minskande populationer på flera platser i världen. Snoken (*Natrix natrix*) är en vanligt förekommande orm i Sverige som tycks vara på nedgång, troligen på grund av en sämre tillgång på äggläggningsplatser. Vanliga äggläggningsplatser i Sverige utgörs av gödselstackar och komposter vilka ger den stabila temperatur som äggen behöver. Dessa har dock blivit både färre och mer svårtillgängliga under det senaste århundradet. Huvudsyftet med denna studie var att undersöka vilka äggläggningsplatser som används i ett område där gödselstackar saknas och hur anpassningsbara snokhonor är till nya områden. Detta gjordes genom att jämföra rörelsemönster och habitatanvändning mellan individer som känner till området och individer som inte gör det. Tre snokhonor flyttades från en population utanför Uppsala till en population på Djurgården, Stockholm och spårades dagligen tillsammans med tre individer från den lokala populationen under 25 dagar med hjälp av externa radiosändare. Studier på snokens rörelsemönster och habitatanvändning har inte genomförts i Sverige på 30 år och mer kunskap behövs om arten för att bättre kunna skydda den. Inga ägg kunde lokaliseras men värmen från ett pumphus kan tänkas ge de nödvändiga förutsättningarna. De förflyttade snokhonorna från Uppsala visade tecken på att inte anpassa sig till den nya lokalen genom en högre aktivitetsgrad samt ovilja att nyttja främmande habitattyper jämfört med de lokala individerna. Dessa resultat bör dock tolkas försiktigt på grund av det lilla antalet individer samt den begränsade studieperioden. Varken ljus eller temperatur var bra förklaringsfaktorer för aktiviteten hos snokarna men indikationer på att de är mer aktiva inom ett temperaturspann mellan 20-25°C observerades. De främsta habitaten som användes av snokarna var vassbälten samt oklippta gräsmarker som tack vare sin strukturella komplexitet skapar en blandning av solbelysta och skuggiga områden där snoken effektivt kan uppnå en bra kroppstemperatur. För den framtida förvaltningen av arten krävs att mer information insamlas under en längre tid. Inventeringar och ökad kunskap om äggläggningen behövs innan man kan börja göra upp planer på om och hur snoken bör skyddas.

Abstract

Movement patterns and habitat use of snakes is a research area where relatively few studies have been done despite signs of declining snake populations around the world. The grass snake (*Natrix natrix*) is a common species in Sweden that seems to be declining, possibly due to a lack of oviposition sites. Commonly used nesting sites in Sweden constitute of manure heaps and composts which provide the thermally stable environment the eggs require. These have, however, become fewer and less accessible during the last century. The initial purpose of this study was to locate natural oviposition sites in an area without any of the commonly used structures. The adaptive ability of grass snakes was also tested by comparing movement patterns and habitat use of grass snakes familiar with the area and individuals unfamiliar with it. This was done by moving three grass snake females from a population outside the city of Uppsala to a population in Stockholm, Sweden. These three individuals were tracked alongside three females from the local population using external radio transmitters for 25 days. Similar studies have not been made in Sweden in the last 30 years and an increased knowledge of the species is needed to better protect it. No eggs could be located even though the heat from a water pumping station might provide the suitable conditions. The translocated grass snake females showed signs of not adapting to the new environment by moving over larger areas and avoiding unknown habitat types. These results should, however, be interpreted carefully due to the small sample size and the limited study time. Reed belts and unmanaged grasslands were used by all individuals to a varying degree while they actively avoided open areas and woodlands. Neither light conditions nor temperature were good predictors of movement patterns although indications pointed to a higher activity at temperatures between 20-25°C. For future management, it is important to gather more information about the species over a longer time period. Surveys and increased knowledge about the oviposition is needed before plans can be made regarding if and how the grass snake needs to be protected.

Keywords: Grass snake, adaptation, movement, habitat use, translocation, conservation.

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1 Introduction

Habitat change has been proposed as one of the main reasons to the decline of many species seen today (Wilcove *et al.* 1998). To cope with these changes, species need to adapt to them or relocate to areas with suitable environmental conditions and habitat structure. Identifying these suitable areas along with the movement pattern in the landscape is thus important for conservation as it gives information about how and when individuals travel between different areas and utilizes different habitat types (Wastell & Mackessy 2011). These areas can then be protected to save threatened species. Snakes are a species group where relatively little research has been done despite signs that several species show signs of declining (Reading *et al.* 2010). This has led to a lack of long-term data for many snake species which makes it difficult to draw any solid conclusions about population structure, status and conservation needs (Gibbons *et al.* 2000).

The grass snake (*Natrix natrix*) is one species of snake that seems to decline in several European countries, including Sweden (Zuiderwijk *et al.* 1998, Hagman *et al.* 2012). The reason for the decline is unknown but one suggested cause is the decreasing availability of oviposition sites which is often made up of manure heaps or composts as they provide a thermally stable environment for the eggs (Hagman *et al.* 2012). As manure heaps gets more dispersed in the landscape, snakes need to travel further to reach them and subject themselves to the elevated mortality risks that often comes with increased movement (Bonnet *et al.* 1999, Plummer & Mills 2000, Yoder *et al.* 2004, Martino *et al.* 2012). The better individuals can adapt to changes in their local area and use unfamiliar habitats and structures, the higher the chance might be that they can handle habitat changes.

Movement rate is often high for animals in unfamiliar environments as they try to locate recognizable sites (Plummer & Mills 2000, Butler *et al.* 2005, Rittenhouse *et al.* 2007, Avgar *et al.* 2013). For snakes however, increased movement is also seen during mating when males search for a female and oviposition when females try to locate a suitable oviposition site (Madsen 1984, Gerald *et al.* 2006). For the grass snake, movement is generally low for gravid females up to a few days before oviposition when it increases to 100m/day on average (Madsen 1984). Temperature on the other hand, has been shown to have a smaller impact on the activity pattern in snakes despite them being ectothermal organisms. However, it has been speculated to be more important in colder environments (Brown & Shine 2002, Butler *et al.* 2005). The importance of temperature for the grass snake is however unknown.

The opportunity to thermoregulate seems to have some impact on the habitat choice made by snakes (Reading & Jofre 2009, Martino *et al.* 2012). Mixed habitats are often preferred with access to both sunny and shaded areas where they accurately can regulate their body temperature (Martino *et al.* 2012). These kinds of habitats are also useful for escaping threats as they have retreat sites nearby (Wisler *et al.* 2008, Madsen 1984). Open areas are usually avoided as they carry a high risk of predation (Isaac & Gregory 2004). Similarly, forested areas are often avoided by the grass snake, possibly due to the limited basking opportunities offered by the canopy cover (Reading & Jofre 2009). The degree of preference for different habitats might however differ between individuals which mean it is important to identify all different habitat types that can be used to better manage a population (Wisler *et al.* 2008). Grass snakes seem to use the same sites during subsequent years which indicate that they might suffer when these habitats are removed. Translocated hognose snakes (*Heterodon platirhinos*) and tiger snakes (*Notechis scutatus*) have earlier been shown to adapt poorly to new environments and suffer a high mortality when searching for localities they recognise (Plummer & Mills 2000, Butler *et al.* 2005). If the same is true for grass snakes, care should be taken when physically altering the environment as this might force the snakes to relocate.

In this study I will test the adaptability of female grass snakes by moving a few individuals to an unfamiliar site. Translocation studies have never been done on this species before and studies on their movement has not been made in Sweden in the last 30 years (Madsen 1984) which is why it is important to investigate how it responds to severe habitat changes. Movement rate and habitat choice will be compared between translocated grass snake females and individuals from the local population. I will further investigate how temperature and light conditions affect their choice of habitat and their activity. Only females will be tracked here as I will attempt to locate oviposition sites to further see if the translocated females can find these sites. Movement rate is expected to be high for all of the tracked snakes if they are gravid as they will try to locate a good nesting site. The translocated individuals will probably be more active as they might try to locate familiar areas. Movement rate and habitat use are expected to be positively correlated to the temperature as gravid females are often basking more when gravid (Madsen 1987). Habitat use will probably also be affected by how protected the individual snakes are in the different habitat types. Good nesting sites will probably be difficult to find due to the relatively cold climate in Sweden but suboptimal sites may be used such as inside rotting stumps and logs, sites that are used in warmer countries (Luisella et al. 1997, Löwenborg et al. 2011).

2 Methods

2.1 Species description and conservation status

The grass snake is a non-venomous snake belonging to the family Colubridae and is distributed over most of Europe except Ireland, Scotland and several of the Mediterranean islands. In Sweden it can be found throughout the southern and central parts and along the northern east coast (figure 1). Several subspecies has been proposed to exist but the focus here lies on the nominate form, *Natrix natrix natrix*. It is one of three snake species found in Sweden along with the European adder (*Vipera berus*) and the smooth snake (*Coronella austriaca*) and the only oviparous reptile in the country along with the sand lizard (*Lacerta agilis*) (Kreiner 2007). Its prey consists of toads (*Bufo bufo*), other amphibians and occasionally fish (Reading & Davies 1996). Main predators are herons (*Ardea cinerea*), and storks (*Ciconia ciconia*) as well as medium sized mammals such as badger (*Meles meles*) and fox (*Vulpes vulpes*). Cats, dogs and hens are also known to kill grass snakes (Kreiner 2007, Kärvemo unpublished data).

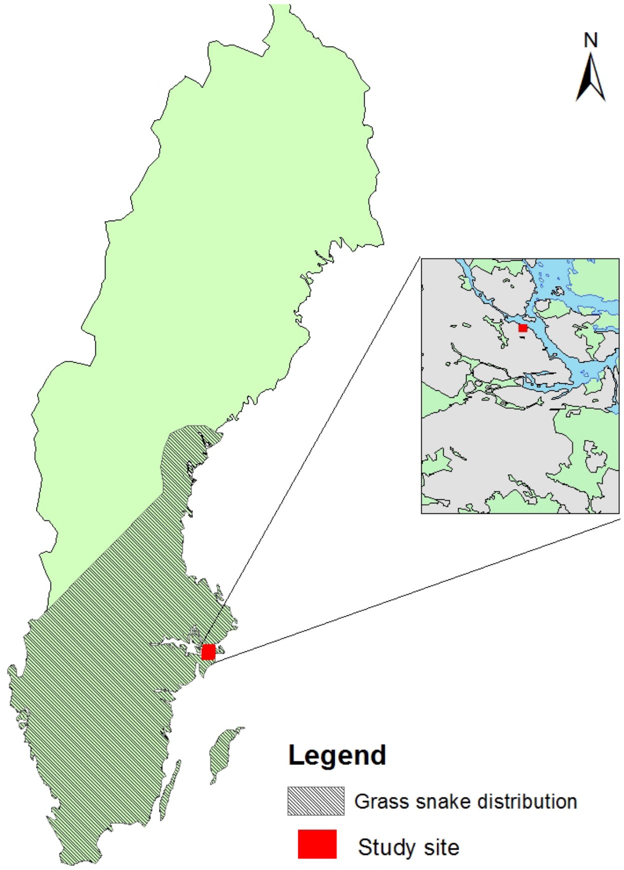


Figure 1. The distribution of grass snake in Sweden with the inset showing the study site in Stockholm. Distribution data is based on reported observations in the Swedish project Artportalen during the years 2000-2014.

The grass snake display sexual size dimorphism with females being on average 120 cm long and males being 70 cm (Gregory 2004, Kreiner 2007). According to studies from other parts of Europe, the grass snake is typically found in heterogenic habitats with access to safe basking sites and nearby calm waters where they can forage (Reading & Jofre 2009, Hutinec & Mebert 2011). In Sweden, the grass snake emerges from hibernation in March and April with males emerging earlier than females. Mating occurs shortly after the females emerge, towards the end of April but it can occur later as well (Madsen 1984). Approximately two months after mating the females lay between 11-50 eggs in a warm, moist environment. In countries with a warm enough summer temperature, eggs are often laid in stumps and natural piles of decomposing vegetation (Madsen 1984, Kreiner 2007, Edgar *et al.* 2010).

The grass snake is classified as least concern (LC) according to the international and national red list but the international classification has not been updated since 1996 (European Reptile & Amphibian Specialist Group 1996). The national classification was updated 2010 but the lack of new data makes it difficult to be certain of the status of this species. Reports are emerging from different parts of Europe concerning how the grass snake population seems to be declining, indicating the need to update the IUCN classification (Zuiderwijk *et al.* 1998, Reading *et al.* 2010, Hagman *et al.* 2012).

2.2 Area descriptions

2.2.1 Stora Skuggan , Stockholm (Study site)

Stora Skuggan in Stockholm is a recreational green area in the northern part of central Stockholm (N 59.365569, E 18.077552; WGS 1984) and part of the National city park Djurgården, an area protected from exploitation (figure 2). It is mainly made up of open lawns with patches of groves and ponds. No manure heaps exists in the area and as such it is unknown where the local grass snake females oviposit. In the northern part of the area is a larger forest mainly consisting of aspen (*Populus tremula*), birch (*Betula* sp.) and scots pine (*Pinus sylvestris*). The forest floor is dominated by heath land with mostly blueberry (*Vaccinium myrtillus*) and, in more open areas, common bracken (*Pteridium aquilinium*). The Norway spruce (*Picea abies*) gets more common towards the western part of the forest but is mainly prevalent in the lower parts of the forest. The eastern part of the forest is more open and is largely dominated by oak (*Quercus robur*) and maple (*Acer platanoides*) and a few other hardwood trees such as small-leaved linden (*Tilia cordata*), rowan (*Sorbus aucuparia*) and bird cherries (*Prunus padus*). The ground floor consists of mainly wavy hair-grass (*Deschampsia flexuosa*).

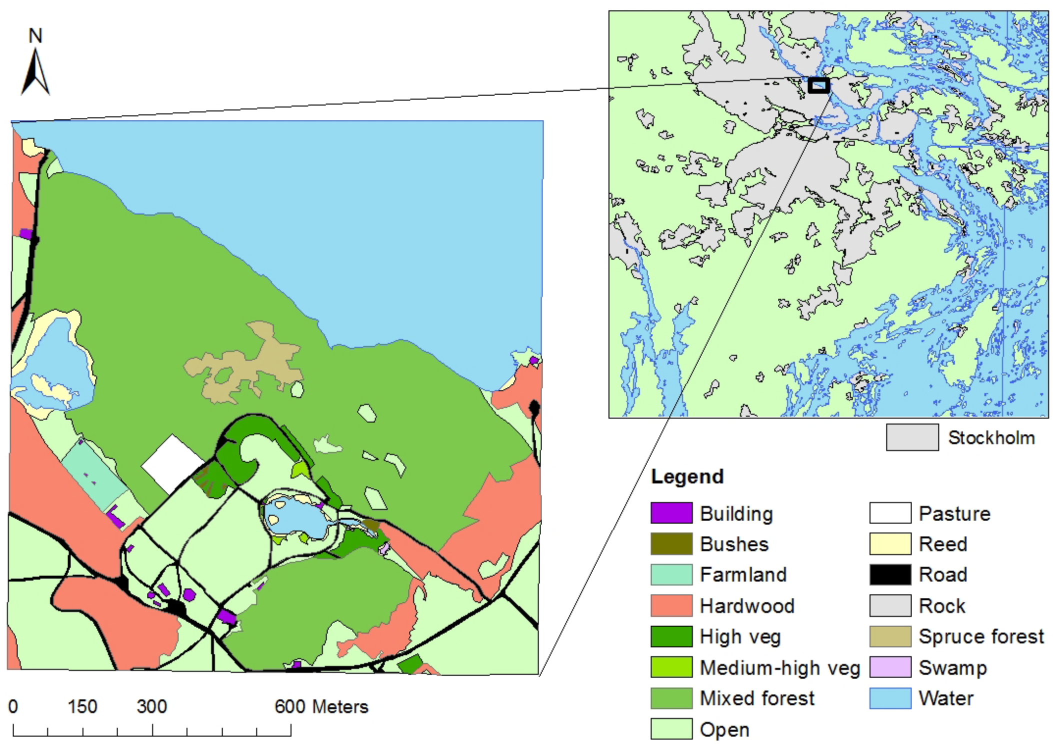


Figure 2. Map depicting the study area of Stora Skuggan, Stockholm.

The open areas consist mostly of a regular lawn that is being cut once every 1-2 weeks and is commonly used by people. There are a few unmanaged areas where the vegetation can grow freely, reaching heights of between 0.5 m and 1 m. The main vegetation here is made up of different species of grass such as orchid grass (*Dactylis glomerata*) and meadow oat-grass (*Helictotrichon pratense*). Holes dug by water vole (*Arvicole amphibious*) are common in the unmanaged areas, especially in the eastern part. The grassy vegetation becomes less common on the top of the hills where it is thinned out and mainly consists of spear thistles (*Cirsium vulgare*), yarrow (*Achillea millefolium*) and ox-eye daisy (*Leucanthemum vulgare*). One of the hills has a few rows of bird cherry and wild cherry (*Prunus avium*).

The main pond (located in the centre of figure 2) has an area of 1.1 ha, is 2 m deep at most and surrounded by common reed on all sides and a few areas where common club-rush (*Schoenoplectus lacustris*) is prevalent. Towards the western part of the pond are two small islands completely covered in reed and occasionally lesser bulrush (*Typha angustifolia*) and common bulrush (*Typha latifolia*). Around a water pumping station located on the western side of the pond grows a dense stand of dwarf elder (*Sambucus ebulus*). The water surface is open and a few bird cherries and beeches grow close to the water. The pond is inhabited by several pairs of breeding coot (*Fulica atra*) and mallards (*Anas platyrhynchos*) and grey heron is a common visitor. The waters are otherwise inhabited by smaller fishes, and several amphibians such as the common toad, newts (Salamandridae) and possibly different species of frogs (*Rana* spp.).

2.2.2 Uppsala, Dalkarlskärret (origin site of the translocated grass snakes)

Dalkarlskärret is situated approximately 50 km northwest of the site in Stockholm and is a nutrient rich fen located south of the city of Uppsala (59°46'38.4"N 17°34'58.9"E; WGS 84; figure 3). Most of the fen is covered with common reed (*Phragmites australis*) and towards the shores they produce large floating bog mats where reed sweet-grass (*Glyceria maxima*) and sedges (*Carex* spp.) are common. The area surrounding the fen is made up of an open, grazed area to the northwest and plantations of scots pine around the rest of the fen. The forest becomes more mixed towards the eastern parts. The fen is inhabited by several species of ducks such as mallards and common teal (*Anas crecca*) as well as grey heron. Common toad is breeding in the fen alongside other amphibians. Other reptiles observed here are viper and common lizard (*Zootoca vivipara*).

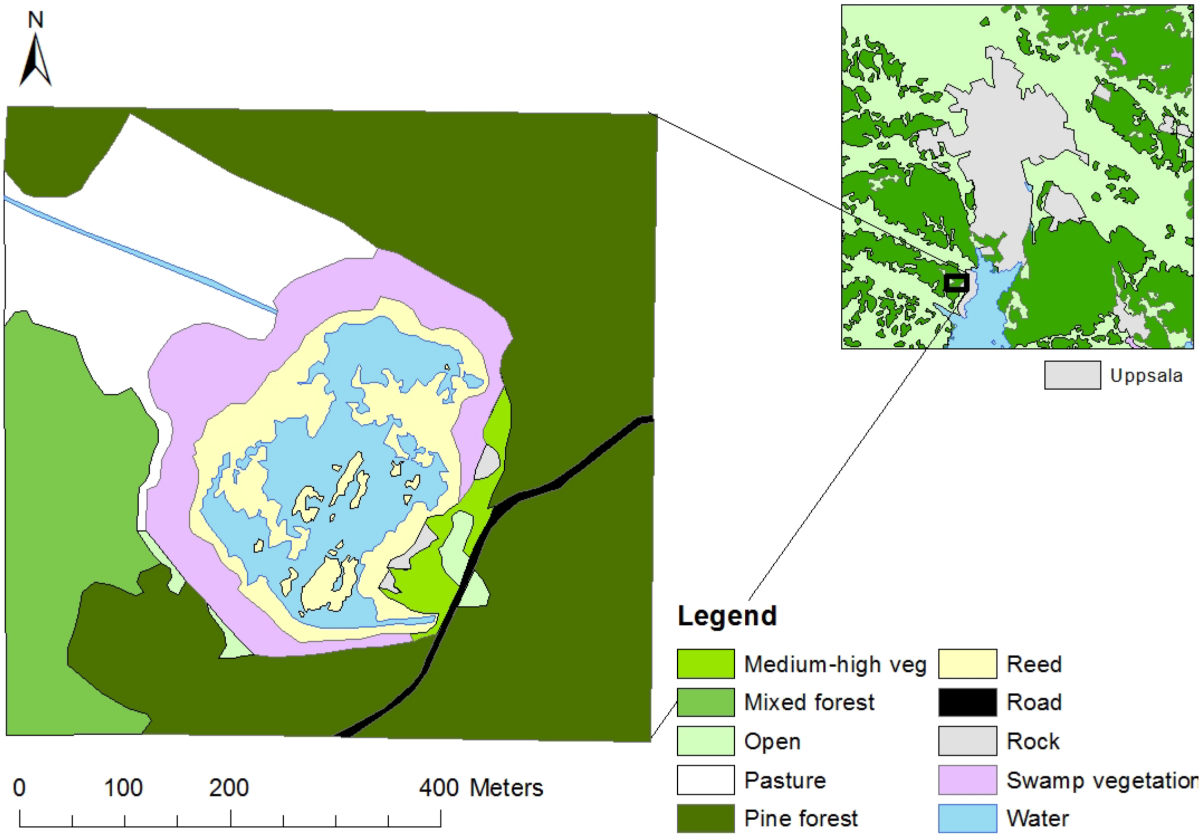


Figure 3. Map showing the area of Dalkarlskärret, Uppsala

2.3 Methodology

Female grass snakes were caught by hand in spring of 2014 at the sites described above and stored in plastic boxes (50 l) with a layer of heat pellets and access to water, shelter and food in the form of herring. Heat mats were put under the boxes providing the snakes with a constant temperature of 27°C. Three females from each population were chosen (labelled as S1, S2 and S22 from Stockholm and U16, U21 and U25 from Uppsala). Snakes were weighted before being equipped with a radio transmitter (model PIP 3, Biotrack Ltd, Dorset, Wareham, UK) featuring a thermistor which increased the pulse rate when the temperature rose. This could be used as an approximation of the relative body temperature of the snakes. The transmitters were fastened behind the cloaca to not hinder egg-laying or food passing through the gut. They were glued on to the scales using cyanoacrylate glue (super glue, Loctite®) a method used in previous studies (Tozetti & Martins 2007, Madrid-Sotelo & García-Aguayo 2008). A thin thread was wrapped around the snake and the transmitter several times and glued to make the structure rigid enough to prevent it from falling off. Two layers of duct tape (Pattex®) were then wrapped around the transmitter to further secure it. Lastly, the package was covered in two layers of surgical tape (3M®) to prevent dirt to enter beneath the duct tape and loosen it (figure A1). The resulting transmitter package weighted 4 g which was below the threshold of 5 % of the body weight used by most snake researchers (Blouin-Demers & Weatherhead 2001, Tozetti *et al.* 2009, Conelli *et al.* 2011). The package was no larger than the thickest part of the snake as to not hinder the snakes' movement. Furthermore it did not hinder the blood flow to the tail as the snakes could still move it when the transmitter was fastened. The females were left together in room temperature for six hours to make sure the package did not fall off and give the glue time to dry. This method of attaching the transmitters is reminiscent of previous studies on other snake species with the exception of the glued thread (Tozetti & Martins 2007, Madrid-Sotelo & García-Aguayo 2008).

The use of external transmitters was chosen here instead of the more commonly used method of surgically implanted transmitters used in most other snake studies as the handling time is generally low and reduces the stress put on the animals (Blouin-Demers & Weatherhead 2001, Gerald *et al.* 2006, Figueroa *et al.* 2008, Pattishall & Cundall 2008). The method is however unsure as few previous studies have used external transmitters on snakes and it is mostly suitable for short-term studies (Ciofi & Chelazzi 1991, Tozetti & Martins 2007, Madrid-Sotelo & García-Aguayo 2008, Wylie *et al.* 2011). Methods such as mark-recapture would not work here as it does not give the high resolution data needed to find oviposition sites and accurately determine movement pattern and degree of activity. GPS tracking could have been performed but the location errors due to variation in connectivity between different days would have been too large on the small scale studied here.

The six snakes were released at Stora Skuggan in the beginning of June. They were then tracked two to three times each day for 25 days using a sika receiver (model SIKARX4, Biotrack Ltd, Dorset, Wareham, UK) and a three-element Yagi antenna (model LINFLEX3, Biotrack Ltd, Dorset, Wareham, UK). The snakes were tracked using a homing-in technique where the signal is followed until the individual is seen. This technique is suitable for tracking animals that do not move over larger areas (White & Garrott 1990). Each individual was tracked during approximately the same time period each day (10:00 AM, 12:00 PM and 14:00 PM). It was not always possible to see the snakes as they sometimes hid in burrows or under dense vegetation but the equipment could locate the snakes down to the nearest decimetre and a location could always be recorded. When they were found, care was taken to minimize disturbance. At each location, I measured the ambient temperature close to the ground, the body temperature of the snake (measured as the pulse rate from the transmitter) and the amount of light the snake was subjected to. Light levels were measured using a light meter (Velleman DVM1300). The micro- and macrohabitat was assessed visually and recorded alongside the activity of the snake. Lastly the coordinates for each site was recorded on a handheld GPS (Garmin 60CSX). The females were recaptured one to two times each week to make sure the transmitter was still attached and to see if they had or were close to oviposit. Eggs can sometimes be felt on the belly of the snakes as they take up a large portion of the females' body. After oviposition, the skin tends to be loose towards the posterior part of the body, a characteristic that can be seen several days after oviposition (pers obs.).

The package did not restrict movement to any high degree as the snakes were seen swimming and moving through vegetation with little problem. On occasion, the snakes got stuck in denser parts of the vegetated areas but they were able to get loose with little problem. I helped untangle them a few times to reduce the stress put on the snakes when I was nearby and reduce the pressure exerted on the transmitter package when the snake tried to free itself through force. Movement was limited around the package as they could not bend this part as well but this stiffness seemed to disappear after a few days in the field. This method did not hinder oviposition as some of the collected females laid eggs in captivity with the transmitters still attached and no discernible damages on the eggs or the snake. These females were not used in the study. One problem experienced with this attachment was that it led to smaller abrasive wounds anterior of the package. After tracking, the translocated snakes were returned to their original site.

The methods used here were approved by the Swedish ethical board (Stockholms Norra Djurförsöksetiska Nämnd; ethics approval number N77⁄14) and the snakes were collected with permission from the county administrative board of Uppsala and Stockholm.

2.4 Analysis

2.4.1 Movement

A vector map was created over the study area in ArcMap v. 10.1® using an ortophoto provided by the Swedish Ordnance survey. The recorded data points were imported from the GPS into Microsoft Excel® using the supplied program (MapSource®) and later transferred into ArcMap where they had to be transformed from the WGS84 coordinate system to RT90 to fit with the ortophoto. As the connection of the GPS varied between different locations in the field, the accuracy of the unit was not always correct. This led to several data points being displaced meaning they had to be moved manually using notes on macro- and microhabitat I had collected at each point. The error from the GPS was never more than five meters off from the true site. This had to be done because of the small scale of the study area as the errors in the habitat choice probably would have been high. The limited amount of locations along with the sedentary behaviour of the snakes minimizes the errors that might have arisen when manually moving the points. Using the tracking analyst toolkit in ArcMap I calculated the Euclidean distance between each point for the six individuals. By using the Euclidean distance, the true distance travelled is underestimated as animal seldom move in a straight line but given the low movement distances and the short time period between relocations, the errors are probably small.

The distribution of movement distances were strongly skewed towards values close to zero and it was not possible to transform the data into a normal distribution. Thus many of the models used were non-parametric. Furthermore, the small sample size made it difficult to draw any solid, statistical conclusions regarding the behaviour of the snakes in the different populations. Therefore, the analysis is restricted to comparing the different individuals to each other, mainly through regression analyses and correlations with movement distances as the main response variable.

Home range sizes for the individual females were estimated using the Alphahull package in R using the spatial coordinates for each individual (Pateiro-López & Rodríguez-Casal, 2010). Movement distances were summed for each day and correlated against average light conditions during the day and the pulse rate of the transmitters. Linear relationships were correlated using Spearman's correlation test. The analyses were done in Minitab® v.16 and R® v. 2.14. Friedman's test was used to compare the movement distances between the different individuals as it is the non-parametric equivalent of the repeated measures ANOVA which accounts for individual variation as well as total variation. As Friedman's test assumes equal sample size between the different individuals, some days had to be removed as the individual snakes where recaptured at different times. Friedman's test was also used to test differences in movement during different times of the day as they were located during the same time periods each day. This creates measurable distances made between morning-midday, midday-evening and evening-morning (the non-tracking period).

2.4.2 Habitat use

The vector map was divided into 14 habitat classes based on the different habitats the snakes had used and how they might differ from the snakes' point of view. Grasslands were divided into two categories depending on the height; areas with vegetation up to ~0.5 m were classified as medium-high vegetation and areas higher than that was classified as high vegetation. To analyse the different habitats used by the snakes I narrowed down the study area to only encompass the main area where they had moved (figure 4). Three points from snake 21 where not considered when doing this as these were located far away from the core area where the locations from the other tracked individuals were and it would make the resulting image include a non-representative amount of forested area.

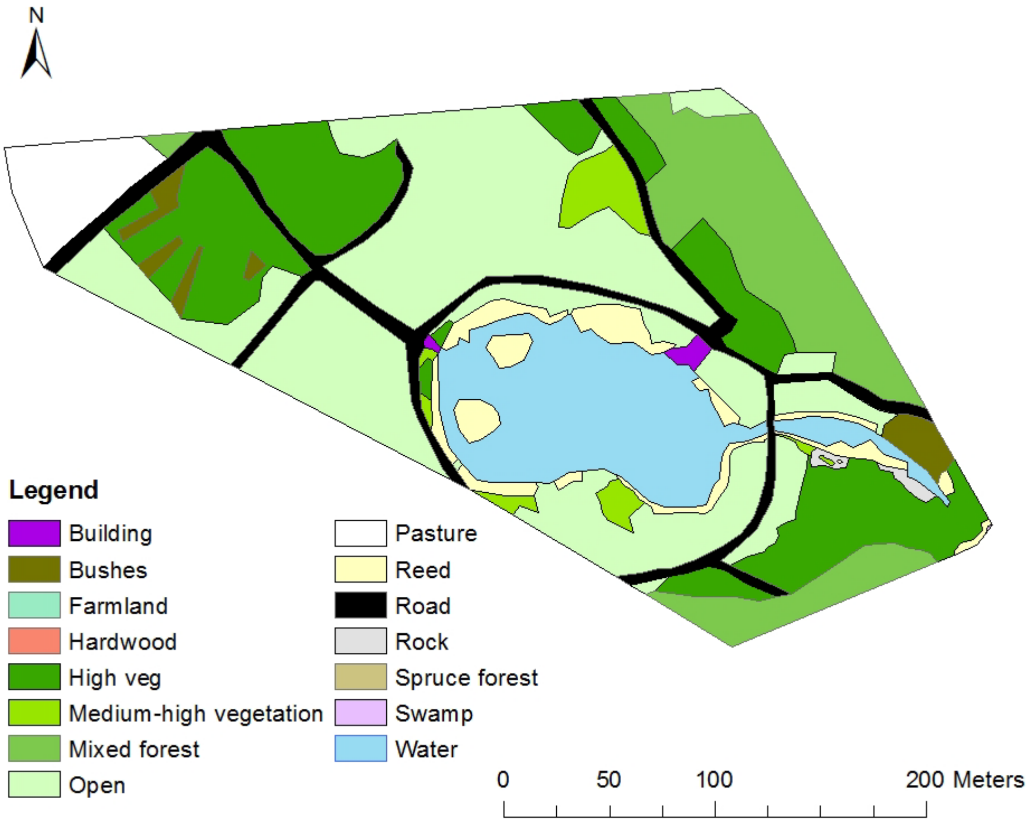


Figure 4. The main study area where the snakes were located during the tracking period.

The number of points within the different habitat classes was counted and the proportion of points in each habitat class calculated for each individual along with the total proportion available of the different land classes. To find a potential preference or avoidance of a certain habitat I used Jacob's index, calculated as $D = \frac{r-p}{r+p-2rp}$ where r = the proportion of a specific habitat used and p = the available proportion of that habitat (Jacobs 1974). To see how the temperature differed in the habitat classes I calculated the difference between the air temperature and the ambient temperature, the relative temperature.

3 Results

One of the six transmitters stopped working after four days which led the final data to consist of records from five individuals (S1, S2, U16, U21 and U25). Two transmitters fell of when the snakes shed after 17 and 24 days. Tracking was cancelled after 25 days as only three individuals remained. Four individuals were successfully tracked between the 17 June and 11 July and one from 17 June to 3 July. Despite the intensive tracking, no oviposition sites were found.

3.1 Differences in movement patterns

Mean daily distances varied between 19.11 m (S2) to 71.93 m (U21) with an overall mean of 43.83 m (figure 5). No discernible difference in movement distances could be seen between local and translocated individuals. S2 was the only one that moved significantly less than any other snake (Friedman test; S1, $\chi2(1)=10.67$, $p=0.001$; U16, $\chi2(1)=5.26$, $p=0.022$; U21, $\chi2(1)=11.64$, $p=0.001$). Home range sizes varied from 0.28 ha (S2) to 4.99 ha (U21) with a mean size of 1.81 ha (figure 5). Two of the translocated snakes had larger home ranges than the local individuals.

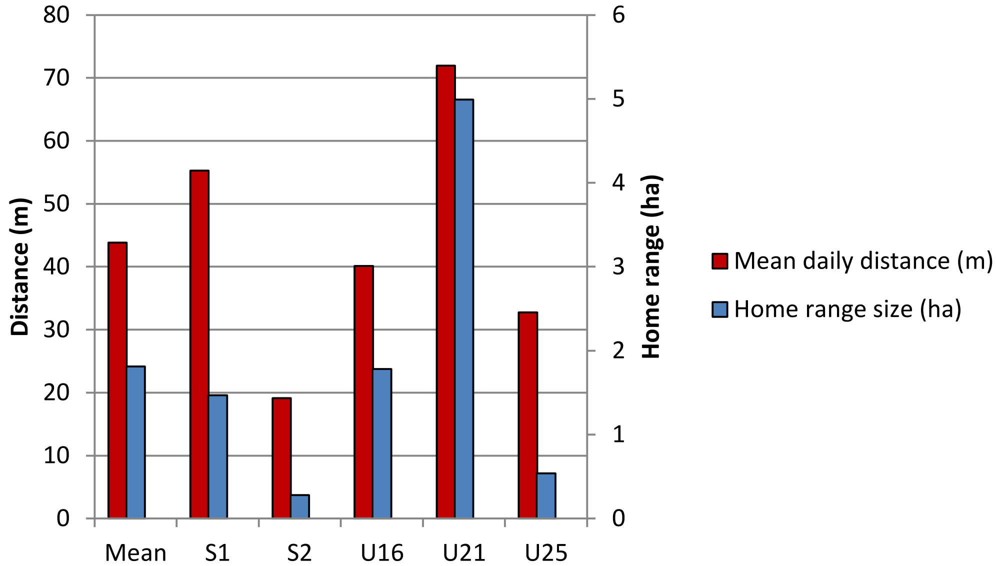


Figure 5. Mean daily distance moved for the five grass snakes along with their home range sizes.

Even though the difference was not significant in all cases, two individuals, S2 and U25, tended to move less than the other three individuals (figure 6). The movement pattern between the different individuals was, however, similar as they were more active around the same time periods, most notably between June 24th and June 27th as well as around July 4th. Periods of longer travels were often followed by more sedentary periods but U21 showed a more restless behaviour than the other individuals as she was less prone to stay in the same place for long.

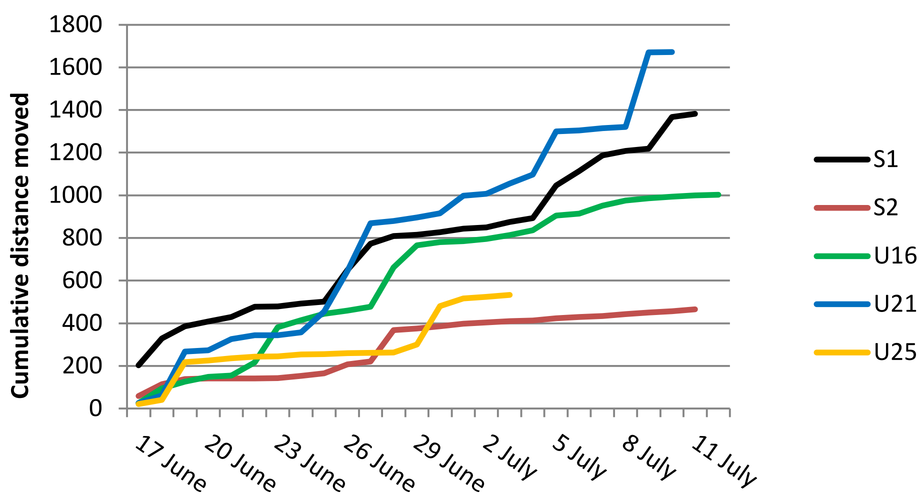
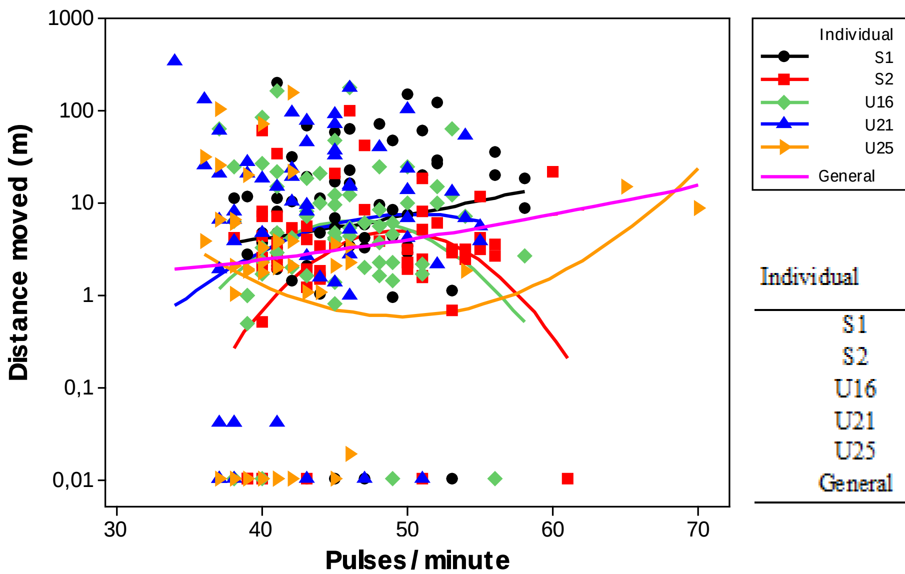


Figure 6. Cumulative distances moved for the five studied grass snakes over the study period.

3.2 Factors behind movement patterns

Both body temperature and light intensity explained little of the movement patterns although temperature was a slightly better predictor (figures 7, 8). The variance between the different individuals were however large for both of these factors. Generally, movement distances increased at higher temperatures but some individuals seemed to have an optimal temperature where they moved over longer distances (S2, U16 and U21) while S1 showed a more linear relationship (figure 7). U25 moved over greater distances at lower and higher temperatures as opposed to the others. Activity was further seen increasing for all individuals when the day-time temperature increased to approximately 20°C (figure A3).

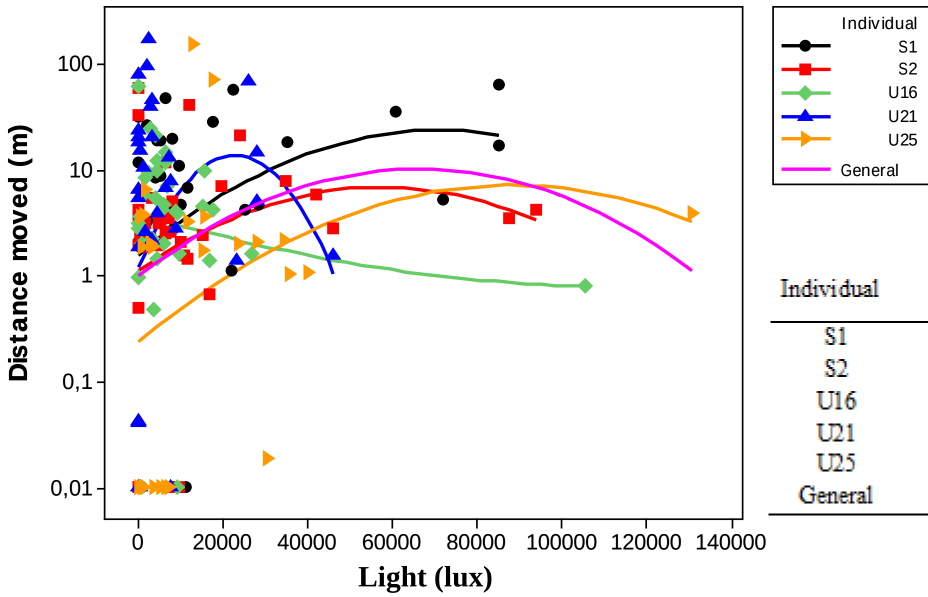


Individual	R^2
S1	1.8
S2	14.2
U16	6.3
U21	4.2
U25	5.3
General	17.5

Figure 7. A quadratic relationship between the movement distances for the individual snakes and the pulse rate of the transmitter used here as a proxy for body temperature with 46 ppm~22°C. A general trend is also displayed. The relationships are generally weak and ambiguous. 0.01 was added to zero values before log-transforming them to enhance visibility of the plot.

Temperature was further shown to have some effect on movement distances as the individuals generally moved more during the day than during the night (Kruskal-Wallis; $\chi^2(1)=5.3686$, p=0.0205). However, movement tended to be lower during midday, although not significant and with some variation between the different individuals. Dividing the day time tracking period into two groups, movements made between morning-midday and midday-evening, revealed that movement was generally significantly higher during the non-tracking period between the evening and morning next day (Friedman test; $\chi^2(1)=8.19$, p=0.017). This is not suprising as this time period spanned approximately 19 hours. Looking at the different individuals however, reveals that only U21 displayed this behaviour (Friedman test; $\chi^2(1)=6.04$, p=0.049) while the others showed indications to move more during the nn tracking period.

Similar to the relationship to body temperature, movement was generally higher around an optimum value of light intensity (figure 8). The optimal level differed between the different individuals but most individuals showed this pattern. The only exception was U16 who showed a decreasing activity at higher light levels. The snakes were seldom found basking in direct sunlight on clear days as indicated by the few, high values of light intensity.



Individual	R^2
S1	11.0
S2	5.1
U16	2.4
U21	6.6
U25	8.3
General	9.2

Figure 8. A quadratic relationship between the average light intensity and movement distances for the individual snakes as well as a general trend. The relationships are generally weak. The ambiguous results could be a consequence of the different habitats utilized by the individuals. 0.01 was added to zero values before log-transforming them to enhance visibility of the plot.

3.3 Differences in habitat use

Reed was the most preferred habitat class for most of the grass snakes tracked here as between 36 and 74 % of the points were located in this habitat for four of the five individuals (U16 resp. U25). S1 used this habitat in proportion to its occurrence and instead showed a higher preference for unmanaged grasslands than the other individuals (high vegetation; figure 9). This habitat class was preferred to a smaller degree by the other local snake, S2, and actively avoided by two of the translocated individuals (U21 and U25) while U16 did not show any relationship at all to it (figure 9). Few points were located in forests and open areas indicating a general avoidance of these habitats despite how common these classes were (14% resp. 33% of the study area in figure 4). Generally, snakes tended to be close to good hiding places, either burrows or dense vegetation and were generally close to habitat edges. This was especially seen with U25 who, for the most time, was found in a reed belt on the edge to the open road and lawn (figure A8).

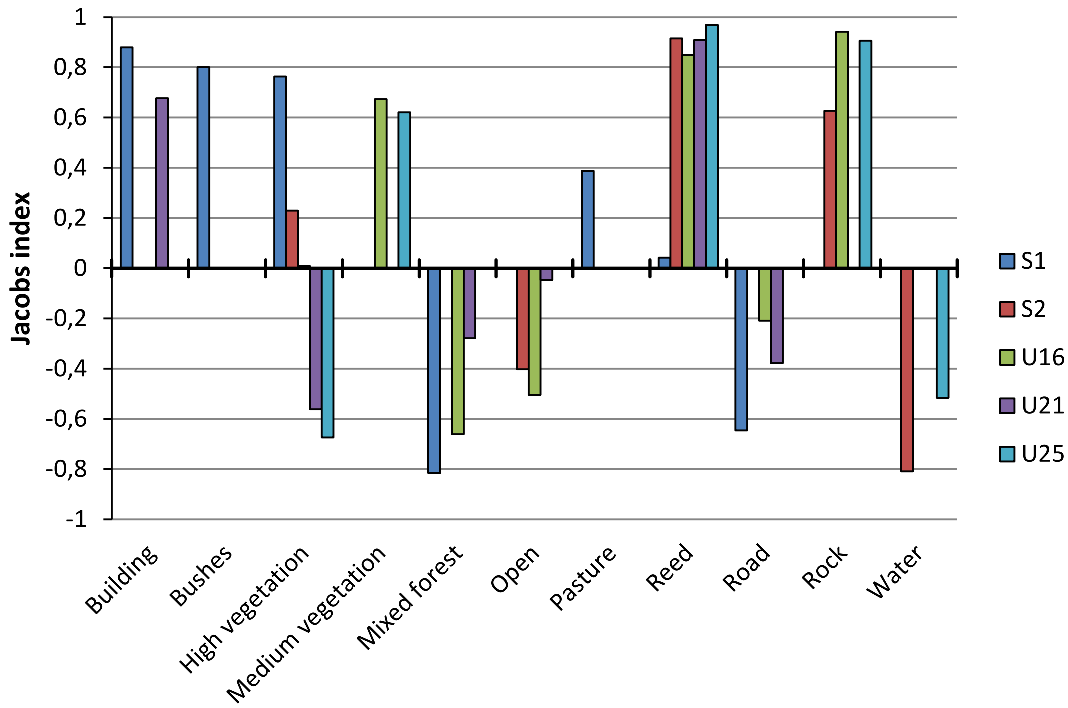


Figure 9. Jacobs index for the different individuals showing relationship to the different habitat classes used here. Values close to 1 indicate preference while values close to -1 indicate avoidance for the specific habitat.

Most of the habitats used were warmer than the average air temperature ranging from 3 °C (water) to 7.8 °C (pasture) degrees above air temperature. Burrows and buildings were the only classes that were colder than the surrounding air (0.26 and 0.925 °C below air temperature; figure 10). On average, the different habitat classes were 3.68°C warmer than the air. The variation in some classes was large but few of the classes ever reached temperatures that were below or equal to the average air temperature.

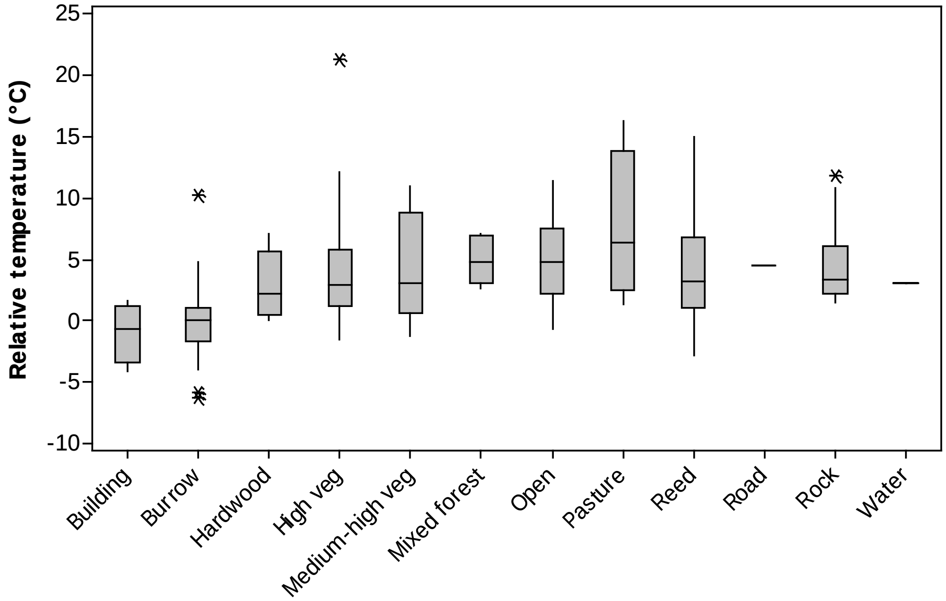


Figure 10. Box plot of the relative temperature of the different habitat classes compared to the air temperature. Most habitat classes tended to be warmer than the surrounding air providing the snakes with a special microclimate.

4 Discussion

Here I have studied the adaptability of three grass snake females to a new environment by means of radio tracking in an effort to locate oviposition sites and study how adaptive grass snake females are to new environments. No eggs were found but the translocated snakes seemed to behave differently from the local individuals, despite the low sample size. A general preference for vegetated areas was seen for the snakes although the preferred type of habitat differed between individuals and movement was generally low compared to earlier studies. Temperature was a better predictor to how much the grass snake moved although it only explained a small portion of the variation.

4.1 Adaptation

Even though there was no difference in movement distances between the individuals from the two populations, two of the translocated snakes had larger home ranges than the local individuals which means they moved over a larger area (U16 and U21; figure A2). These two also behaved differently from the other snakes as they were less sedentary than the other tracked snakes. (figures A4-A8). This behaviour is similar to what has previously been shown for translocated animals (Plummer & Mills 2000, Butler *et al.* 2005, Rittenhouse *et al.* 2007). The third translocated individual, U25, was more stationary than the other translocated individuals which could be explained by the fact that she was in a moulting phase for most of the study period. During moulting, movement is limited and the snake usually stays in the same location throughout the process (Madsen 1984). When preparing to moult, grass snakes usually try to locate a protected site as they become vulnerable during this time period. U25 however, chose a suboptimal site in a relatively thin reed belt where the disturbance factor from people and dogs seemed to be high. The reason why she stayed at this site could either be a result of stress as she did not have enough time to locate a better place for moulting when she was rereleased or it could be that she could not find a better place due to being unfamiliar to the area. S2, the other snake that successfully moulted during the study period found a better site where she appeared to be less disturbed. The restless behaviour of U16 and U21 along with the poor habitat choice of U25 could indicate a poorly adaptive capacity of these snakes or at the very least, that they did not have enough time to adapt to the new area. Furthermore, S2 and U16 started preparing to moult around the same time (June 29th) but while S2 hardly moved over the coming two weeks, U16 was more active, possibly due to her being unfamiliar with the area (figure 6).

The reason behind the generally high activity seen for U16 and U21 might however, also be a sign of them being gravid and trying to locate a good oviposition site as movement usually is high during this time period (Madsen 1984, Reading & Jofre 2009). If that was the case, however, it would be assumed that they should cease being active after oviposition and adopt the sedentary behaviour of non-gravid females. Instead they showed high activity throughout most of the study period. U16 became less active towards the end of the study period but this was likely due to her preparing to moult. Furthermore, no eggs were felt on the females but this is not a definite method to recognise a gravid female. However, U21 was difficult to locate and catch which means it is possible that her increased activity was due to nest searching and that her prolonged activity was due to foraging. It is not possible to draw any solid conclusions based on these few individuals as the patterns displayed could be the cause of random variation between individuals.

Another aspect that seems to separate the translocated and the resident snakes is the habitat choice. While almost all snakes (except S1) showed a preference for reed (figure A9), the translocated snake showed an avoidance of high vegetation (unmanaged grassland, figure A10), a habitat class that is uncommon at the site in Uppsala. High vegetation was used to a higher degree by the resident females although S1 was the only one showing a strong preference for it. The translocated females instead kept closer to the reed belts, a habitat that is more common at the site in Uppsala than in Stockholm based on the maps (figures 2 & 3). Staying close to familiar habitats is a trait that has been seen in translocated reptiles further supporting the fact that the females from Uppsala did not adapt to their new site (Rittenhouse *et al.* 2007, Stamps & Swaisgood 2007). Few studies have been made on translocated snakes and neither of them saw any adaptation, even over larger time spans (Plummer & Mills 2000, Butler *et al.* 2005). This might be the case for the grass snake as well but more data over a longer time period is needed to accurately draw any conclusions.

4.2 Movement patterns

Movement distances observed here were generally lower than what has previously been reported for grass snakes during the same time period but the activity pattern is similar to what has been seen earlier (Madsen1984). Movement is generally high for female grass snakes as well as other oviparous snakes towards the end of June and beginning of July as they locate oviposition sites and daily distances can be approximately 100 m (Madsen 1984, Ciofi & Chelazzi 1991, Shew *et al.* 2012). These long movements are often made when resources are unevenly spread out in the landscape and longer excursions are needed to reach these resources (Shew *et al.* 2012). The relatively short distances covered by the females in this study could either indicate that they were not pregnant or that good nesting sites can be found within their home ranges. This last alternative might not be too improbable since many habitat classes were on average 3.68 °C warmer than the air temperature although this temperature needs to be kept during night as well. However, most of the sedentary periods can be explained by the individuals moulting (S2, U16 and U25) or digesting (S1), activities that are shown to lower movement for snakes (Madsen 1984, Plummer & Mills 2000).

Movement was weakly affected by both light levels and temperatures but the latter seems to play a larger role than the light levels as movement distances were longer during days when the temperature rose. This was probably mostly true for a specific range of temperatures as movement was generally lower during midday when it was the warmest and higher during the morning and evening/night. This was especially prevalent during the last week of tracking when the temperature rose to approximately 30°C and movement during the day was lower than seen during colder days. The rapid increases in movement seen for the females happened when the temperature was low and rose to approximately 25°C which probably lies within the optimal temperature range for the grass snake, something that has been observed for other snake species (Butler *et al.* 2005, Hutinec & Mebert 2011; figure A3). This could also explain the increased movement during the non-tracking period between the evening and morning. Even though this time period represents a time span of 19 hours, movement was not much higher than seen during the two hour periods suggesting that movement here is limited. Movement might be higher around the evening as the temperature falls, reaching the proposed optimum and then decrease during the night and morning when temperatures are relatively low. Movements during the evening might be especially prevalent when the temperature has been too high for the snakes during the day as it gets colder towards the night.

4.3 Habitat use

Most of the locations (88%) were recorded in reed, high vegetation and in open areas which was where the strongest preferences and avoidances can be observed. All females but one (S1) had a strong to very strong preference to the reed belts where they mainly used the thicker belt on the northern side of the pond where the sun shone throughout most of the day, providing heat (figure A9). A preference for reed has not been reported for grass snake earlier although this might be due to a lack of this habitat in the study sites of previous studies (Madsen 1984, Reading & Jofre 2009). At the site studied here, reed seems to be important as many local snakes were seen here along with the tracked females. The unmanaged grasslands used by S1 and, to a lesser degree S2, might not be as commonly used as fewer local snakes were seen here but that might be due to the reduced visibility here because of the denser vegetation (figure A10). Structurally, high vegetation resembles reed in that it provides access to good basking areas, shelters and a similar relative temperature (figure 10).

Two females were seen visiting the water pumping station on the western side of the pond on two separate occasions. This building seems to be used by a few individuals as evident by the moults found inside. The heat generated by the machine during the night and early morning might create a suitable resting place for the snakes. Even though building as a habitat class had a generally lower temperature than the air (figure 10), these measurements were taken when the machine was not active. Anthropogenic structures like this is often seen being used by snakes ranging from crevices beneath railroads and in cement walls to manure heaps and composts and is likely linked to the stable temperature often associated to these structures (Pattishall & Cundall 2008, Conelli *et al.* 2011, Shew *et al.* 2012). If the temperature around this building is stable throughout the day it might also make a good oviposition site although this needs to be investigated further.

Similar to previous studies, the females here were often close to potential retreat sites, something that has been observed for several different snake species (Madsen 1984, Durner & Gates 1993, Reading & Jofre 2009, Wastell & Mackessy 2011, Martino *et al.* 2012). It is believed that snakes use this kind of habitat to get the benefits of both the open, sunny area where they can bask and the closed, protected properties of nearby bushes or crevices where they can escape threats as well as the sun when it becomes to warm (Martino *et al.* 2012, Shew *et al.* 2012). This might also be a reason why grass snakes has been observed to use the forest edge when travelling over larger distances, something that was not observed during the short time this study took place (Durner & Gates 1993, Reading & Jofre 2009). Something that seemed to be rather common here was the use of structures created by other species, mainly burrows dug by water voles and trampled areas in the high vegetation created by both humans and local wildlife. Similar behaviour has been reported earlier (Wisler *et al.* 2008).

4.4 Management implications

The main habitats used by the grass snake were areas not commonly used by humans and where little active management is done, reed belts and the grasslands. These areas seem to be important for the local grass snakes, especially the reed where many other grass snakes were seen and as such, disturbances in these areas should be kept low. The thicker reed belt on the northern side of the pond was used more frequently than the other belts around the pond which indicate that this site is especially important, possibly due to it facing the sun throughout the whole day. Reed around the entire pond was, however, used to some degree, as both tracked and non-tracked snakes were seen around the entire pond but thicker belts (>2m) might offer more protection as it is easier to hide there. Parts of the reed belts are removed every year to prevent overgrowth (pers. comm.) and care should be taken when deciding what parts are to be removed. If larger actions are planned close to the reed belts, they should preferably be made during times when the habitat is not used, either during early spring before they emerge or after they enter the hibernacula during autumn. These belts are also used by the birds nesting in the pond which further shows the importance of the reed.

No movement was seen between the different ponds in the area and it is unknown how much movement is made between them. As it is now, dispersing snakes must cross the open areas to get to new areas, a habitat that is dangerous for the snake as they are unable to protect themselves if attacked. To facilitate the exchange of individuals between these ponds and possibly increase gene flow between them, patches or corridors of higher vegetation could be constructed, preferably close to the forest patches. Forest edges have previously been observed to be used by grass snakes and might increase the opportunities to travel between ponds in the area (Wisler *et al.* 2008, Reading & Jofre 2009).

Where the females from this population lay their eggs remain a mystery as there are no known composts or manure heaps close to the area, sites that are commonly used by grass snake (Madsen 1984, Kreiner 2007). It is also unknown if the population at Stora Skuggan suffers from this or if there are sites with the necessary requirement for the eggs. It is possible that that the population is acting as a sink population, gaining new individuals from subpopulations closer to a good oviposition site further away. However, given the relative high temperature in many of the habitats, it might be possible that there are sites close to the pond area that works well enough for the individuals in the area. Especially the water pumping station might be important even though it needs to be investigated further. Recently, reports have surfaced of eggs being laid in stumps and rotting wood, sites that are used in warmer parts of Europe but which seem to be too cold for the eggs in Sweden (Löwenborg *et al.* 2011, Kärvemo, unpublished data). It is possible that similar places are used at Stora Skuggan as some snakes, both tracked and non-tracked where seen in the forests. If structures like this are used they need to be saved, preferably with unmanaged vegetation around them where the hatchlings can hide when they hatch.

4.5 Future studies

More data is needed regarding the grass snake, especially what sites provide a good environment for the eggs when manure heaps are not available. Future studies should focus on finding these sites and evaluate how optimal they are. Furthermore, studies aimed at investigating how habitat use and movement pattern differs between different climate zones should also be performed. The environmental conditions in Sweden are different from those in other parts of Europe and knowing how they differ and how the grass snake adapts to these differences might enable us to make better management decisions. These kinds of studies should be made over a longer time period, preferably over several years, following individuals of different age and sex to see how their spatial patterns differ from each other. Internal transmitters should be used to better follow individual snakes for such a long time. Long term population surveys are also needed as they can tell us something about population structure and fluctuations. These should be made on both a local and national level and should start as soon as possible since the species already seems to decline in several countries. Options for artificial oviposition sites should also be researched as the lack of nesting sites is widely regarded as one of the main threats for this species.

4.6 Conclusion

The grass snake is not a threatened species according to the IUCN red list but as shown by Hagman *et al.* (2012) they might be decreasing in Sweden because of the lack of oviposition sites. Little else is known about the grass snake and no proper surveys have been made on population structure and fluctuations which make it difficult to say if something needs to be done to preserve the species. Snakes in general are tricky to study because of their cryptic lives and a body form that makes it difficult to accurately track them over longer time periods. In this report I have presented a partly new method for attaching radio transmitters on snakes and the first data on movement and habitat use for grass snake in Sweden in 30 years. The small sample size and the limited time tracking was performed prevent any general conclusions to be drawn about the species but some indications are fairly prevalent. Sunny areas with high vegetation seem to be important for the grass snake as they stay in the same place for long periods of time, mainly for moulting and digesting and need to stay hidden during these times while still having access to sunlit areas. Long distance movements seem to primarily be made when the temperature is rather low, possibly to avoid overheating although many other factors likely affect the movements.

5 Acknowledgements

I first would like to thank my two supervisors, Marcus Hedblom and Simon Kärvemo at SLU who provided valuable advice throughout this project and also made it possible. I would also like to thank Erik Öckinger and Teresa Soler at SLU who provided some valuable advice on this project as well. Kristin Löwenborg Di Marino at Stockholm University also helped with knowledge of the snake population at Stockholm. A big thank you also goes to the people at Kungliga djurgårdsförvaltningen who helped with information about the area and its current management and access to the pumping station. The people at the 4H farm at Stora Skuggan helped with access to the pastures and information about how they manage the manure from the area. Lastly I would like to thank Johannes Forkman and Jonas Knape at SLU who helped with understanding the statistical procedures used here.

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Appendix

Figure A1. The transmitter attached to U21.

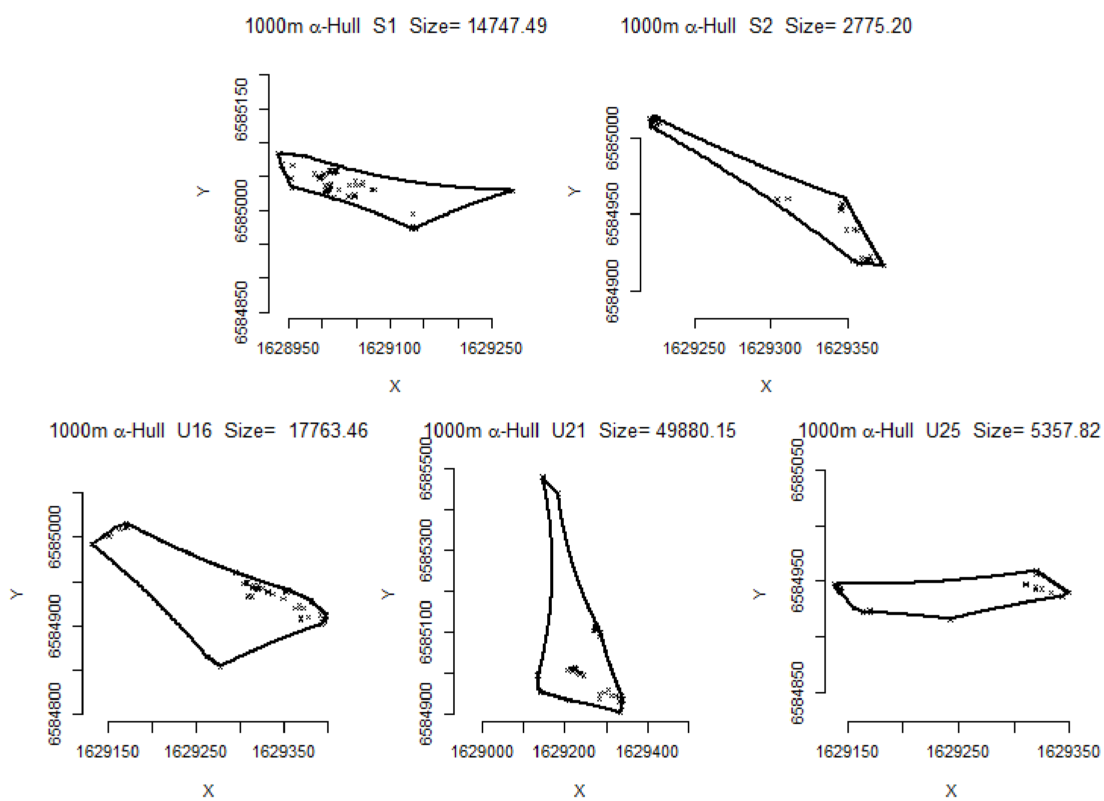

Figure A2. Home range sizes for the five tracked grass snakes.

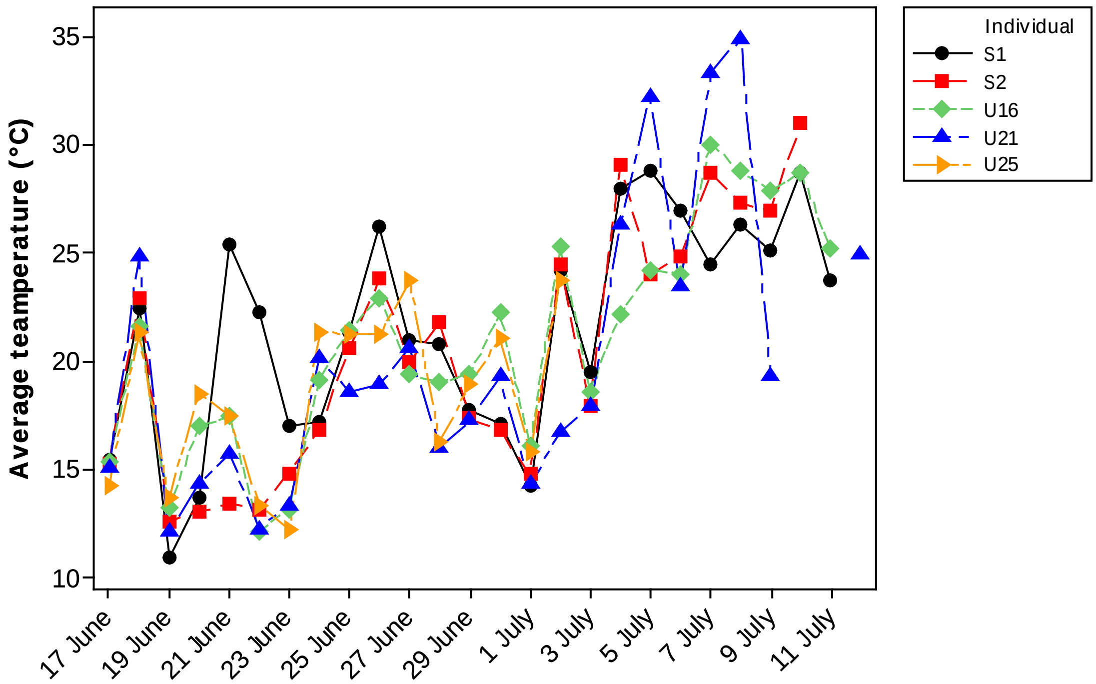

Figure A3. Average temperature for each point where the females were found.

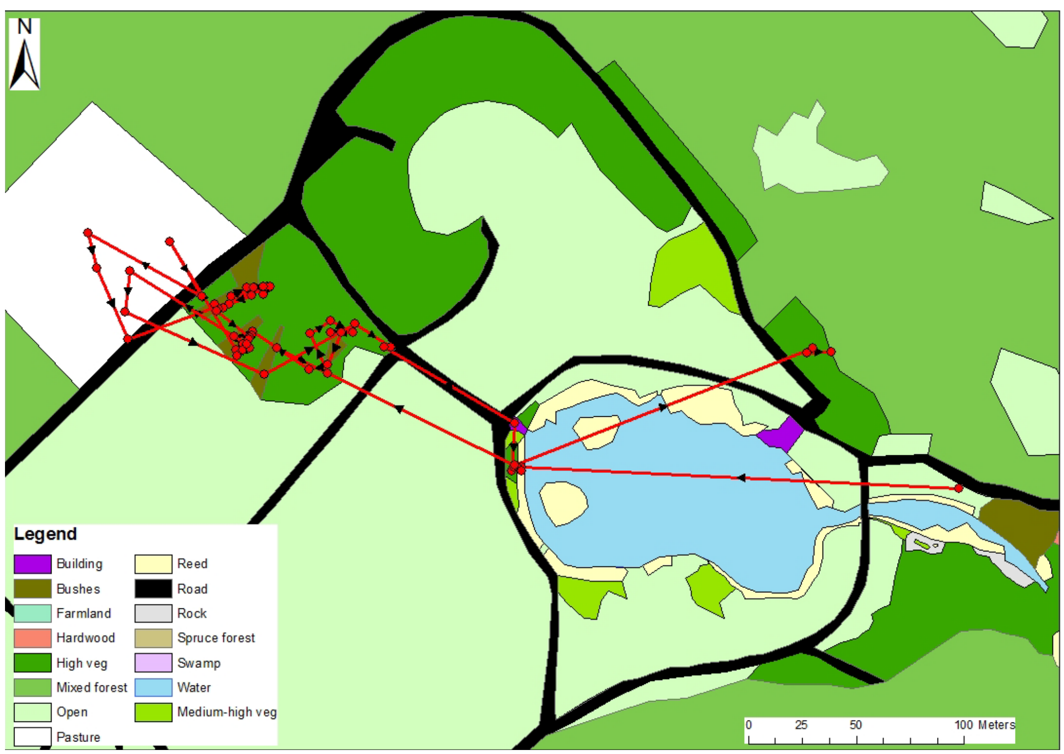

Figure A4. Movement of S1

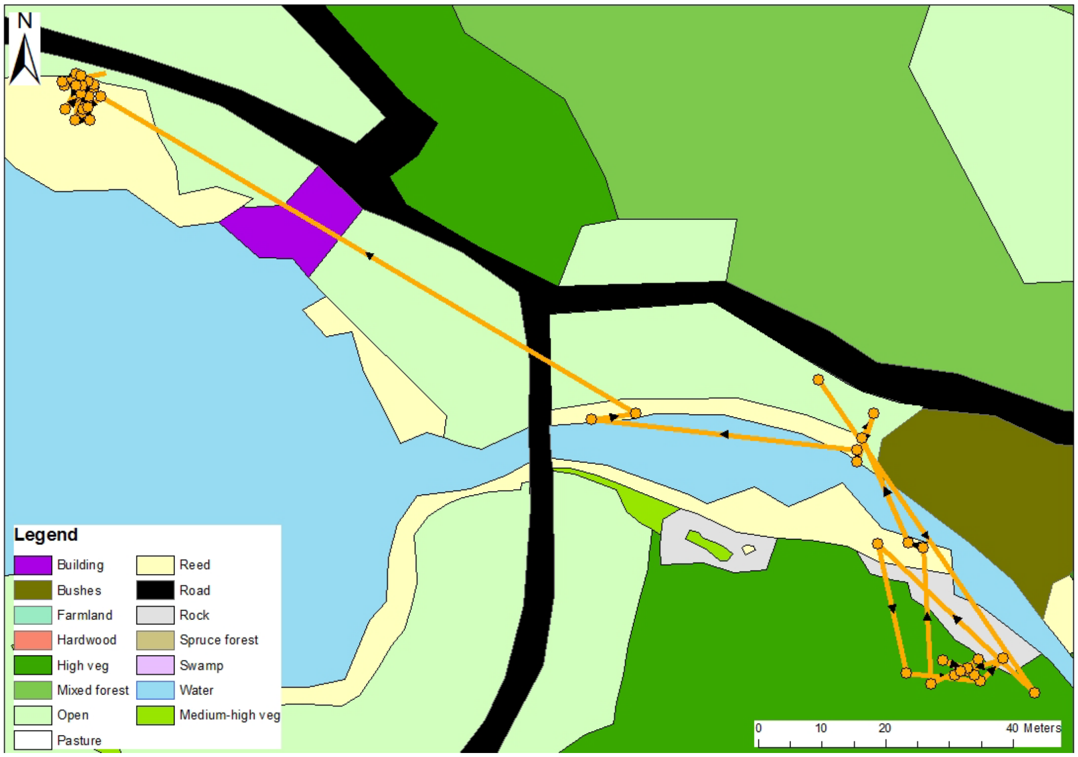

Figure A5. Movement of S2

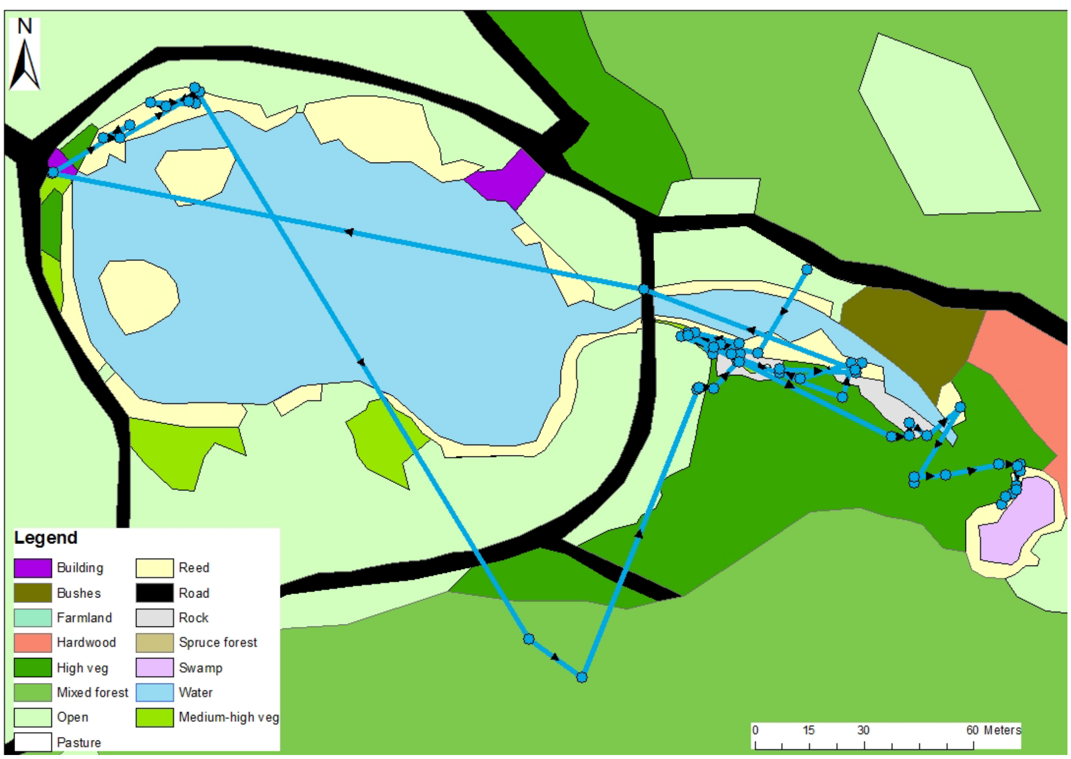

Figure A6. Movement for U16

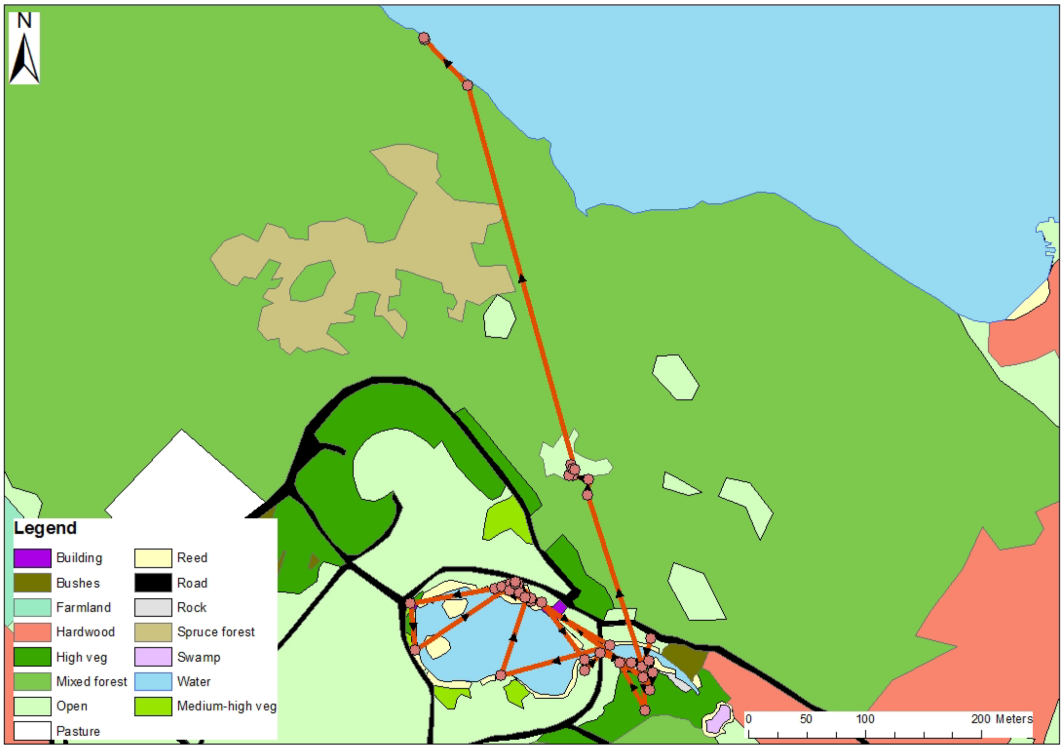

Figure A7. Movement of U21

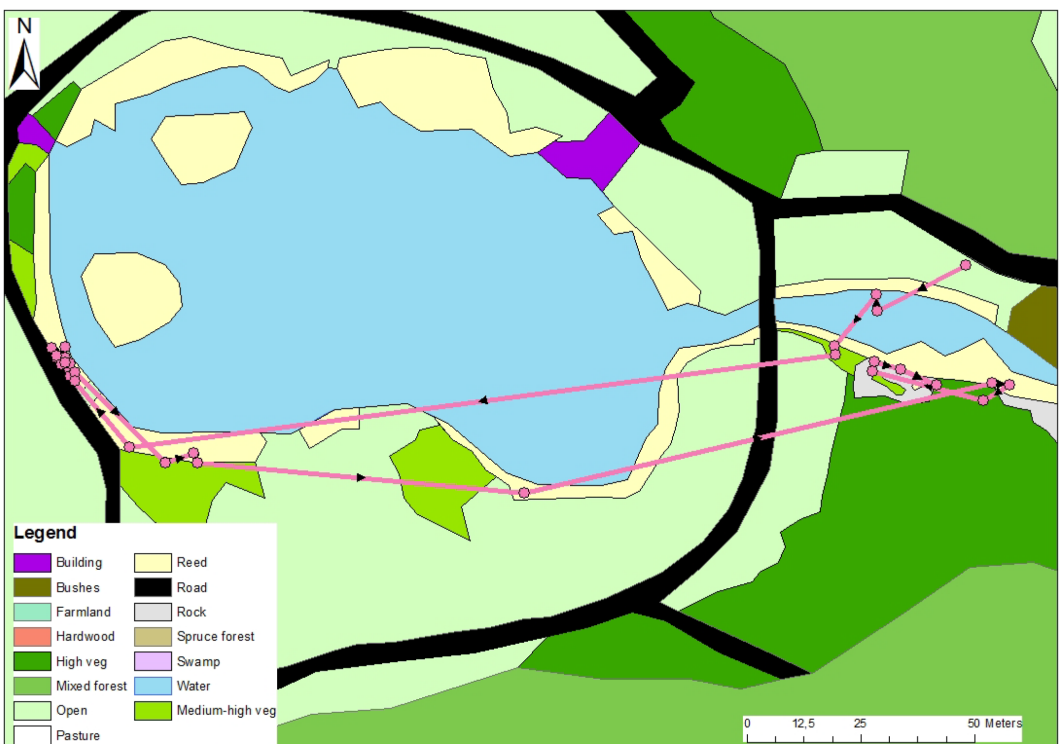

Figure A8. Movement of U25

Figure A9. Picture of the large reed belts in the northern and western part of the pond.

Figure A10. Picture showing the unmanaged grassland