“Distribution of the Tree frog in Southwest Drenthe in relation to landscape quality

August, 2016

Hannah Böing & Fraukje Sportel
HOGESCHOOL VAN HALL LARENSTEIN UNIVERSITY OF APPLIED SCIENCES

RESEARCH REPORT

"Distribution of the Tree Frog in Southwest-Drenthe in Relation to the Landscape Quality"

This report is written in the context of the Bachelor of Animal Management majoring in Wildlife Management as a thesis research project during the fourth year at the Van Hall Larenstein University of Applied Sciences.

Leeuwarden, August 2016

Hannah Böing
Student number: 900813002

Fraukje Sportel
Student number: 910120002

Supervisors: Marcel Rekers, Ignas Dümmer & Henry Kuipers

Cover picture: *Hyla arborea* (©Fraukje Sportel, 2016, Drenthe, The Netherlands)

Acknowledgements

For the completion of this research project we would like to thank our supervisors, Marcel Rekers, Ignas Dümmer, and Henry Kuipers from Van Hall Larenstein, University of Applied Sciences for coaching us during this thesis project for the Animal Management Bachelors program. Secondly, our thanks go out to Wilbert Bosman, a biologist at RAVON, Reptilian and Amfibieën Vissen Onderzoek Nederland (Reptilian, amphibian, fish research in the Netherlands). We thank Edo van Uchelen, another biologist and private conservationist who provided insight and expertise that greatly assisted the research. Many thanks also go out to members of Landgoed De Eese, especially Sybren Mulder who provided us with access and a guide to the estate to complete our research. The guide, Joost Zwaan has been of great help as he is an expert in the area concerning flora, fauna and conservation. We are very grateful for members of Staatsbosbeheer (State Forestry Commission), especially Wouter de Vlieger, Het Drentse Landschap (The Drents Landscape) and Stichting Maatschappij van Weldadigheid (founding society of kindness) for providing us with permits and giving us access to the areas. Lastly, we would like to thank Peter van den Berg and his wife for accommodating us during our fieldwork period.

August 2016, Leeuwarden

Abstract

The loss of biodiversity is a worldwide problem caused by over exploitation of natural resources, pollution, introduction of invasive species and other problems, human induced (IUCN, 2014). The Netherlands is working on strengthening its natural environment boosting social and economic wellbeing. (Government of the Netherlands, 2016). In Southwest Drenthe, a small population of European tree frogs has been released (person unknown) in the year 2000 near a village called Vledder. Unclear is how the population has dispersed to other pools and what their routes of migration are. This is important as they are a protected species. The European tree frog is listed on Annex II of the Berne Convention and Annex IV of the EU Natural Habitats Directive. The area researched was selected within a 10km radius, around the release point just North of Vledder resulting in a research area of 314km^2. In this research the aim is to find out what the distribution of the European tree frog is in Vledder and the surrounding area concerning pool vegetation quality, pool characteristics and land use qualities, as well as contributing to find out which landscape structure is preferred by the tree frog for migration, which is also referred to as connectivity.

The main research question is “What is the suitability of the landscape and the predictability distribution of the tree frog in Southwest Drenthe and what connectivity opportunities are provided for this species in the near future in the research area?” The research question contributes to more knowledge about the European tree frog species located in Vledder and the surrounding areas in Southwest Drenthe. Sub-questions leading to an answer of the main research question concern occupancy of the tree frog in Southwest Drenthe, relationship between pool characteristics and presence of the tree frog, and the appropriateness of the landscape in terms of migration and connectivity.

Data was sampled by selecting the starting point, North of Vledder, and accordingly creating a 10km radius around this point. First a selection was made in ArcGIS software according to function, water type and size. Starting at 11.080 pools, using many selections in ArcGIS, eventually 150 pools were randomly selected from the remaining 260 pools. During field work, pools were measured during daytime with the help of a (guideline) field form pre-designed, once used for the analysis of the crested newt done by RAVON in the Netherlands. Measurements during daytime included measurement of pool water qualities, and vegetation structure analysis. After darkness the pools were visited to interpret whether or not the tree frog was present. Following data collection, data was analysed in programs as IBM SPSS Statistics 23 to analyse which factors had an influence on the presence of the tree frog, the program Presence was used because each pool was visited three times in order to calculate the detection probability. Data was also analysed and displayed using ArcGIS 10.3.1. For the appropriateness of the landscape in terms of migration ArcGIS was used to calculate cost distance routes according to the frictions calculated according to vegetation structures and altitude.

It was managed to analyse 129 pools of which 29 of these showed the presence of tree frogs. The occupancy resulted in psi =0,2249 meaning in 22,49% of the examined pools, the tree frogs occurred. It was found that water temperature, the presence of the green frog and agriculture had an influence on the presence of the tree frog. The increase of the water temperature has a negative influence of the presence of the tree frog if other variables are constant whereas the dominance of agriculture and the presence of the green frog has a positive influence. The presence of the different aquatic vegetation structures showed no significance on the presence of the tree frog. The connectivity in the research area shows provided opportunities for

the tree frog to migrate. Possible taken routes show a high percentage of forest (>50%) and thus result in low cost for the tree frog to migrate.

Results of this research correspond with the literature study whereas the disproportional stratified random sampling could have influence on the statistical results when looking at the Land use types as land uses are not equally divided. There is a high dominance of agriculture in the collected data which results in the statistical analysis showing a positive influence on the tree frogs presence. Also changes in the weather condition (rain, sun) had influence on the detection of the tree frog and could possibly also influence certain variables (e.g. depth and water temperature). The stream valley in the research area seems to be a possible natural migration limitation (due to a change in land use) for the tree frog to migrate to the South and in the Nature2000 and NNN areas only 3 out of 35 pools did inhabit the tree frog which could be linked with the dominance of heathland.

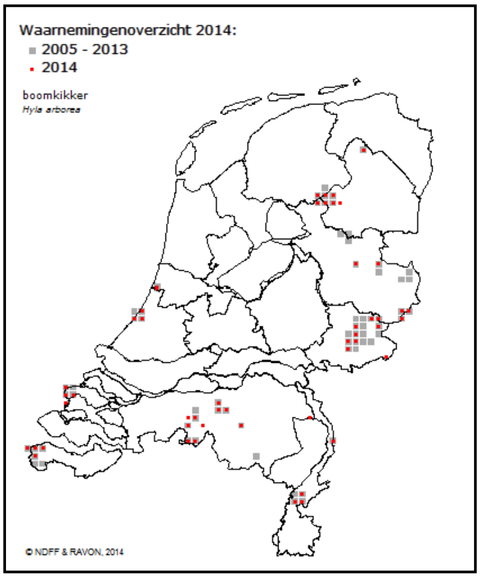
Contents

1. Introduction

1.1. Problem description

The loss of biodiversity is a worldwide problem. The main threats to biodiversity are habitat loss and degradation, the introduction of invasive alien species, over exploitation of natural resources, pollution and diseases and lastly, human's own enemy too, human-induced climate change (IUCN, 2014). According to the convention about life on earth at least 40% of the world's economy derived from biological resources as well as 80% of the needs of the poor. Also, medical discoveries and economic development highly depend on the diversity of life. The richer the diversity, the greater the opportunity. Amphibians are vulnerable to degradation of habitats, physiological constraints, short dispersal distances and high fidelity towards breeding sites (Sinsch, 1990, Blaustein et al. 1994). Habitat loss is one of the main factors which can lead to the extinction of species. Unfortunately, the Netherlands is the most densely populated country of the European Union with a population density of 498 people per km^2. This high population density could be a huge strain on nature conservation as there is (Benedick, 2000).

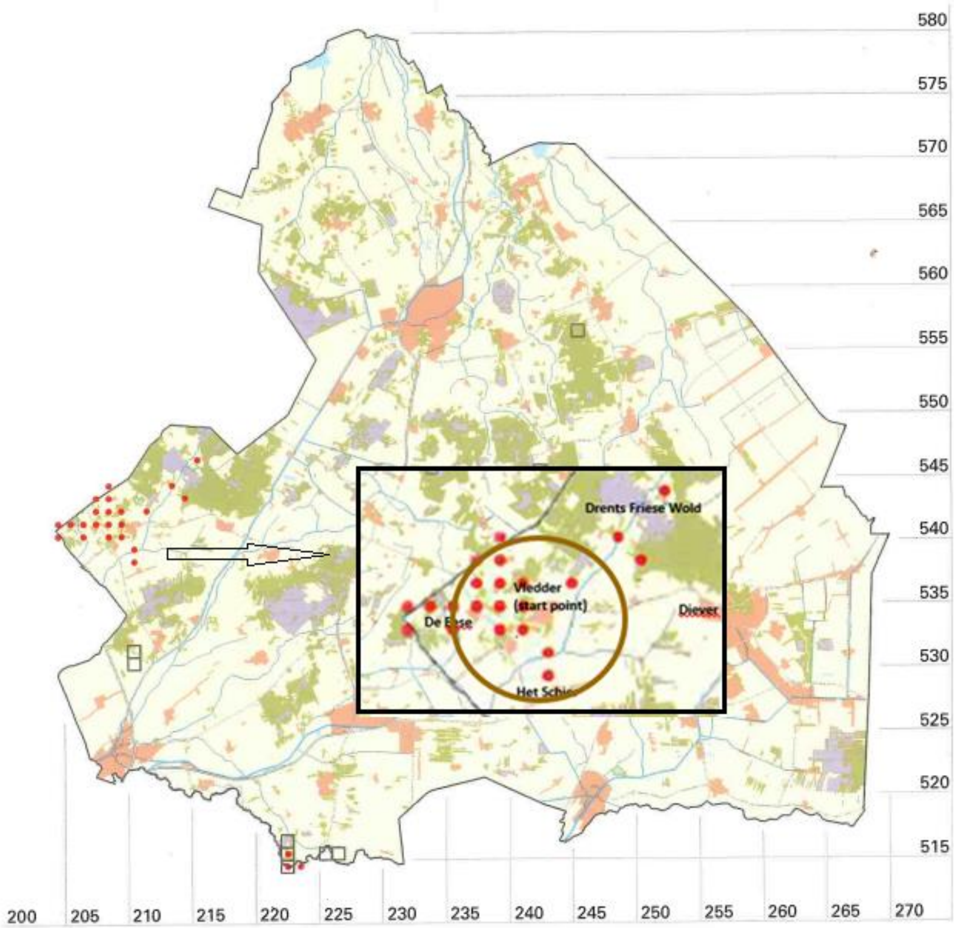
Nevertheless, the Netherlands contains a quarter of all listed habitat types in Europe, namely 51 natural habitat types. Home to 28 species of wild plants and animals mentioned in the Annexes of the EU Habitat Directive (92/43/ECC), and 100 bird species in the EU Birds Directive (79/409/ECC), the Netherlands has a large variety for the minimal space it has to offer (Nilesen et.al, 2003). Unfortunately, biodiversity is degrading worldwide, which is for this reason that the Netherlands wants to preserve and strengthen the natural environment. This also boosts the social wellbeing as humans see different benefits such as cultural, geographical and historical contexts in which societies develop and the economy as a large amount of the researches derive from biological resources (Government of the Netherlands, 2016). As well as protected areas, the farmlands in the Netherlands also contribute to the landscape value. Farmers adopting management in a nature-friendly way qualify for a remuneration from the SNL grant scheme (Subsidy Nature and Landscape). Momentarily, not all farmers have adopted this management system (Government of the Netherlands, 2016) and it remains unknown how many farmers have adopted to this system.



Map 1- Population trend of *Hyla arborea* 2014 (Ravon, 2016)

A typical species sensitive to habitat change, habitat loss and fragmentation of forest is the European tree frog (*Hyla arborea*). According to the IUCN Red list the European tree frog population is currently decreasing. In the Netherlands, there have been sightings of the tree frog in different provinces including Overijssel, Gelderland, Midden-Limburg and locally in Noord-Brabant which is shown in Map 1, page 8.

The European tree frog was released in 2000 near a village called Vledder. (Uchelen, 2010). Today the population of tree frogs has spread out to locations up to 10km from this point. The current status of this population is unknown, however, observations have been made over the years. In the area researched, in and around Vledder, a total of 150 croaking males were counted in 2009 (Uchelen, 2010). In 2009 a breeding population was recorded in a big water pool near Vledder, around the estate called De Eese and the villages known as Boijl, Noordwolde and Wapse. Also that year the tree frogs have been heard (red points Map 2, page 9) in Friesland and Overijssel, two bordering provinces of Drenthe (Uchelen, 2010). Map 2, page 9, shows the known distribution.



Map 2- Known Distribution *Hyla arborea* near Vledder with the research area (circle) (Uchelen E., 2010)

Due to it being a protected species in the Netherlands it is of importance to make the survival and the distribution possible. On the other hand, due to the fact that this population was released, the management has to cope with possible effect on other endemic species like the crested newt and the green frog. These species share a similar habitat and the introduction of another species, in this case the tree frog, may alter the abundance (Nwf, 2016).

As not much is known about the whereabouts and preferences of this tree frog (Ravon, 2016) population it is of good use to do a recent recording of the current situation. Literature points out that pool vegetation quality (percentage of emergent, floating, submerged and riparian vegetation), Land use types (grass, agriculture, urban, forest etc.) and the water quality (presence of fish & amphibians, pH, depth, desiccation, water surface and temperature) could have an influence on the presence of the tree frog and its distribution (Ravon, 2016; Uchelen E., 2010). Research can thus give an understanding of the frogs' needs and give an indication of location, connectivity how it can distribute over the landscape, and reproduction preferences. Gaining knowledge about the species may help conserve the species.

Due to small scale conservationists, this tree frog population has been provided with the opportunity to breed in privately conserved pools. An example of this is from a private biologist/conservationist in the area, Mr. Edo van Uchelen, who lives on the edge of a National Park, the Drents Friese Wold. His terrain and work gives the tree frog a good chance to reproduce successfully. The terrains consist in total of seven breeding pools. During this research Mr. Edo van Uchelen is used often as a valid source to gain more detailed information about the tree frogs habitat, ecology and behaviour.

For the frog to migrate it is of importance to know which vegetation structures it prefers. In this report vegetation structures are used in ArcGIS to measure the connectivity and cost distance. Each vegetation structure is rated in frictions. “Connectivity is defined as the degree to which the landscape facilitates or impedes movement” (Ament et al., 2014). The connectivity is measured according to the rated frictions given to each vegetation structure, after referring to literature to know which vegetation structures are most preferred.

1.2. Aim and Research questions

The aim of this research was to get an overview of the distribution of the European tree frog in Vledder and the surrounding area and its dependence on the pool vegetation quality and landscape structure used by the tree frog for migration (connectivity). This was of interest because the current distribution and connectivity quality is unknown.

The main research question is “What is the suitability of the landscape and the predictability distribution of the tree frog in Southwest Drenthe and what connectivity opportunities are provided for this species in the near future in the research area?” This research question contributes to more knowledge about the European tree frog species located in Vledder and the surrounding areas in Southwest Drenthe. Sub-questions leading to an answer of the main research question are listed below:

1. “What is the occupancy of the European tree frog in Southwest Drenthe?
2. “What is the relationship between the pool characteristics concerning acidity, surface area, vegetation structures, water temperature, desiccation and presence of fish and other amphibians and the presence of the European tree frog?”
3. “To what extent is the landscape appropriate for the tree frogs to migrate in and around the research area?”

2. Materials Methods

In this chapter the layout of this research and a description of the location and the population distribution is given. Furthermore, the data sampling, collection, preparation and analysis are elaborated on.

The model of the analysis process (Fig 1, page 11) gives an overview of the steps undertaken. An explanation is given following the figure.

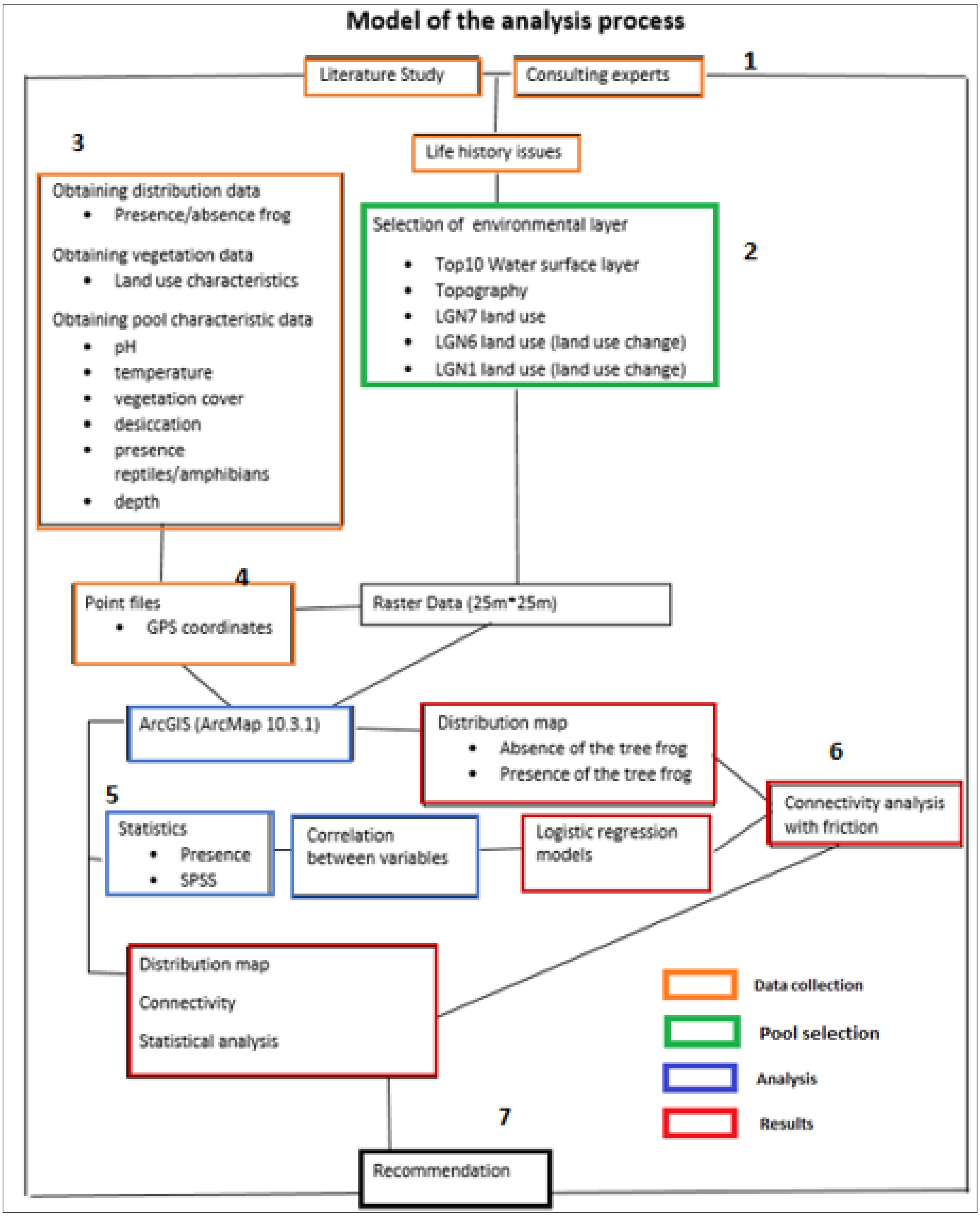


Figure 1- A detailed description of the processes taken to reach final maps in ArcGIS

The first step (1) includes literature research and consulting experts which gave an overview of the research area and the life history issues concerning the tree frog, explained in the following sub chapters.

2.1 Research Area

The location of interest is the area of Southwest Drenthe. The area covers 314km^2, (31.400 Ha.). It is a region in the province of Drenthe and is formed by the cities Hoogeveen, Meppel, Westerveld and De Wolden (Drenthe, 2016). South-West Drenthe holds two National Parks and one Nature2000 area which give place to unique and diverse habitats (Natura2000, 2016). Appendix III Map 14 shows the Nature2000 and NNN areas in the research area which covers 14,6% of it. Nevertheless, this area is also effected by landscape changes due to farming, habitat directives Natura2000guidelines and population growth. Looking at the Land use 8 years after the introduction of the tree frog near Vledder the landscape was dominated by agriculture with a percentage of nearly 56%. Around 20% of the area was covered by forest and 9,3% had the characteristics of urban Land use. In 4 years' time the landscape mainly changed in terms of agriculture. Where the land was then covered with more than 59% of agriculture, the percentage of forest decreased to less than 17%, which also leads to more fragmentation of forest areas. In Appendix I the Land use Maps with the attached Tables 13 and 14 from 2008 and 2012 show those changes.

In the centre of the research area is a privately owned pool, the starting point of the research population, which is 1km in the North of the village Vledder (Map 2, page8). The landscape around Vledder is dominated by open fields, fen and forest (Vledder, 2011). Many parts around the village are part of the State Forestry Commission (Staatsbosbeheer) and belong to the Ecological Network (De Ecologische Hoofdstructuur). On privately owned terrains agricultural activities take place with livestock farming and cropping (Vledderveen, 2016).

The research area is chosen in a radius of 10km around the starting point in Vledder. According to the literature found in "De Amphibien en reptielen van Nederland, Creemers 2009" and personal interviews with Edo van Uchelen, did the tree frog spread in the last years up to a distance of 10km around the starting point.

Within the research area certain land uses are found, which could have an influence on the presence/absence and distribution of the tree frog. Table 1, page13 shows the found land use types and their definition with the percentage of cover in the research area calculated with ArcGIS.

Table 1 - Land use types defined according to categorical terms used in research and showing percentage coverage of research area

Land use	Definition	%
Agriculture	Consist of different types of farmlands in forms of crop fields, meadows used for grazing areas, flower fields and tree nurseries/ orchids	55,83
Forest	Consist of coniferous woodland and deciduous woodland with a tree layer > 3m, shrub layers 0.1-3m, < 10cm herb layer and a moss layer. There will be a subdivision in forest types with a developed herb layer and forest type without herb layer	19,89
Grassland	Is one of the most species-rich plant communities with orchids, variety of grass and different kind of herb layers (Natura2000, 2008)	6,37
Heathland	(H4010) consists out of heathland with more or less acidic and nutrient-poor soil with bog heather (*Erica tetralix*) and magellanic bog-moss (*Oxycocco-Ericion*) (Natura2000, 2016)	5,24
Marshland	Is characterized as wetlands which are frequently flooded and have a well-developed emerged vegetation with Reed (Phragmites australis), bog heather (Erica tetralix). This Land use is an important shelter for many kinds of amphibians, fish and bird (EPA, 2016).	1,01
Reed	Is formed by a high hydrology and communities of T*ypho Phragmitetum calthetosum* species like Marsh marigold *(Caltha palustris)*	0,64
Sand	Consist out of areas with shifting sands and/or river shorelines. These areas are characteristic for the dry grounds with some moss layers and plants like whitish hair-grass (*Corynephorus canescens)* and Scotch heather (*Calluna vulgaris)*. Land use types belonging to this category are Inland dunes (H2330) and dry sand heath (H2310) (Natura2000, 2016)	0,24
Surface water	Consists out of any kind of water as slopes, pools, rivers and ponds and are noted all individual	1,40
Thicket	Consists out of an area with more than one line of hedges, small trees (< 3m) and shrubs. There will be a subdivision in types with a developed herb layer and without herb layer	Categorized under forest
Urban	Consists of houses with gardens and farms with grass fields. Everything is connected through city roads and country roads	9,38

(Pictures of these specific Land uses are found in the Appendix II – Land use types.)

2.2 Study Species

Chapter 2.3. Study species gives an explanation of the biology, distribution, habitat and ecology of the European tree frog.

2.3.1. General Biology

The tree frog (*Hyla arborea*) belongs to the class of the Amphibians. In this class the *H. arborea* is classified in the order Anura with the Family Hylidae and the Genus Hyla (IUCN, 2016). Table 2, page 14 shows the detailed classification of it. The *H. arborea* is a small green frog of the size up to 5cm and 5-15g (see Photo 1). The tree frog has small acetabulum at the tip of its fingers and toes providing it the possibility to climb. *H. arborea* is the only species in the Netherlands which is able to climb. Typical for this frog is the brown line over its side started by the eyes. The males do have a big vocal sac under the chin. Tree frogs are capable to change colour from light brown to dark green. Only during the mating season, the tree frog can be sighted in water. Most of their time is spent living on land (Uchelen, 2010; Creemers et al, 2009).

Photo 1 - Hyla aborea (©Boing, 2015)

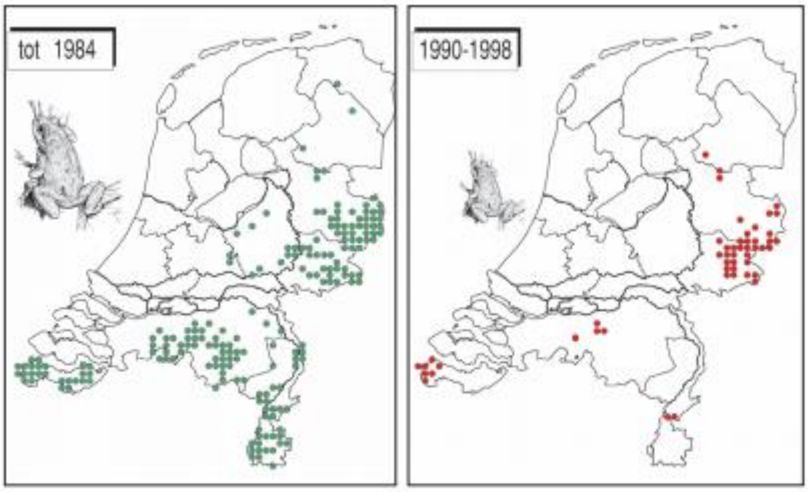
Table 2 - Categories Hyla aborea (IUCN, 2016)

Kingdom	Animalia
Phylum	Chordata
Class	Amphibia
Order	Anura
Family	Hylidae
Genus	Hyla
Species	*Hyla arborea*

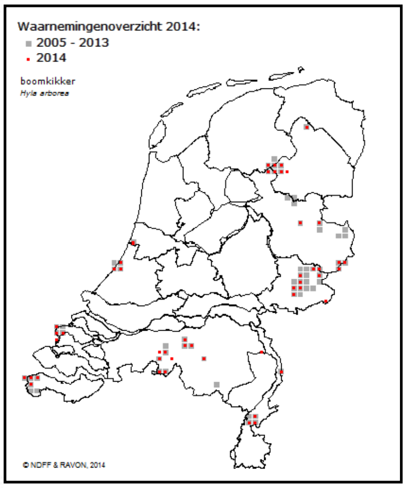
In the middle of April, the males start their journey to small waters and pools for reproduction. The males form a chorus of croaking frogs, which attracts the females in the area for breeding. Depending on the weather these scenarios could start at the quiet early, at the end of March, when sunny and warmer, and takes up to mid-July (Creemers et al, 2009). This chorus can be heard up to half an hour prior and after sunset. (Schneider, 1971). During cold nights and rain the chorus declines, and may even disappear completely. As soon as the females are attracted by the chorus the mating takes place. After the fertilization the female lays a bunch of eggs (up to 1200 eggs) in multiple clutches in a water depth of 10cm (Uchelen, 2010). These eggs develop into small larvae which leave the water after metamorphosis in July and August (Creemers et al, 2009; Strumpel, 2004).

2.3.2 Distribution

In Europe the *H. arborea* is a wide spread species. It is found from Iberia (Spain) up to the West of Russia and from Sweden down to the South of Greece. The distribution and the population size after 1991 decreased rapidly in the Netherlands due to habitat destruction (Map 4, page 15) (IUCN, 2016; Soortbeschermingsplan, 2001). From 1997-2012 the population increased from 54 populations to 334 (NEM, 2015). Nowadays the tree frog is sighted in the East of the Overijssel, Gelderland, Middle-Limburg and North Brabant, Zeeuws-Vlaanderen. The trends from 1984 until 2014 are shown in the Maps 3, page 15 and 4, page 15.



Map 3- Population trend of the tree frog 1984-1998 (Soortbeschermingsplan, 2001)



Map 4- Population trend of the tree frog 2014 (Ravon, 2016)

2.3.3 Habitat and Ecology

For survival and reproduction, the tree frog needs an area characterized by shrubs, hedges, resting water and developed vegetation like herb and shrubs layers (Bergmans & Zuiderwijk, 1986). The preferred location for reproduction should not be too acidic with a big surface, shallow and sunny. Changing water level or desiccation avoid fish population, which gives the chance for better survival of the species (Grosse, 1994).

Examples for those waters are swamps near streams, pools surrounded by meadow and shallow sand pits. Plants with little spread leaves are very characteristics for those pools, which have a big surface/depth proportion. The bank is characterized by an herb layer without bushes and trees. The presence of flowers and fruit plants increases the insect population and so there is feeding success for the tree frog. The pools should have a sunny position, so that the water heats up fast (16-25°C) for good reproduction condition and should not inhabit any species of fish and other amphibians to avoid competition and predation of eggs. The surface of those pools is between 500 m^2 and 2000m^2 (up to 20 meters' diameter) with a marginal depth of around 30 centimetres (Soortbeschermingsplan, 2001). The pH of the pools may variate between 5.5 and 8, which means a slight acid to an alkaline value. In case of a too low pH the eggs would not develop (NABU, 2015). Observations show that the pools are in a distance of ca. 300 meter from their land habitat, which is characterized by scrubs and smaller trees (Creemers et al, 2009; NSR, 2015). The Figure 2, page 16 and Photo 2, page 16 show the general characteristics of those pools.

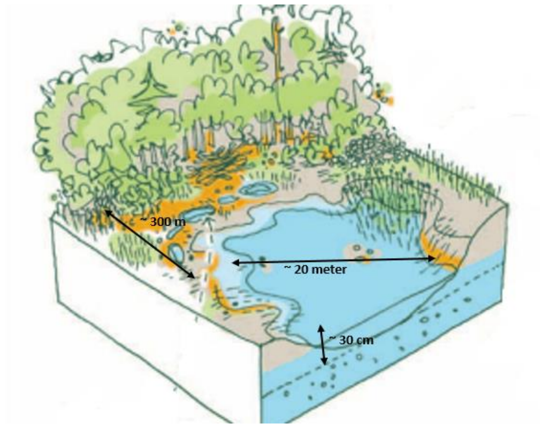


Figure 2 - Drawing of a suitable habitat for Hyla aborea (Pro Natura, 2015)

Photo 2 - Suitable habitat for Hyla aborea in Vledder (©Sportel, 2016)

Generally, tree frogs do not move around too much and tend to be capable at finding the same spot daily. This is because a large part of the 'safety feeling' is on home grounds where the breeding and reproduction waters are located. However, it is possible that the tree frog can move to another pool nearby (FOG, 1993). Only a small part of the population is mobile and capable to cover a large distance. (Stumpel & Hanekamp 1986). Juveniles are capable to migrate in the year they metamorphose, varying from 100m to 1km distance from the breeding pools whereas adult frogs are able to migrate up to 12km in one year (Mattison et al.,2011). This contributes to population stimulation (Creemers et al., 2009). Important passages for tree frogs are forest edges with ground vegetation, hedges and thicket and along ditches (Uchelen, 2016).

Pools which adhere to the correct criteria explained previously are further referred to as Edo layers or Edo's management systems. This includes pools with a pH ranging from 5.5 to 8, surface areas ranging between 500m^2 and 2000m^2, pools which are desiccated in the late summer season, a depth of around 30cm with, at the edges a depth of around 10cm in order to reproduce successfully with the possibility to reach 16°C during breeding season.

2.3.4 Threats

According to the IUCN (International Union for Conservation of Nature) the worldwide population of the tree frog is decreasing (IUCN, 2016). The main threats are habitat destruction, fragmentation and the drainage and pollution of wetlands (IUCN, 2016). In the Netherlands the destruction of copse, hedges and stagnant waters mainly led to the decrease of the population (Creemers et al, 2009).

2.3.5 Protection

On the global scope the tree frog is listed in the red list as "least concern" and through the Convention of Bern categorized as "strong protected species" (IUCN, 2016). Since 2007 it is listed as "Threatened" in the Netherlands. In the Flora and Fauna legislation it is categories as "strongly protected" and listed in the Table 3. Through the Habitat Directives Appendix 4 the protection of their natural resting – and breeding areas are regulated (Ravon, 2016; Creemers et al, 2009).

Nature2000 areas consists of a network of protected areas which offers breeding and resting sites for rare and threatened species and gives unique vegetation the chance to develop with the aim of long-term survival of these species including the tree frog which are listed under the Birds and Habitats Directives (EU Commission, 2016). The Nature Network Netherlands (NNN) is a network of already existing and newly built

nature areas which aims for the connection with its surrounded agrarian characteristics (Rijksoverheid, 2016).
The NNN gives an important foundation for the future of the tree frog in case of maintenance of nature and its protection. Together with the Nature2000 it covers 14,6% of the research area which is shown in Map 14, Appendix III.

2.3 Data Sampling

This Chapter will give an understanding of data which was needed for this research.
Before the data collection there have been some preparations made beforehand.

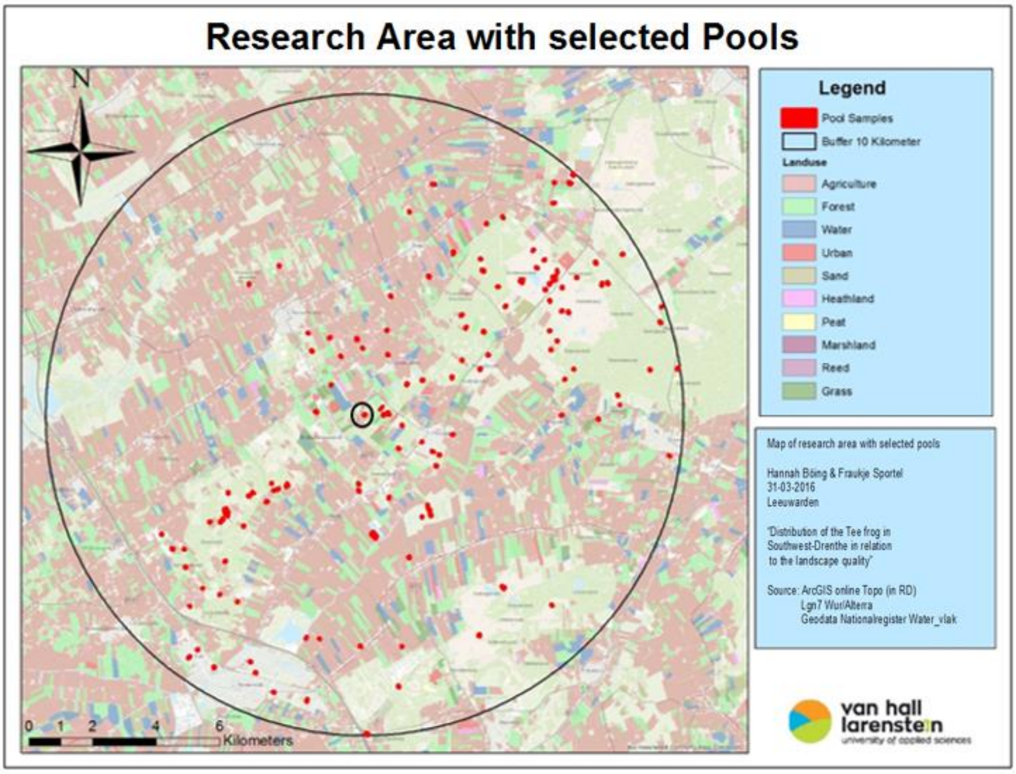
After the first step (1) in Figure 1, page 11 which includes literature research and the consultation of experts about the general/ background information of the tree frog and its current status the first calculation for the sample size was done. With the formula **N = 10 k / p** (Peduzzi et al., 1996) the sample size (N) was calculated. K gives the number of independent variables whereas p gives the smallest of the proportions of negative or positive cases in the population. This means that it is the fraction amount of pools with the possibility of the presence of the tree frog. With the calculated sample size (N= 10*7 / 0.5), resulting in valid and reliable results.
The sample size is calculated by 10 times the 7 variables of which the last variable, presence of fish/amphibians was split into 3 categories (crested newt, fish and the green frog) at a later stage in this research. The 7 variables are pH, temperature, desiccation, depth, surface area, vegetation and presence of fish/ amphibians, as clarified under 2.3.3 Habitat and Ecology as these are important survival and reproduction factors. This value is then divided by 0.5 as 50 % of the pools are expected to contain tree frogs, which is based on the literature used above and expert knowledge but still stays as an estimation. This value is an estimation according to the expected amount of pools containing tree frogs. The answer for N= 10*7 / 0.5 is calculated to be 140 pools. This amount was rounded up to 150 pools for this research to get a bigger sample size and thus increase the accuracy of the results. To have a sufficient big sample size to calculate the occupancy of the tree frog with an error of 5%, a coefficient level of 95%, a response distribution of 50% (expectation to find the tree frog at a pool) and the sample size of 260 pools resulted in the ArcGIS selection. Of the 260, 150 were selected using disproportional random stratified sampling. The calculated sample size would be 156, because of a quite high response distribution, meaning that the sample size of 150 pools would be sufficient to still get a viable result, also considering possible setbacks.

To answer the main question and the 3 sub questions the following steps were of essential.
Using GIS software ArcGIS 10.3. a categorizing of the landscape was done using a classification (Tool "Reclassify") in 14 groups of the Land use Map of 2012- LGN7 (agriculture, forest, urban, grass/meadow, garden/parks, sand, marshland, thicket, roadside, brushwood, peat, reed, heathland and water) which is shown in step 2 Figure 2, page 16. These types of the landscape are of the most influence for the tree frog regarding to the literature research done before (Chapter 2.3) and expert opinion. This generalised Land use Map (Appendix I) was used for the selection of the sampled pools. By adding characteristics of pools in ArcGIS 10.3. by choosing the tool "select by attribute" in the surface layer "top10_NL waterdeel_vlak" the preselection was done (see Map 5, page 19). Characteristics taken into account during this preselection were the function of the water type, if it I a main sewerages or not, the water type and the surface area Table 3, page 19 shows the characteristics added during the preselection. This step means a selection of pools which have another function than river, fishing pools or ditches, are no main sewerages and are a water type referred to sea or lake. Furthermore, the surface size was limited between 500 and 2000m^2 which could have an influence of the tree frog presence, based on the literature explained in Chapter 2.3.

Table 3 Pool characteristics added in ArcGIS for the preselection

Attribute	Selected by the category
Function of the pool	Other (means no rivers, fishing pools and ditches)
Main Sewerages	No
Water type	Sea and lake
Size	Size of the surface water between 500 and 2000m^2 (Soortbeschermingsplan, 2001)

This analysis resulted in a selection of 2000 pools out of 11.080 pools. With a buffer of 10km radius around the starting point of the tree frog population the research area was selected. According to the literature found in "De Amphibiën en reptielen van Nederland, Creemers 2009" and van Uchelen the tree frog has distributed in the last years up to a distance of 10km around the starting point. After this stage pools are selected by location within the buffer which resulted in an outcome of 260 pools.



Map 5 Research area and pools to be sampled (#150 red dots) showing the selected radius (large black circle) and the starting point near Vledder (small black circle)

After the preselection the last step of the data sampling was done by using a disproportional stratified random sampling method where pools are selected randomly within each Land use. It is important to have every Land use represented in the research to get a valid result about the influences of each Land use on the tree frogs´ presence/absence. In total the Map shows 150 selected pools.

2.4 Data Collection

The following chapter will give an overview of how the data was collected. The data collection took place from 20.4.2016 – 31.5.2016. Using an adjusted field form of RAVON (see Appendix IV) the data of the frogs' presence/absence, the characteristics of the pools, vegetation and Land use was collected which is shown step 3 in Figure 1, page 11.

2.5.1 Presence of the Tree frogs (occupancy)

The tree frogs were tracked only by their sounds after sunset hours and evening, while the tree frogs were feeding and reproducing (Creemers et al, 2009). This was between 21:30-24:00. Because of their unique croak they are distinguished easily from other frogs. By listening from different angles around the pool the tree frog could easily be recognized.

By having a sound sample of the croak on a device, frogs were motivated to croak. In general, every pool was visited 3 times based on the analysis used later in this research for calculating the probability of occupancy, whereby detection probability is considered. However, weather conditions could influence the frogs' activity and the ability for the researchers to detect the frogs. Due to this reason, weather is our control variable. By starting with the pools where presence of the tree frog had been already confirmed, the conditions of suitable time and weather could be checked prior to checking new pools.

Per attempt a call index was used 0 (no frogs calling), 1 (frogs calling). The numbers were collected in categories from 1-5, 6-9, 10-50, 50-99 and 100+ individuals. In cases of 100+, which only occurred at Mr. van Uchelen's pools this was told. "These pools contain hundreds." (van Uchelen, 2016)

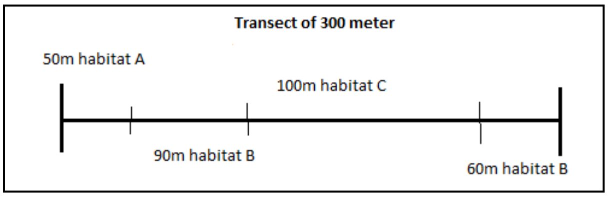
2.5.2 Characteristics of the pools

The pools were reached by car or by bike, which depended on the weather condition and the distance. Each day pools were selected in clusters near each other (per village) to analyse. With the help of a Map the coordinates in RD_new (in meter) and the street names the pools were located.

The area size of the sampled pools was directly calculated in ArcGIS 10.3. The depth was measured by having intervals of 2 meters along the bank, most suitable for the tree frog over a distance of 1m from the water edge (3 times) (Mazerolle, 2004). The pH strips were used at 3 locations (preferably along the shoreline where tree frogs were present) 30cm of the water column and in a depth of around 10cm (Tahvanaien, 2003; Mazerolle, 2004) and the average pH calculated. The temperature average of the water was calculated by using the same location where the depth measurements were done. Also the presence of fish, green frogs and the crested newt is very important as research shows a decrease of the tree frog population with presence of these species (Grosse, 1994). Presence of fish was analysed by visual observation and by asking owners or being informed by local people. As soon as fish were observed the pool was marked with fish presence. The presence of the green frog and the crested newt were done by checking for eggs and/or seeing and hearing the individual as well as gaining information from local people and owners. The eggs of the crested newt are inside a folded leaf, which makes it quite easy to recognize in clear waters. In general, the whole water outline was reviewed step by step by looking between the plants near the bank (1m). To know whether or not pools are desiccated during the summer owners, experts and locals were interviewed. The vegetation of the pool was estimated visually in percentages whereas every category could cover up to 100%. Reason for this is that the tree frog does not choose specified individual plants or a specific plant species, but a group of plants like floating plants, which gives the pools a usable structure for the tree frog to live and reproduce. Studies have shown that the vegetation structure of the pools are more important than the individual plant (Creemers et al, 2009). Aquatic vegetation was categorized as submerged, floating, and emergent and riparian vegetation.

2.5.3 Land use in a radius of 300m around the pool (connectivity)

The Land use (listed in Chapter 2.1 Table 1, page 13) around the pool was be measured through 4 transects each 300-meter-long in the direction of North/East/South/West. The reason for choosing 300 meters is because it is the tree frogs natural behaviour to only choose pools with a suitable land habitat in a radius of 300 meters from the reproduction waters (Creemers et al, 2009; NSR, 2015).



Land use A: 50m – 16,7%
Land use B: 150m- 50%
Land use C: 100m – 33,3%

Figure 3- An example of how the Land use transects was categorized

At each transect the Land use was categorized and converted into a percentage which is shown with an example in Figure 3, page 21. The Land use outside the radius of 300m was characterized with the help of ArcGIS and the Land use classification done before (Map 5, page 19).

During the data collection a distinction was made between forest with little or no underground/herb layer and forest with underground/herb layer as well as thicket with a lot of underground and with little underground/herb layer. This distinction was done due to the importance of the vegetation structure (Chapter 2.3) (DBU, 2016). No distinction was made between forest and thicket types (deciduous or coniferous) because of the tree frog dependence on vegetation structure and no single plant species. Because tree frog is using hedges and different vegetation for migration it could have an influence of its movements (Uchelen E., 2016).

2.5 Data Preparation

After the data was collected on paper, the first step to undertake was the digitalization of the data, which is clarified in this chapter as data input.

A database shown in the form of an Excel Table. The collected data was divided into four categories (pool characteristics, water, vegetation and Land use) shown in sheets. An Example of each sheet is shown in Figure 4, page 22.

General sheet

Pool nr.	Date	Time	Temperature (°C)	Wind	Rain (	Address	X-Y co-ordinates	Picture number	Presence tree frog
1	21.4.2016	10:38:00	12	1	1	Middelweg 5BB	52860998 6194737	27	1
2	21.4.2016	11:22:00	12	1	0	Reeweg after Edo	52860059 6179066	28-32	1

Water sheet

Pool nr.	Water surface (m2)	Mean depth (cm)	Presence of fish	G. Frog (	Presence of C.Newt (	pH	Av. Water temp (°C)	Desiccation in summer(
1	1328,79	15	0	1	0	4,00	8,8	0
2	464,08	26,6	0	1	0	5,90	13,2	0

Vegetation sheet

Pool nr.	Floating aquatic plants (%)	Submerged aquatic plants (%)	Emergent vegetation (%)	Riparian vegetation (%)
1	0	0	0	80
2	0	80	0	30

Land use sheet

Pool nr.	Gras/Meadow	Forest with	Forest little	Wooded bank	Thicket with	Thicket little	Brushwood	Agriculture
1	0	0	64,42	0	0	15	0	20
2	0	0	42	0	0	27	0	31

Figure 4 - Data organisation in Excel sheets

To gain an understanding of the sheets there are some special codes (numbers) used for different variables. Temperature and picture numbers are simply given in scale form, vegetation and Land use are given in percentages. The percentages of the Land uses were calculated by adding the found Land use types of all 4 transects (North, East, South, West) together. The wind speed is measured using system of the Beaufort-Scale. This was inserted daily by checking the weather forecast, using the same website daily. Furthermore, dichotomy (0/1) was used to categorize the following variables.

- Rain 0= No rain 1= rain
- Presence of tree frog 0= No 1=yes
- Desiccation 0= No 1=yes
- Presence of fish 0= No 1=yes
- Presence of green frog 0= No 1=yes
- Presence of crested newt 0= No 1=yes

To create tales and a better overview of the gained data, the variables were categorized in 3 groups.

Land use (L) - Agriculture, brushwood, deforestation, grass/meadow, garden/park, forest with developed herb layer (further as forest with), forest with little or no herb layer (further as forest little), heathland, marshland, new forest, reed, sand, shrub, thicket with developed herb layer (further as thicket with), thicket with little or no herb layer (further as thicket little), urban, water and wooded bank

Pool characteristics (G) - Water surface, depth, pH, water temperature, desiccation, presence/absence of fish, presence/absence of the green frog and presence/ absence of the crested newt

Pool vegetation (P) - Floating plants, submerged, emerged and riparian vegetation

After categorizing, the differences in percentages of (Land uses, vegetation) and values (temperature, depth, fish etc.) between pools with the presence and pools with the absence of the tree frog were calculated (Tables 4, 6 ,7, pages 26, 28 and 29)

To create a good basis for further study a factsheet per pools was created which contained all collected data with pictures (example in Appendix V).

2.6 Data Analysis

This chapter clarifies the way of the data analysis done during this research after the data collection and preparation. A chart showing the steps of the analysis in general is found in the Appendix VII and in Figure 2, page 16 steps 4-6.

The first step was to get a general overview of the collected data by analyzing the ranges and standard deviations and the mean per variable and counting the number of pools with the presence and absence of the tree frog (Tables 4,6,7, pages 26, 28 and 29)

To gain an estimation of occupancy known as Psi (ψ) and the detection probability (P) of the tree frog, its presence/absence data was inserted. Each pool was visited three times resulting in a (presence) 1/0 (absence) dichotomous dataset. For this data a simple single-season test was run in Presence Program, resulting in an AIC.
The Collinearity test was used to gain VIF values. Each variables' VIF appeared lower than 2,5 so all factors were taken into account for the next analysis.
Each variable in the groups pool characteristics (G), pool vegetation (P) and Land use (L) were tested on their P- value <25% and thus resulted in a selection of variables (Table 9, page 33) taken further into account for the next analysis, linear regression. For nominal variables (1/0) the Chi^2 test was used. For scale values the Mann-Whitney test was used, shown in Table 8, page 32
Presence ranks models in terms of Akaike's Information Criterion, also referred to AIC, for the selection of statistical models. It estimates the measure of quality of each model relating to one another resulting in a top model (the model consisting of the best possible combinations).
After that the significance of each variable belonging to the top model group were tested.
Significant variables ($P<0.05$) are then characterized by having influence on the presence of the tree frog weather it is negative or positive.
The results of the models are further displayed in chapter 3 "Results".

By using ArcGIS 10.3.1 the collected coordinates of the study sites (pools) were inserted in a Map which shows the distribution of the tree frog around Southwest Drenthe (step 2 Appendix VII).
Tree frogs are known to disperse up to 12km per year from their reproduction area (Mattison et al., 2011).
Vegetation structures were compared according to literature studies, data collection and in ArcGIS in order to observe the possibilities for the tree frogs to migrate from one pool to another using connectivity. "Connectivity is defined as the degree to which the landscape facilitates or impedes movement" (Ament et al., 2014). With the help of the friction (resistance of the Land use for the tree frog to move) based on the literature research and the opinion of the expert Edo van Uchelen (see Chapter 2.3 and friction Table 18 Appendix VI) the connectivity opportunities are shown in the form of a Cost Path Map (Appendix VII step 6). This Map was created with the help of a Cost Distance analysis (Appendix VII step 4) and the creating of a Cost Back Link (Appendix VII step 5).
The cost distance shows the cost of movements based on the distance whereas the Cost Back Link shows the cost of movements from one cell to the neighboring cell (ArgGIS, 2016).
The more connectivity, the more chance for species to migrate and survive (Ament et al., 2014).
The Cost Path Map was used to display 6 possible routes between interlinked pools and the surrounding areas with the suitable vegetation structures. The 6 destination covers following criteria. 3 pools inhabit the tree frog and three pools does not. Furthermore, the pools where selected by different Land use types

and most distance to the starting point. After the paths were analyzed with the help of the 3D analysis tool in ArcGIS 10.3 the percentages of covered Land use and its composition was shown.
A Table of all metadata used during this research and a model of the steps is found in the Appendix VII.

3. Results

In the following chapter the collected data is described and the research questions are answered.

3.1 Data description

This chapter gives an overview of the collected data and gives a short summary of general findings of the fieldwork. In the following abiotic pool factors including the presence of fish and amphibians, pool vegetation quality and quantities, and land use types around the pools are shown.

The data collection took place from 20.04.2016 – 31.05.2016 during daily hours and again after sunset, to ensure the active state of the male tree frogs. In total data was collected from 129 pools whereas 100 pools did not inhabit the tree frog. During the data collection the temperatures ranged from 8-26°C and a wind speed ranged from 0 – 5 Bft (up to 8,0-10,7m/sec).

3.1.1 Pool characteristics with abiotic factors, the presence of fish and amphibians

Table 4, page 26 gives an overview showing the variation of the different pool characteristics for pools where tree frogs were present as well as pools where the tree frogs were absent. Abiotic pool factors included water surface, water depth, pH, water temperature and desiccation. Aside from these abiotic factors, the presence/ absence of the green frog, crested newt and fish were investigated.

Table 4 – Table showing collected data including maximum and minimum values for each section and their standard deviations.

Pools / **Characteristics**	All pools (n=129) Min-max	Pools with absence of the tree frog (n=100). Min-max	Standard deviation / Mean	Pools with presence of the tree frog (n=29). Min-max	Standard deviation / Mean
Abiotic pool characteristics: Water surface	14-181.088 m^2	14-181.088 m^2	20423,6 m^2 / 5136,4m^2	51-13.264 m^2	3072,8 m^2 / 1776,5m^2
Abiotic pool characteristics: Depth	6-150cm	6-150cm	21,49 cm / 26,9 cm	6-50cm	9,54 cm / 20,2 cm
Abiotic pool characteristics: PH	3.9-9.00	3.9-9.00	0,75 / 6,5	4-7.8	0,74 / 6,5
Abiotic pool characteristics: Water temperature	5-22°C	5-21°C	3,61°C / 14,4°C	7-22°C	4,26°C / 14,9°C
Abiotic pool characteristics: Desiccation	24	15	-	9	-
Presence/absence: Fish	33	31	-	2	-
Presence/absence: Green frog	75	50	-	25	-
Presence/ absence: crested newt	5	1	-	4	-

Examining the data per variable the water surface shows a wide range from $14m^2$-$181.088m^2$ with a standard deviation of 20423,6 and a mean of $5136,4m^2$. Whereas the range of the pools with the presence of the tree frog is smaller (51-13.264 m^2) with a standard deviation of 3072,8 and a mean of $1776,5m^2$ showing the deviation of all observations.
Sampled pools resulted in a mean depth of 6-150cm with a standard deviation of 21,49cm and a mean of 26,9cm. Only 1 pool had a mean depth over 100cm. Pools with the presence of the tree frog again had a smaller range in mean depth with only 6-50cm and a standard deviation of 9,54cm and a mean of 20,2cm respectively.
The pH generally ranges from 3,9-9,00 (Sd 0,75). Pools with the presence of the tree frog, however, only showed pH from 4-7.8 with the Sd of 0.74 respectively. Looking at the mean it does not differ at pools with does not inhabit tree frogs and pools which does inhabit tree frogs (6,5).
The average water temperature at pools with the presence of the tree frog (14,4°C) does differ slightly from the pools which inhabit the tree frog (14,9°C) and is thus a bit colder.
Only a minor amount, 24, of the 129 pools desiccate during summer whereas 15 out of these 24 pools did not show the presence of the tree frog. Out of 33 pools which inhabit fish 31 pools did not inhabit the tree frog. Outstanding is that tree frogs were only found twice in pools where fish were present.
Out of 29 pools with presence of the tree frog 25 pools also inhabited the green frog. In only 5 pools the Crested newt was found whereas 4 of the pools also inhabit the tree frog.
In Table 5, page 27 the different findings for presence/ absence of the tree frog is summarized to give a clearer overview. Standard deviations were as follows:

Table 5- Standard deviations of variables to show clear distinctions between presence/ absence means of abiotic variables.

Variable	St. Dev. absence of tree frogs	Mean absence of tree frogs	St. Dev. presence of tree frogs	Mean presence of tree frogs
Water surface (m^2)	20423,6	5136,4	3072,8	1776,5
Depth (cm)	21,49	26,9	9,54	20,2
pH	0,75	6,5	0,74	6,5
Water temperature (°C)	3,61	14,4	4,26	14,9

3.1.2 Quality and Quantity of the pool vegetation characteristics

In Table 6, page 28 the range of the water vegetation divided in the groups of floating, submerged, emerged and riparian vegetation is shown.

26/29 pools where the tree frog occurred contained a low percentage of floating plants (0-9%). The majority of the pools containing 71-100% riparian vegetation whereas 77 pools have 100% riparian vegetation. 90% of the pools had 100% riparian vegetation. 82% of the pools with a 100% riparian were home to the tree frog.

Table 6 – Range of water vegetation cover in percentages with Sd and mean values

Number of pools / Vegetation	All pools (n=129)	Absence of the tree frog (n=100)	Standard deviation / Mean	Presence of the tree frog (n=29)	Standard deviation / Mean
Floating plants			14.52		17.6
0-9%	114	88	4,5	26	5,2
10-30%	11	9		2	
31-60%	0	0		0	
61-70%	0	0		0	
71-100%	4	3		1	
Submerged			31.9		33.7
0-9%	43	35		8	
10-30%	34	27	26,7	7	40,4
31-60%	16	15		1	
61-70%	12	5		7	
71-100%	24	18		6	
Emerged			23.02		34.05
0-9%	34	27		7	
10-30%	58	49	24,9	9	37,8
31-60%	18	14		4	
61-70%	5	4		1	
71-100%	14	6		8	
Riparian			39.94		28.46
0-9%	16	15		1	
10-30%	14	12	70,9	2	83,6
31-60%	11	7		4	
61-70%	2	2		0	
71-100%	86 of which 77 have 100%	64 of which 59 have 100%		22 of which 18 have 100%	

3.1.3 Land use types 300m around the pools

In Table 7, page 29 shows the mean percentage ($\bar{x}$) of Land use presence at the pools. Dominant Land uses (fat numbers) around pools where the tree frog was absent are forest, grass/meadow, urban, heathland and agriculture. Where the tree frog was present the following Land uses were dominant: grass/meadow, forest, agriculture and urban. Notable is that there were no pools with the Land use type reed and low percentages of brushwood, wooded banks, deforestation, new forest and sand. A reasonable amount of pools with the presence of tree frogs were found in grass/meadow, followed by forest with little/no underground and agriculture.

Table 7 - Percentages of the Land use characteristics in the 300m transects around the sampled pools (dominant Land uses shown y big numbers)

Land use in % / **Land use**	All pools (n=129)	Pools with absence of the tree frog(n=100)	Standard deviation	Pools with presence of the tree frog (n=29)	Standard deviation
Agriculture	9.35	**7.31**	18.52	**16.38**	18.56
Brushwood	0.19	0.25	2.50	0	0
Deforestation	0.24	0.27	2.58	0.14	0.77
Grass/Meadow	15.61	**14.06**	24.08	**20.97**	21.79
Garden/Park	4.40	4.65	15.5	3.54	10.19
Forest with herb layer	13.87	**13.45**	29.47	**15.3**	27.71
Forest little/no herb layer	22.15	**23.44**	29.73	**17.73**	26.49
Heathland	11.79	**13.88**	3.54	4.56	13.43
Marshland	2.02	2.41	10.77	0.66	3.25
New forest	0.38	0	0	1.17	9.19
Reed	0	0	0	0	0
Sand	0.45	0.57	27.04	0.04	0.23
Shrub	0.08	0.10	0.91	0	0
Thicket with herb layer	1.27	1.53	8.69	0.37	0.97
Thicket little/no herb layer	1.72	1.24	4.25	3.38	7.54
Urban	15.28	**15.49**	30.28	**14.52**	24.74
Water	1.03	1.10	3.60	0.79	4.26
Wooded bank	0.19	0.25	2.50	0	0

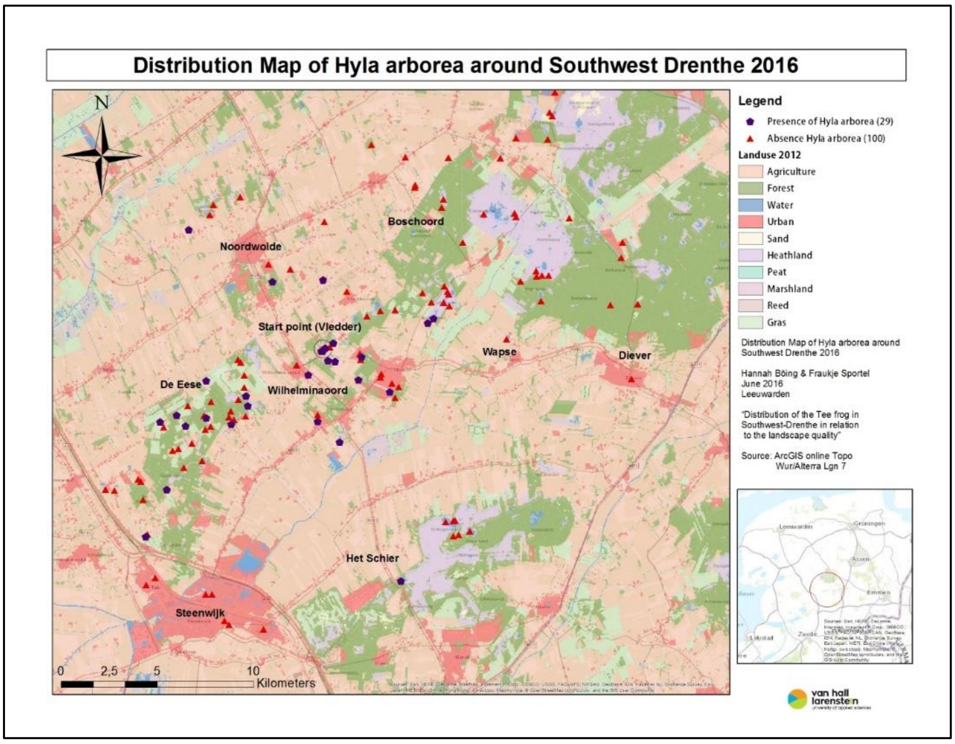
In Appendix VIII boxplots of the variables are shown.

3.2 Occupancy

The occupancy of the tree frog in the study area with 129 sampled pools has a Psi (ψ) value of 0.2249 (+/- 0.0368) and the detection probability; $p = 0.9307$. This was calculated in presence excluding all variables. This means that there is an occupancy of 22.49 %, and a 93.07% probability detection meaning that if the tree frog is spotted once at this study site that there is a 93.07% chance that it will also be detected during second and third attempts at the same study side.

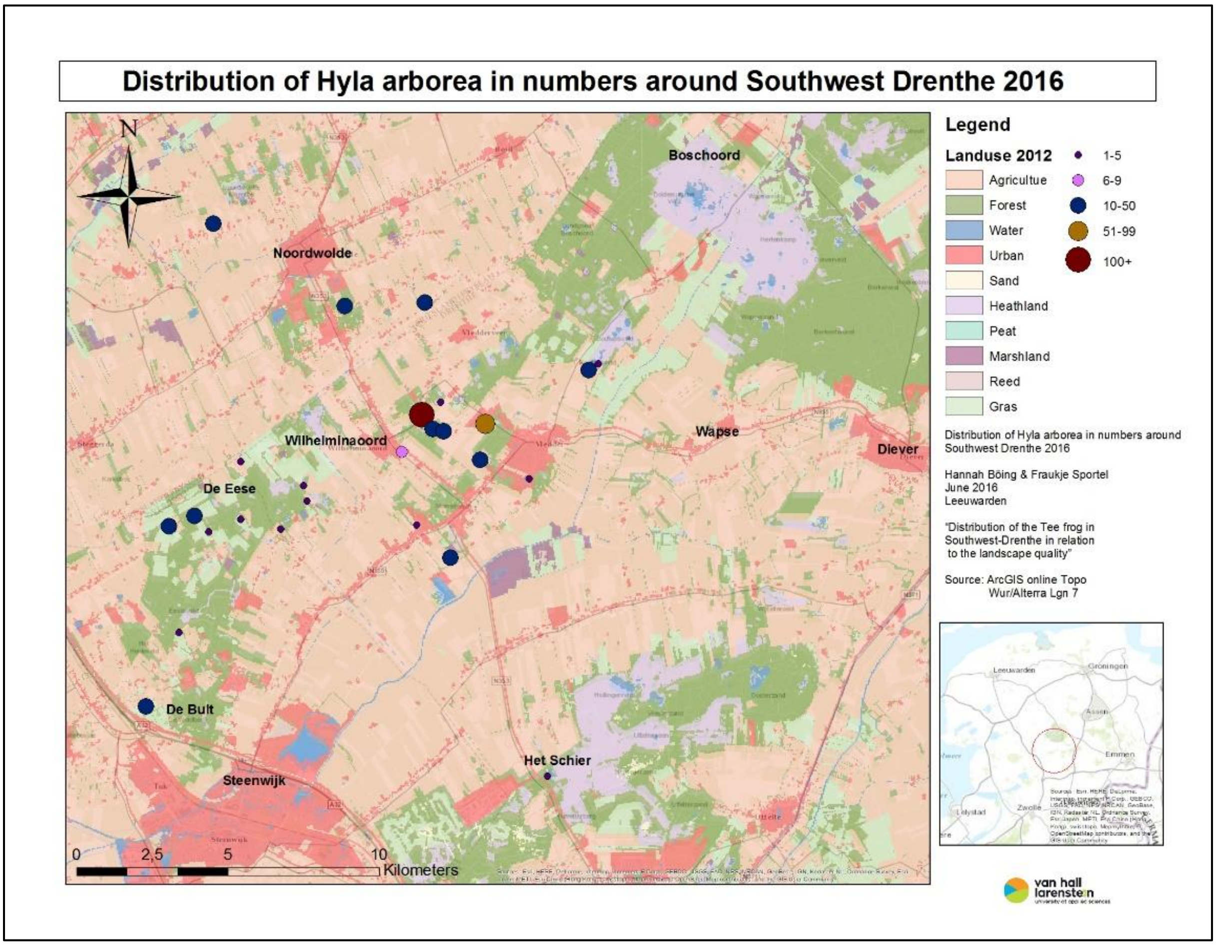
Map 6, page 30 displays the distribution of *Hyla arborea* around Southwest Drenthe in 2016. Each point represents one study site (pool). From the 129 pools 29 pools were marked with the presence of the tree frog (purple pentagon) and 100 pools were marked with absence (red triangle). There is a clear indication that the distribution of the tree frog has strongly spread to the West and densely populated areas are located around Vledder (starting point) and in the area of Eesveen, also known as De Eese.

Outstanding is one pool with the presence of the tree frog located in the South of the starting population near the village Het Schier, located about 9km in the South from starting point, and one pool in the Northwest from the starting population near Noordwolde. Pools sampled in the Land use type heathland do not inhabit tree frog whereas in less than 1km pools where sampled with the presence of the tree frog (Northeast of Vledder). Looking at Urban places, for example Steenwijk all sampled pools showed an absence in tree frogs. It is clearly visible that most of the sampled pools with the presence of the tree frog in the West occurred in or near forest (green areas), whereas sampled pools in the East within the Land use type forest and heathland does not inhabit the tree frog. Looking at the North of the research area a big part of the Land use is covered by agriculture which also results in a lower pool density than for example in the area around De Eese. De Eese is also a very important area to look at due to the fact that there is a high density of pools, which are very close to each other (few hundred meters apart) and the fact that there is a high number of tree frogs but still some pools which do not inhabit the tree frog which are further analyzed in the discussion chapter 4.2.



Map 6 Distribution Map of *Hyla arborea* around Southwest Drenthe 2016 showing the presence and absence of the tree frog in the sampled pools

Looking at Map 7, page 31 the approximate numbers of tree frogs found at the pools are given. The starting point also represents the biggest population with more than 100 individuals spread over 4 pools. Nearby, to the East of the starting point is also a population found with a number of individuals between 51-99. In the area of De Eese where the density of pools is quite high, as stated before, only two pools showed a population bigger than 10 individuals whereas 6 pools inhabit a number of individuals from 1 to 5. Looking again at the two outstanding pools far away from the starting point the pool in the Northwest of Noordwolde has a population of 10-50 individuals and is thus quite a large population compared to the others. During the research of the pool near Het Schier (in the South of the starting point in Map 7, page 31) only a number of 1-5 individuals were recognized.



Map 7-Distribution of Hyla arborea in numbers

3.3. Relationship of pool characteristics with presence of tree frog

The relationships of pool characteristics were analysed statistically using simple single-season tests, Chi^{2}and Mann-Whitney, which where only valuable variables were processed into Logistic regression SPSS Statistic 23.

Table 8, page 32 shows an overview of the selected variables taken into account in the further analysis (logistic regression) which is based on the theory of Bendel & Afiffi (Bendel & Afiffi, 1977).

Table 8- All variables included in the research. Yellow shading according to statistical analysis and green shaded according to literature are taken into account for further analysis

	Test	Variables	Absence	Presence	Z	P-value
Pool Characteristics (nominal)	Chi^2	Green frog	50%	86,2%	-	0,027
Pool Characteristics (nominal)	Chi^2	Crested newt	1%	13,8%	-	0,287
Pool Characteristics (nominal)	Chi^2	Fish	31%	6,9%	-	0,459
Pool Characteristics (nominal)	Chi^2	Desiccation	15%	31%	-	0,135
Pool Characteristics (scale)	Mann-Whitney	Surface	$\bar{x}$ = 5136,4	$\bar{x}$ = 1776,5	-0,824	0,092
Pool Characteristics (scale)	Mann-Whitney	Depth(cm)	$\bar{x}$ = 26,9	$\bar{x}$ = 20,2	-0,327	0,557
Pool Characteristics (scale)	Mann-Whitney	pH	$\bar{x}$ = 6,5	$\bar{x}$ = 6,5	-0,074	0,115
Pool Characteristics (scale)	Mann-Whitney	Temperature(°C)	$\bar{x}$ = 14,4	$\bar{x}$ = 14,9	-3,443	0,252
Pool vegetation	Mann-Whitney	Floating	$\bar{x}$ = 4,5	$\bar{x}$ = 5,2	-0,919	0,358
Pool vegetation	Mann-Whitney	Submerged	$\bar{x}$ = 26,7	$\bar{x}$ = 40,4	-1,828	0,067
Pool vegetation	Mann-Whitney	Emergent	$\bar{x}$ = 24,9	$\bar{x}$ = 37,8	-1,28	0,201
Pool vegetation	Mann-Whitney	Riparian	$\bar{x}$ = 70,9	$\bar{x}$ = 83,6	-0,973	0,33
Land use	Mann-Whitney	Grass/meadow	$\bar{x}$ = 14,1	$\bar{x}$ = 20,9	-2,308	0,021
Land use	Mann-Whitney	Forest with herb layer	$\bar{x}$ = 13,5	$\bar{x}$ = 15,3	-0,791	0,429
Land use	Mann-Whitney	Forest little/no herb layer	$\bar{x}$ = 23,4	$\bar{x}$ = 17,7	-0,686	0,492
Land use	Mann-Whitney	Thicket with herb layer	$\bar{x}$ = 1,5	$\bar{x}$ = 0,4	-1,426	0,154
Land use	Mann-Whitney	Thicket little/no herb layer	$\bar{x}$ = 1,2	$\bar{x}$ = 3,4	-3,304	0,001
Land use	Mann-Whitney	Agriculture	$\bar{x}$ = 7,5	$\bar{x}$ = 16,4	-3,564	0,0001
Land use	Mann-Whitney	Urban	$\bar{x}$ = 15,5	$\bar{x}$ = 14,5	-0,027	0,978
Land use	Mann-Whitney	Water	$\bar{x}$ = 1,1	$\bar{x}$ = 0,8	-1,867	0,062
Land use	Mann-Whitney	Garden/park	$\bar{x}$ = 4,7	$\bar{x}$ = 3,5	-0,467	0,641
Land use	Mann-Whitney	Marsh	$\bar{x}$ = 2,4	$\bar{x}$ = 0,7	-0,408	0,684
Land use	Mann-Whitney	Heath	$\bar{x}$ = 13,9	$\bar{x}$ = 4,6	-1,661	0,097

The variables green frog, desiccation, surface, pH, submerged vegetation, emergent vegetation, grass/meadow, thicket with and thicket with little/no herb layer, agriculture, water and heath are taken into account in the further analysis (logistic regression) because of their value P<0,25. Whereas the variables water temperature, floating vegetation, riparian vegetation and forest with and with little/no herb layer are also taken into account which are of importance according to literature studies.

Using the AIC_C calculated as a result of the logistic model the results were displayed in a ranking model (Table 9, page 33). The outstanding value of the "exp(-$\Delta AIC_c/2$) of G" resulted in $1{,}4^E$-17.

Table 9: Final model containing viable data and results

Categorie	ACCI	ΔACCI	exp(-ΔACCI/2)	Weight (exp(-ΔACCI/2)/total)
Landuse+pool characteristics (L+G)	53,99	0	1	1,00
pool characteristics (G)	131,6	77,61	1,4E-17 (0,0)	0,00
Vegetation (P)	133,6	79,7	0,00	0,00
Landuse (L)	140,5	86,6	0,00	0,00
Total	-	-	1,00	1,00

Table 9 shows the final model. The results in this table resulted in the top and final model concluding Land use + Pool characteristics with a total weight of 100%. This means that the selected Land uses in combination with the pool characteristics has a 100% chance of being the best model showing the group of variables which have the most influence on the tree frog. This means that if these two categories are combined, chances are high of detecting the tree frog in habitats with these characteristics.

Figure 5 page 34 shows the results of the test of significant done with the variables of the top model Land use + pool characteristics. Significant variables can be recognized when the variable results in p<0,05 (yellow shading).

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	GrasMeadow	,013	,011	1,318	1	,251	1,013	,991	1,034
	Forestwith	,006	,010	,357	1	,550	1,006	,986	1,026
	Forestlittle	-,020	,012	2,968	1	,085	,980	,958	1,003
	Thicketwith	-,106	,085	1,539	1	,215	,900	,761	1,063
	Thicketlittle	-,003	,025	,016	1	,898	,997	,948	1,048
	Agriculture	,028	,014	3,826	1	**,050**	1,028	1,000	1,057
	Water	,007	,054	,016	1	,899	1,007	,905	1,120
	Heath	-,023	,017	1,736	1	,188	,977	,944	1,011
	G.Frog1yes0no(1)	1,533	,573	7,152	1	**,007**	4,633	1,506	14,253
	Desiccationinsummer1yes0no (1)	,097	,779	,015	1	,901	1,101	,239	5,072
	Watersurfacem2	,000	,000	3,692	1	**,055**	1,000	,999	1,000
	pH	-,359	,381	,889	1	,346	,698	,331	1,473
	Av.Watertemp°C	-,299	,093	10,305	1	,001	,742	,618	,890
	Constant	5,020	2,661	3,559	1	,059	151,371		

Figure 5- Results show which variables have positive and negative influence on the presence of the tree frog (shaded)

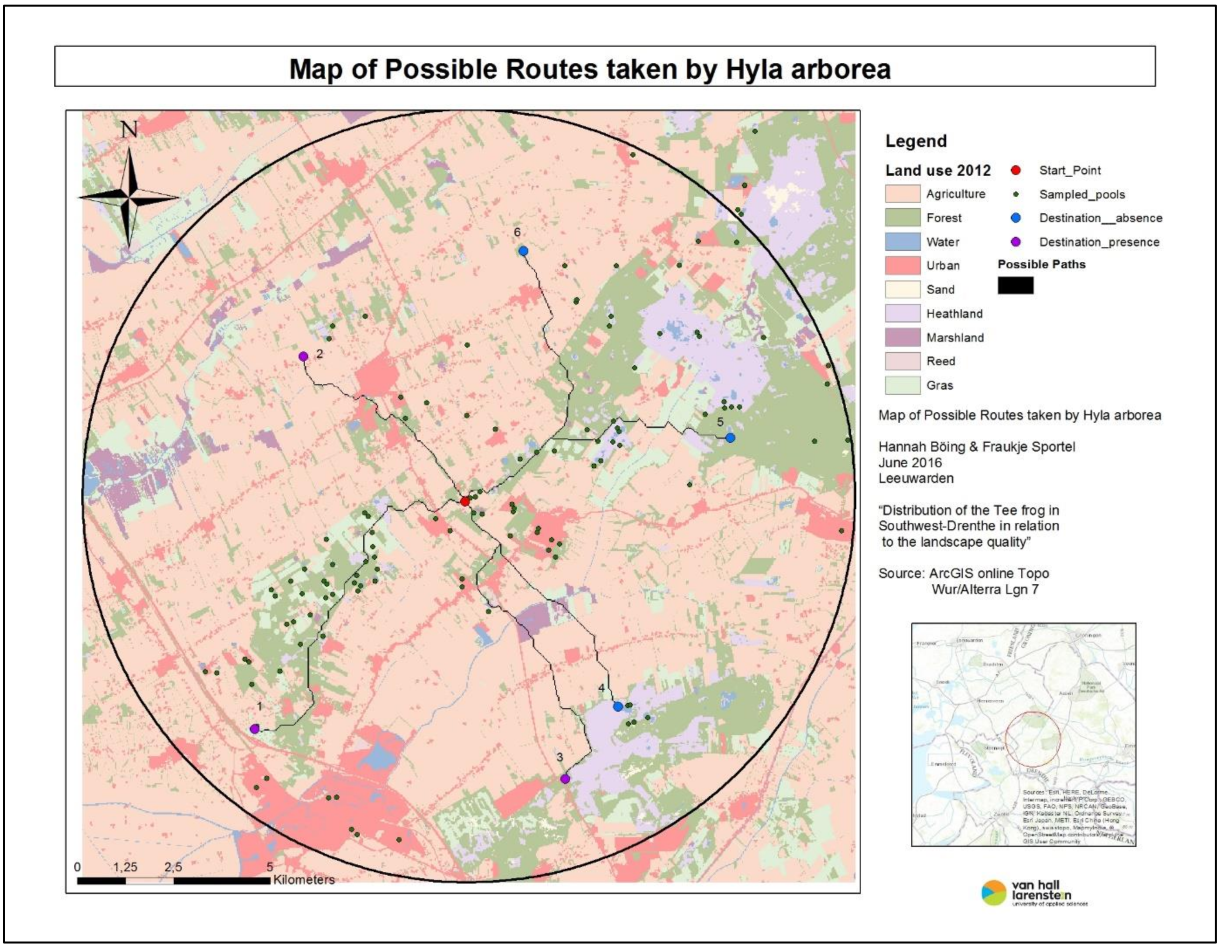
This analysis has shown that for the pool characteristics category, the presence of the green frog has an impact on the tree frogs' presence and water temperature seems to have a negative impact with increased temperature. In the category of Land use only agriculture is significant and has a positive influence on the tree frogs' presence if present.

These results are further discussed in chapter 4, Discussion.

3.4 Land use and Connectivity

The following chapter the illustrate Map shows the result of the tree frogs' connectivity opportunities.

Map 10, page 35 shows 6 possible routes taken by the tree frog from the starting point to different pools. 3 of those destination pools are known with the absence of the tree frog (blue point) and at 3 pools the tree frog was present during the research (purple point). The routes (black lines) are correlated according to the least cost of the path which were calculated with the help of ArcGIS and the friction Table (Appendix VI Table 18). The Friction gives the possibility for the tree frog to move within certain Land uses. As stated before, the higher the friction the higher the effort to move.



Map 10: Possible routes taken by *Hyla arborea*

Clearly, as can be seen in Map 6, page 30 the tree frogs are not found in the heathland areas. Heathland area waters have a pH ranging from 4.0 to 4.5 (De Graaf et al., 1994). This is caused by reduction processes, and is not suitable for the tree frog as the tree frog relies on waters with a pH ranging from 5.5 to 8.0 (Uchelen, 2010). The two points to the South-East (purple and blue), both show no pools along the route. In the discussion chapter 4.1 these points are further discussed.

The analysis (see Figure 6, page 36 and Table 18 in Appendix X) of the taken paths shows that 4 out of the 6 routes the tree frog would move through more than 50% forest.

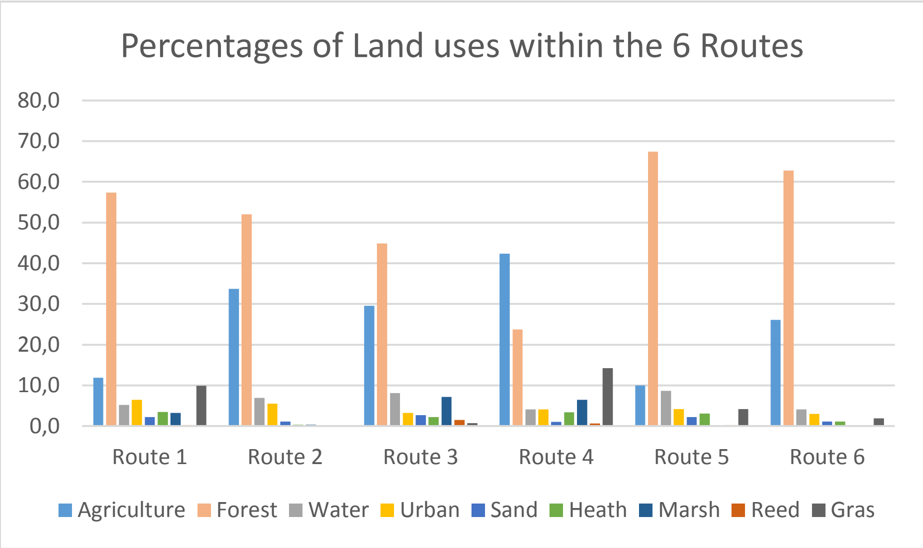


Figure 6- Percentages of Land uses within the 6 Routes

The 6 routes consist out of at least 10% more agriculture Land use, which is thus the second highest value. Marsh, reed, and grass/meadow are the least present Land uses within the routes. Figure 7, page 36 gives an example of Route 1(blue) and Route 4 (orange) and shows the path in meters through the different Land use types. Within the route 1 in the distance of nearly 10km the Land use changes 67 times (count of the peaks) whereas the Land use type forest is entered 23 times agriculture 10 times and water 7 times. Changes from agriculture to forest always refers to less than 1km.

Having a look at route 4 (absence of the tree frog) with a distance of around 7200 meter the Land use changes 48 times. The Land use type forest is entered 15 times as well as agriculture and water 2 times. Also here the changes from agriculture to forest always refers to less than 1km. Looking again at Figure 6 it is seen that the percentage of agriculture is quite high (above 40%) compared to all the other routes.

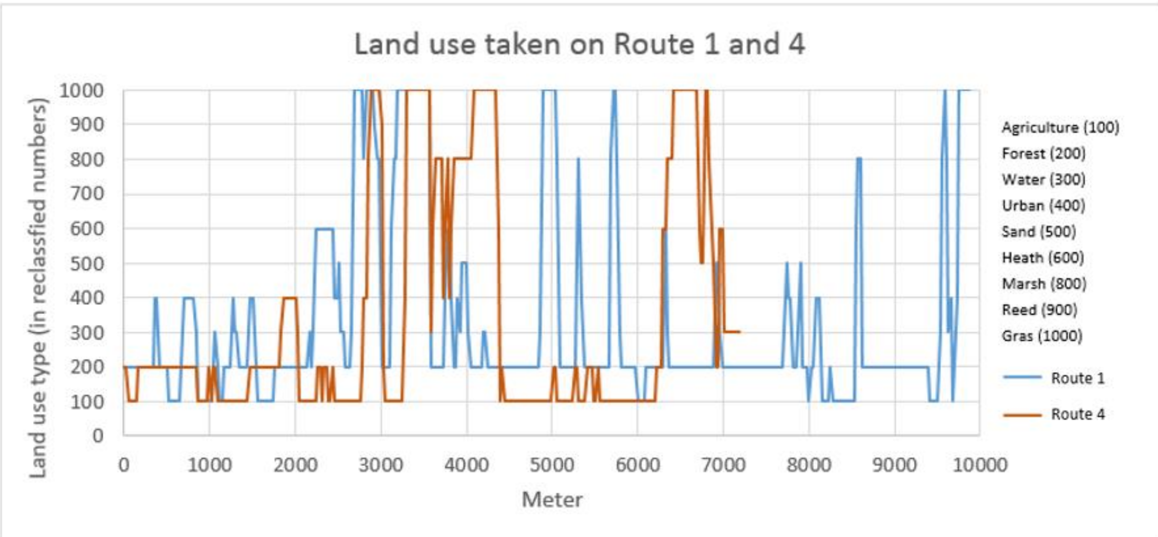


Figure 7- Land use taken in Route 1

These two Land uses also represent the highest cover of the route. There is no part of land which is occupied by more than 1km of the same Land use type.

4. Discussion

Certain factors appeared to have an influence on the results of this research. These factors are discussed in the following chapter. Furthermore, there will be a focus on the results and their comparison with literature studies and predictions for the presence of the tree frog.

4.1 Estimation of the tree frog population and distribution

During the first two weeks of April, breeding season for the tree frogs had begun. This happened sooner than expected due to high temperatures. During this period, the male tree frogs were in their peak of breeding intentions because of the buildup of the hormonal system (Uchelen, 2016). The first few weeks are of essence due to this peak. Unfortunately, due to a delay in data preparation it was not possible for the field research to take place during this time. By the time data preparation was complete, the temperatures dropped again leaving the fieldwork to not much choice but to wait for higher temperatures, when the tree frogs would be active again. Counting this as a missed opportunity, good weather was endured during most of the rest of the days of fieldwork, even though the peak had been missed. Also, it is an observation depending on the right moment, through which the counting could differ if taken place on other days, hours. This could lead to an underestimation of calling males and their distribution.

Also an underestimation of the distribution of the tree frog is possible due the fact that the research area only covers a radius of 10km from the starting point. Looking at the Map 6, page 30 we can conclude that it might be possible that the tree frog migrated further to the West (De Eese) than this research covers. Because there is a lack of pools which inhabit the tree frog in the very East of the research area we can resume that the distribution does not go further than the 10km radius to the East. Also, according to Mattison et al. adult tree frogs are known to travel up to 12km per year (Mattison et al., 2011) and juveniles may travel up to 10km (Uchelen, 2010). Therefore, a radius of 10km is too minimal, also concerning that the tree frogs were released in the year 2000, which is now 16 years ago and observations were done near the border to Friesland and Overijssel which is outside the 10km radius (Creemers et al, 2009; Ravon, 2016). Therefore, the advice would be to extend the research area by sample pool starting at the border of the 10km.

Comparing the distribution of the tree frog from 2014 (Map 2, page 9) and the literature based distribution (Chapter 2.3) with the results of this research it gives a strong accordance. The chance (93,07%) to detect the tree frog is increased due to checking the activity of the tree frog in a pool where the presence of it is already detected before researching unknown pools.

There is at least one place “De Bult” near Steenwijk were it is known that the tree frog was released in +/- 2009 (Meijners, 2016). This means that the distribution of the tree frog not only occurs through migration but also through support of human intervention. More places with released tree frog population in the research areas are not known but still could be present. Because of missing data about populations which are released it is not further taken into account. There is only one pool (near Het Schier/De Bult) which inhabits the tree frog. This population is said to be once set out by a biologist many years ago (De Vlieger, 2016). Unfortunately, more information about dates and quantity on this topic is unknown. However, we can assume that the tree frog did not migrate from the starting point to this area. Only with a DNA test the relation between these populations could be clarified. As other pools in the area do not inhabit the tree frog we can say that there was only limited migration to the South of the area (Uchelen, E., 2010).

4.2 Measuring of variables and literature comparison

Unfortunately, the selected pools were not always accessible due to being private terrains, having barriers such as electric fences, animals or simply the non-existence of the pools according to the recorded coordinates. For this reason, pools which were 'found during travelling' were measured and analyzed. From the list of 150 pools selected in our GIS process, 34 pools were not located/ accessible and thus resulted in a smaller sample size of 129 pools. To get even more reliable results the sample size could be increased.

The results of this research has not been affected by the fact that some of the selected pools were not found due to the disproportional random sampling method. In fact, researching random pools during traveling which differed in surface size (mostly smaller pools <500m2), tree frog was found in pools were they were not expected (according to literature). This resulted in a wider range of results where the tree frog was present and provides for discussion compared to literature studies.

According to literature, the tree frogs are located in pools ranging from 500 to 2000m2 (Uchelen E., 2010). However, the tree frogs were detected in pools ranging in surface area from 51m2 to 13.264m2. Private conservationist, Edo van Uchelen, also quoted, "Although the typical pool size ranges from 500 to 2000m2, the tree frogs can be found where they are not expected" (Uchelen E., 2016). As literature states that surface area has an impact on the tree frog population, the findings of this research cannot confirm this statement. The water surface does not show a significant (0,055) also if variables (higher $p>0.8$) are eliminated and the linear regression is repeated (water surface $p=0.053$), see table x, page x, appendix, x.

By using pH strips instead of a multi-meter the pH value is not as accurate as it could have been (Johnson K.Y et al., 2007). However, this does not have big influence on the results as the tree frog seemed to occur in pools with a pH range of 5.5-8.0 (NABU, 2015). Pools hosting the tree frog had a pH range from 4-7.8. This differs slightly from the norm.

The pH showed no significance in the results resulting in a P-value of 0,346, as this value is below 0,05. Based on literature this means a pH insight the range of 5.5-8 does not influence the presence of the tree frog and has thus no linear effect.

The detection of fish and the crested newt may include an underestimation of their presence. Known that the crested newt is widespread in the research area (Uchelen, E., 2010) we did not know the exact location and pools which inhabit the crested newt so that we were reliant on own observations and knowledge of people. Based on marking each pool with a presence or absence of fish, each pool was only analyzed once for this. This means there is a chance of not detecting the species while it may be present. With the support of literature, it was possible to make an estimation of the presence of absence of fish if not visually detected. Important factors were pools containing medium to high nutrition with sufficient plant structure, which are desiccated and have a depth of no more than 50cm (Perner A., 2016; Sportvisserij Nederland, 2016; Kroes M.J. et al., 201). To correct possible errors a research with three attempts per pool would be advised to calculate the probability detection. This would give a more accurate estimation on the reliability of the detection of the species.

In 25 pools (out of 29) the green frog was present and shows a significant influence ($p=0.007$) on the presence of the tree frog. If the competition and predation has a big influence on the reproduction success of the tree frog, which cannot be answered with this research, it should result in the following statement. There is a high chance of presence of the tree frog if the green frog is present, due to the fact that the green frog is wide spread in this area and has common habitat preferences (Uchelen E., 2010). Having a look at the situation of the crested newt, which is not a significant influence and shares the same

habitat as the tree frog (Ravon, 2016), a main reason for migration problems is the fragmentation of its habitat (Ravon, 2005). Due to roads such as highways, farm roads and large scale agriculture with multiple patches the migration for species like the crested newt and the tree frog increases and distribution gets difficult (Rijksdienst, 2014; Provincie Drenthe, 2016). Based on this information we can assume that the tree frog population also suffers from fragmentation which clarifies why it has difficulties to distribute. Detection of fish and the crested newt resulted in an insignificant contribution to the presence of the tree frog.

During this research pools were analyzed according to whether or not a pool is desiccated as one of the variables. This was of importance because according to literature, desiccation plays an important role in the conservation of the tree frog (Creemers et al, 2009; Uchelen E., 2010). On the long run it will only be possible for the tree frog to survive and reproduce if pools dry up in the late summer season (Uchelen E., 2010). The analysis of desiccation or no desiccation was based on asking local owners and local people. As this characteristic is easily seen we can say that there is a high chance that these results are reliable.

Referring to Map 6, page 30 we can see large-scale agriculture in the South of the starting point.
Back in the 1960's years the biodiverse grasslands and livestock fields separated by wooded banks and hedges offered the so called 'perfect summer habitat' as well as parks and gardens containing various well-developed vegetation. Gardens/parks in this research resulted in no significance. This contrasts with previously done research, could be a result because garden/ park of nowadays is very much limited in terms of biodiversity (Biodiversiteit.nl, 2016). Agricultural fields with poor water drainage and so called livestock drinking pools and garden ponds offered the reproduction pools. (van de Bund, 1964). If Van de Bund's research is correct, this can support the results given in this research and explain why agriculture appeared significant in this research, resulting in a significance of 0,05 and thus a positive influence of the presence of the tree frog.

Also, many pools were located in different Land uses, like forest or thicket, but were often surrounded by agriculture. This means that only a percentage of the vegetation would be forest, and the other percentage would consist of agriculture. It is thought that agriculture seemed to have a significant effect on the presence of the tree frog, as mentioned before, a significance of 0,05. Table 10, page 39 shows pools located in an agricultural area, but surrounded by forest little and thicket little, as well as garden/park. The pools are located in an agricultural area, and therefore are example of why agriculture has occurred to be a significant variable with a positive effect for the presence of the tree frog.

Table 10- Dominance (high dominance grey shaded) of agriculture showing 4 pools with the presence of the tree frog and their Land use coverage of pools 54-57

Pool #	Presence	Forest with	Forest little	Thicket with	Thicket little	Agriculture	Garden/Park	Marsh
54	Yes	0,00%	0,00%	0,00%	6,67%	24,16%	2,50%	0,00%
55	Yes	0,00%	0,00%	0,00%	7,50%	54,00%	14,33%	0,00%
56	Yes	0,00%	36,60%	0,00%	3,16%	63,91%	5,60%	0,00%
57	Yes	0,00%	22,91%	0,00%	0,00%	23,59%	2,08%	0,00%

The Land use urban resulted in no significant influence on the presence of the tree frog. In a research from 2004 it has been concluded by Pellet et al that tree frogs are negatively influenced by the presence of roads and urbanization (Pellet et al. 2004). This is not in agreement with the results of this research but could be a result of the unequal sampling per Land use type.

Although forest showed no significance, it was further taken into account as a large percentage of our sample size consisted of forest (19,89%). According to research from 1964 (Van de Bund, 1964), tree frogs are attracted and appear to habituate in compact forests. These forests were transformed into grass land and agricultural pads during colonization of humans. The interaction of the different variables and the unequal sampling of Land use could result in this research in no significance of forest.

There is still the uncertainty of why some pools within the forest type do inhabit the tree frog and some of them do not, especially in the West of the research area. Comparing pool number 37 and 38 (no tree frogs) with pool numbers 98 and 118 (presence of tree frogs) there is no big range in the variables of surface, mean depth, pH and water temperature. The water vegetation is quite dense at the pools 98 and 118 whereas there is hardly any vegetation at the pools 37 and 38. Also outstanding is that the dominant Land use is heath (at pools 37 and 38) and forest around the pools 98 and 118. Resuming this data, it seems that the heathland has no influence on the presence of the tree frog, concerning migration opportunities it might be a limitation. This also shows in the statistical analysis where heath shows no significant, with a P-value of 0,188. The dominating factor for heathland is its low pH which can also be seen by examining the pools in the Doldersummerveld (see Map 9 red circle in the East of the starting point) where pools are located within a dominating Land use of heath and grass. The pools do not inhabit the tree frog, whereas in the area nearby, approximately 500m further, pools within the consisting other Land uses did inhabit the tree frog. These pools are surrounded by grass, marsh and little thicket and do inhabit the tree frog. Contrasting findings show that the pH at Doldersummerveld taken from 6 pools (#124-129) all had a pH ranging from 5,8 to 6. Pool #67-71 also consisted mainly of heathland, but here too, the pH was not outrageously low, ranging from 5,1-6,2. These are appropriate pH's for the tree frog to survive and reproduce, however, not one frog was located in these pools. Pool #5, however, had a very low pH at 3,9, here there was no presence of the European tree frog. The results show that, even though the pH may be just right, the tree frogs are nowhere to be seen in these pools. Clarification for this would be the vegetation structure around the pool.

Looking closer to the estate of De Eese there are also pools which do not inhabit the tree frog while some pools with tree frogs are located directly next to them (pool # 98, 118 and 108). The only difference seems to be in the mean depth (68cm) and very few submerged plants (pool #108) where no tree frogs were found. These characteristics are shared with some other pools around this area which do not inhabit the tree frog (#106: 95cm and #114: 64cm). Statistics support these results; depth has no significant influence on the presence tree frog.

Resuming there is a wide range of different variables which could influence the presence and absence of the tree frog and differ per pool and per surrounding it is important to look at different scales (vegetation, water, Land use) to show a good understanding of effects. As stated in the results the Land use agriculture have a significant influence on the presence of the tree frog as well as the presence of the green frog and the water temperature.

When water as a form of Land use was analyzed it also appeared to be no significant variable concerning influence on the tree frog. A research from 2011 stated that more than 20 small pools in complex formation in the same area suggests to be a positive influence on the presence of the tree frog (NLWKN, 2011). This means that a cluster of pools in an area would support the presence of the tree frog and its migration. This difference of both researches could be based on the small sample size in areas with clusters of pools in this research and thus shows no significance.

For pool vegetation all vegetation types were included as literature states: Well developed vegetation is of importance for the tree frog's habitat (Uchelen, 2010). Outstanding was that this research shows no significance of all of the four variables. Even though they were included for further analysis due to supportive literature statements.

During this research, out of the amount of pools containing tree frogs (29), only 9 were found to be desiccated during the late summer. This research resulted in no significant of desiccated pools. However, it may be so that this quantity of data collected is insufficient for the statistical analysis to result in significant values to show fair results.

Tree frog were found in pools with a mean water temperature of 7-22°C which differs from the literature research where it is stated that tree frogs are mainly found in mean water temperature of 16-25°C. Because of the quite shallow pools and the weather condition the water temperature can change very fast. Pools measured at the beginning of the field research were in general a bit colder than pools measured at the end of the research were the weather was generally sunnier and warmer. Thus the mean water temperature of each pool can differ strongly depending on the outside temperature and the daytime.

After analyzing all factors, it was found that the tree frogs occur where they were expected (measured according to literature), whether it concerns pool characteristics, Land use structures or pool vegetation. Even so, some pools contained fish and the crested newt as well as hosting the green frog. Generally, the tree frogs occurred where they were expected according to literature studies. More details are to be found in Tables 4, 6 and 7, pages 26-29.

Table 11, page 42 shows a summary of the literature statement compared with the finding of his research.

Table 11- Comparison of literature statement and research findings

Variables	Statement Literature	Findings research
Desiccation	Desiccation to avoid predator (Uchelen E., 2010)	9 out of 29 pools desiccate during summer
pH	pH of pool were tree frogs are found is in the range of 5.5-8 (NABU, 2015)	Tree frogs were found in pools with a pH range of 4-7.8
Water depth (cm)	depth of around 30cm (Soortbeschermingsplan, 2001)	Tree frogs were found in pools with a mean depth of 6-50cm
Water surface (m^2)	between 500 m^2 and 2000m^2 (Soortbeschermingsplan, 2001)	Tree frogs were found in pools with a surface of 51-13.264 m^2
Water Temperature	16-25°C (Creemers et al, 2009)	Tree frogs were found in pools with a mean water temperature of 7-22°C
Vegetation & Land use	For survival and reproduction, the tree frog needs an area characterized by shrubs, hedges, resting water and developed vegetation like herb and shrubs layers (Bergmans & Zuiderwijk, 1986).	Important Land uses types for the presence are Grass/meadow, Forest, Thicket, Agriculture, Water and Heath. Furthermore, does the pool vegetation also has influence but not as strong as the above named variables.
Presence Fish/green frog/crested newt	should not inhabit any species of fish and other amphibians to avoid competition and predation (Creemers et al, 2009; Uchelen E., 2010)	Only two pools inhabit fish, 4 pools inhabit the crested newt and 25 pools inhabit the green frog

4.3 Analysis in ArcGIS 10.3.1

During pool selection the steps undertaken did not result in an equal number of pools shared over the different Land uses. Appendix VI shows an ArcGIS 10.3.1. flow chart of the processes undertaken to result in the final selection of pools. At stage 1 (Figure 9 in appendix VII), where the selection was left with 260 pools, 150 were chosen using random stratified sampling. There was no selection according to Land use, pools were chosen using the random stratified sampling method and by doing so it was thought that the selection would be spread over the whole radius assuring that each Land use would be included but not in equal amount because of too less existing of some Land uses (see Table 1, page 13).

Another dilemma concerning ArcGIS during the selection of the pools is that the water levels may rise and fall within a short period of time. The top10_NL_watervlak (water level Map of the Netherlands) used is a Map from 2015. Seeing this fieldwork took place in April 2016 it may be so that water levels are not the same as previous years or will not be the same as water levels in the future. Also, water levels may differ between winter and summer seasons. In the Netherlands, during the summer there is more rainfall than during the winter period.

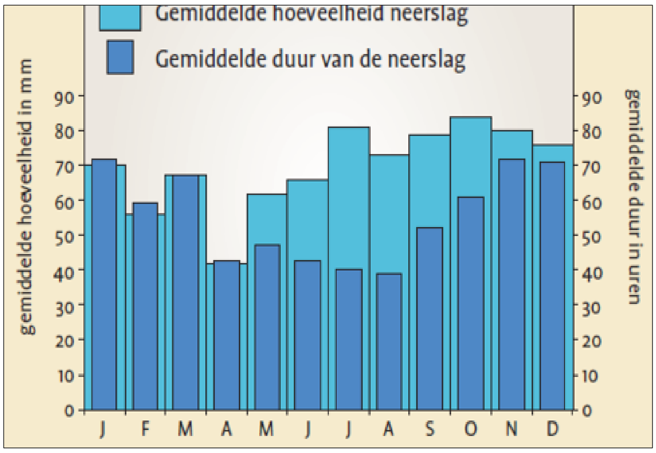


Figure 8- Amount and duration of rainfall annually in the Netherlands (KNMI, 2016)

In Figure 8, page 43 the rainfall per month is shown. From January to March the rainfall is relatively high varying from 65 to 80. As the research took place from the end of April till mid – end May the rainfall was relatively low. As this research took place during the beginning of spring time and quite high temperature at the end of the research, this could be a reason that some smaller pools with low depth could not be found in the field as they may have dried up by the time they were to be analyzed.

The Land use Map (Lgn 7) used during this research is based on data from 2012. It is stated that the Map has an accuracy of more than 80% and that during the years 2007/2008 -2012 only 0.77% of the Land use changed within the categories. Based on this it can be said that the LGN 7 Map is a good base with a high accuracy for this research (Hazeu G.W. et al., 2014) and that there are no big changes within the Land use types agriculture, water, forest, heath, marsh, sand, urban, reed and grass.

During the connectivity analysis in ArcGIS a raster grid size of 25m*25m was used. This grid size is relatively oversized compared to the movement of the tree frog, which mostly moved up to 300m from the pool (Creemers et al, 2009; NSR, 2015). One grid covers approximately than 8% of its route and could thus result in a too general view on the landscape. To specify, a smaller grid size and a more detailed Land use categorization must be used for more suitable results if looking at the path of the tree frog.
Data from the last years (2014-2016) reveal that the tree frog has spread up to 10km from the starting point, which is in agreement with the collected data where tree frogs where found up to distances of 9km from the starting point. Also, at the named villages (Noordwolde, Vledder, Wilhelminaoord) (Ravon, 2016) the tree frog was found (Map 4, page 14 & Map 6, page 30).
A sensitivity analysis is implemented to stimulate error propagation resulting in weights that are expressed through probability density functions (IJGIS, 2014). Taking a sensitivity analysis into account by increasing the friction for forest type from 1 to 3 the Cost path analysis still shows forest as the highest (36.8%) and agriculture as the second highest (24.4%) percentages in route 1.

Table 12- Land use change according to friction

Route / Land use	Low forest friction 1	High forest friction 3
Forest	57,3%	36.8%
Agriculture	11,9%	24.4%
Grass	9,9%	12.2%
Urban	6,5%	5.7%
Water	5,2%	11.2%
Heath	3,5%	4.2%
Marsh	3,2%	1.2%
Sand	2,2%	3.3%
Reed	0,2%	1.0%

Table 12, page 43 shows the percentage changes in the route. Outstanding is that the percentage of forest decreased significantly (over 20%) whereas agriculture increased from 11.9% to 24.4%. The Land uses Water and Grass also increase whereas Urban decreases. The Figure 9, page 44 shows both routes and their way in meters through the different Land use types. There are 3 parts (black squares) which represent the biggest difference in the both routes. Here the Land uses changes for more than 1km compared to the first route with a low forest friction. Taking this change into account by looking at the possibilities for the migration of the tree frog it still results in good possibilities for the tree frog to migrate through areas with low friction. Land uses changes occur within 1km as also the next Land use with lower friction.

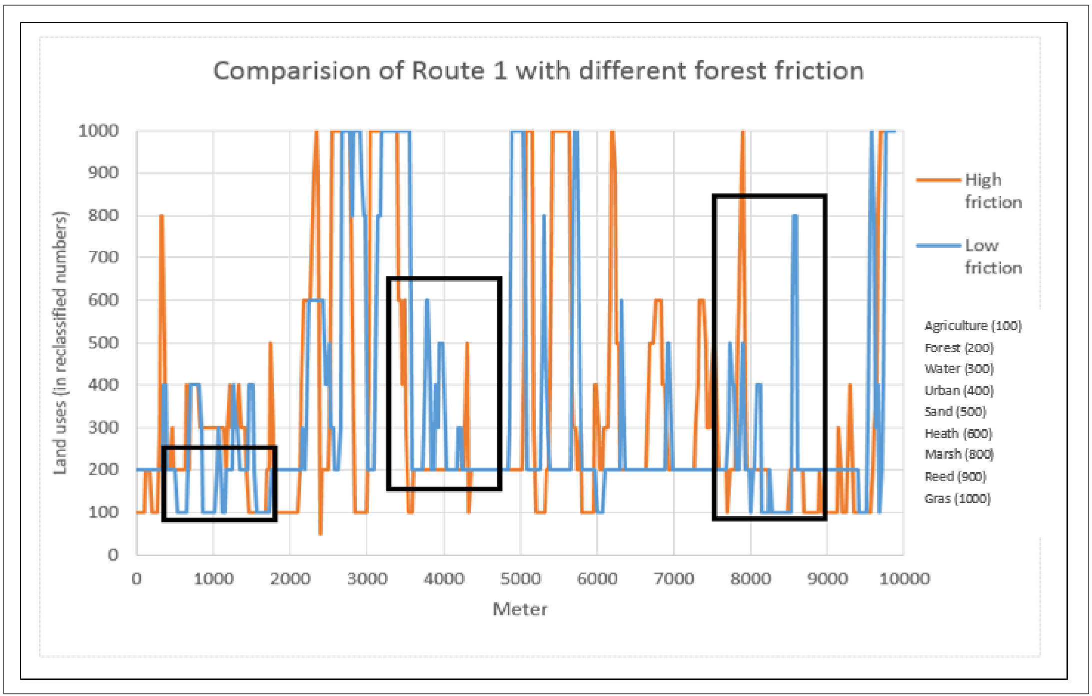


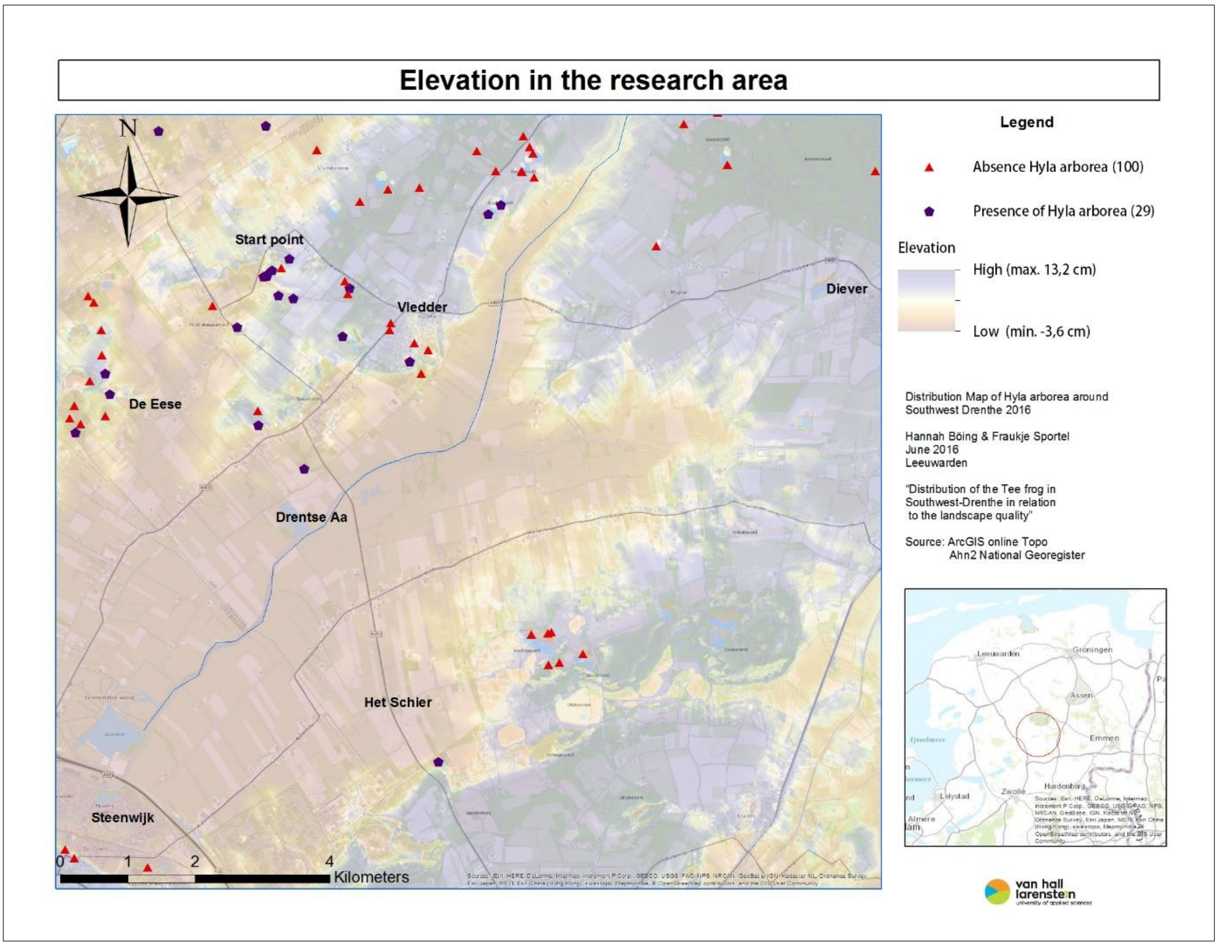
Figure 9- Graph showing the Routes with different forest friction

The change of Land use, and thus, the borders are formed by vegetation structures such as hedges, ditches and forest edges which could give the tree frog more possibilities to migrate from one place to the other due to the fact that the tree frogs depend on the structures (Uchelen, 2010). We could thus resume that fragmentation of Land uses could result in positive effects for the tree frogs´ migration.

4.4 Influence of elevation and the stream valley

Another factor could be the elevation in the South of the starting point (see Map 11, page 42). Around the Drentse Aa there is a stream valley and a decrease in elevation (beige). The elevation seems to be of influence on the tree frogs' presence as they are found on the edges of the valleys (Uchelen, E., 2010). Map 11, page 45 shows an elevation difference of 13,2 (purple shading) to -3,6cm (beige shading). The most pools which inhabit the tree frog (purple points) are found at the edges of the stream valley. The stream valley and the Drentse Aa seems to form a migration limitation for the tree frog. In the South of the river less pools were found and only one did inhabit a tree frog population (Het Schier) which refers to a quite old one which was already discussed in chapter 4.1.

Compared to Map 2, page 9 which shows the known distribution of the tree frog in Southwest Drenthe, the South of the Drentse Aa shows one population of the tree frog. In this research another population was found and so results in only two tree frog populations in the South of the river. Therefore, it could be that the stream valley and the Drentse Aa are a possible migration limitation for the tree frog (Uchelen, 2010).

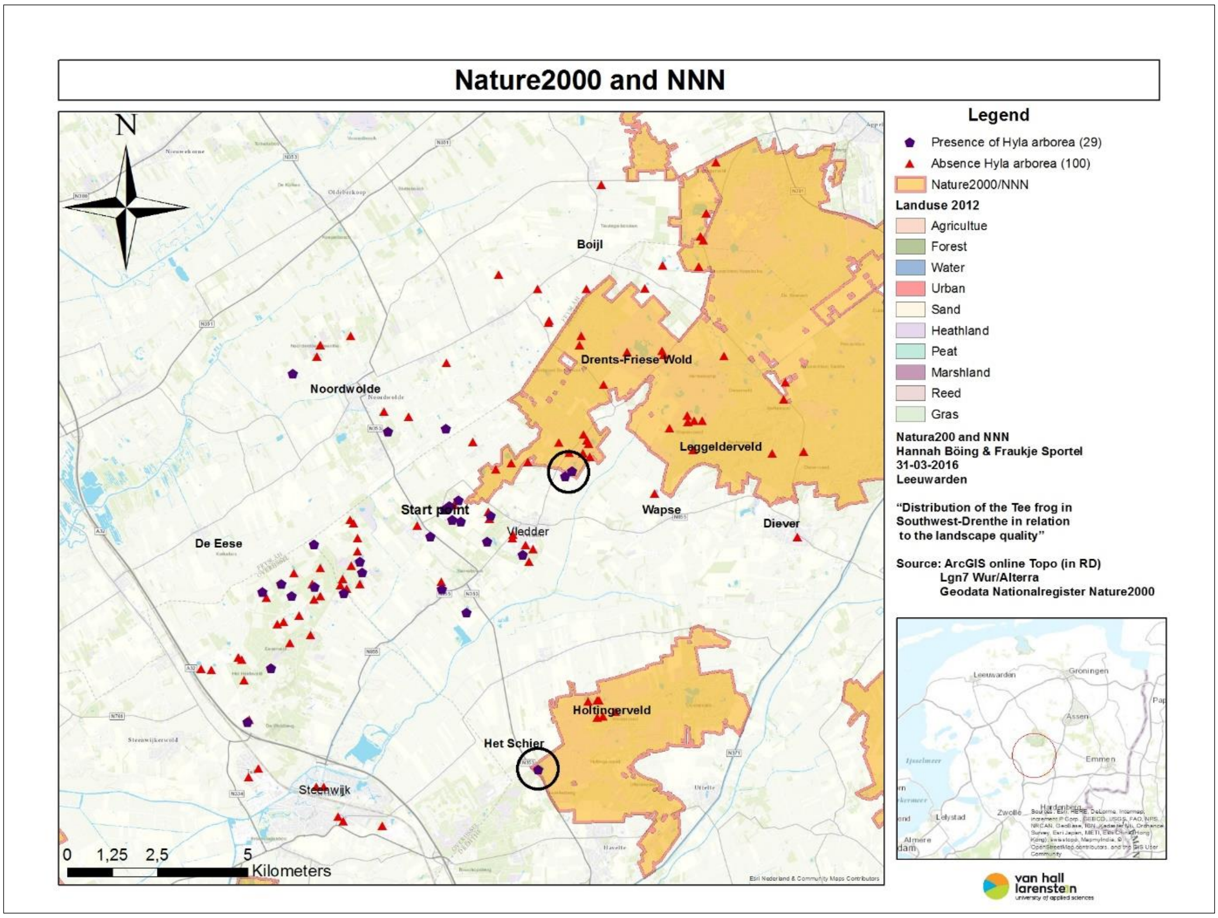


Map 11 Elevation in the research area

4.5 Influence of Nature2000 and NNN areas

Nature2000 areas, the NNN and the Flora and Fauna legislation and also the habitat directive on the landscape and the future management have an influence on the tree frog, but how so?

As mentioned before, 14,6% of the research area belongs to Nature2000 and the NNN (Map 12, page 46). This could be of great importance for the tree frogs' connectivity. When pools were judged in these areas it can be said that in total only three pools where found which does inhabit the tree frog, whereas 35 pools within the Nature2000 areas where researched. This might correlate with the supported vegetation types in this areas (mainly Heath) (Natura2000, 2016), which seems to have a negative correlation with the presence of the tree frog, but the Land use seems to be very important for their distribution. Due to the fact that the crested newt is found in the Nature2000 (Drents Friese Wold, Dwingelderveld and Doldersummerveld) areas it seems to have suitable pools (pool characteristics and vegetation) for the tree frog to live, as it has similar habitat preferences as the crested newt. It is mainly found in pools with water vegetation, shallow to deep and no fish. The surrounded area mainly has the characteristics of agriculture, grass land and forest. The crested newt mainly lives near the reproduction water and prefers a distance of only 100m. Compared to the population of the tree frog the crested newt is generally wider spread and less critical. In total 5 pools were found with the presence of the crested newt whereas 4 of the pools also inhabit the tree frog (# 8, 65, 98, 119) which are all located outside the Nature2000 and NNN areas. Reason for the absence could be the migration limitation due to the Land use as stated before. (Ravon, 2011; Uchelen E., 2010)



Map 12- Nature2000 and NNN areas (pools within Nature2000/NNN areas are marked with a circle)

Through the Flora and Fauna legislation and the habitat directive the tree frog is protected as a native species (FFW, 2016; Ministerie van Economische Zaken, 2016).
Referring to the tree frogs' introduction in this area, this does bring about consequences for other species. A possible interaction with other endemic species like the crested newt and the green frog, which share the same habitat, means that the introduction of another population of a different species, in this case the tree frog, may alter the abundance of already existing populations (Nwf, 2016). Because the green frog and the crested newt are bigger species a chance of predation on the tree frog is given which may alter the population negatively. During this research there was no analysis of this interaction but that the presence of the green frog correlates positively (0,027) with the presence of the tree frog. This does not eliminate possible negative influences of the tree frog on the abundance of the green frog and vice versa.

5. Conclusion

The landscape in the research area West of Vledder appears to be a very suitable habitat for the tree frog in terms of survival and reproduction, whereas the Eastern part of the research area shows to be less suitable to host the tree frog. The reason for this is that the Land use mainly consists of urbanized areas and large scale heathland. To the North and to the South the dominant Land use is agriculture followed by urban, whereas some population were found in the North near the forest and only one population was found in the South.

The Western area provides suitable reproduction pools and allow migration from one pool to another. Looking at the distribution of the tree frog from 2014 in chapter 1.1 Map 1, page 8 with the results of this research, it seems to be a suitable population without further distribution in the last years. Without any further management for the tree frog population sizes and survival within the research area would be questionable.

Out of the 129 sampled pools, 29 pools appeared to host tree frogs. This means that the occupancy of the tree frog at a pool is said to be 22.49%, with a 93.07% detection probability. However, pools with the presence of the tree frog are mostly clustered around the starting point and West of the starting point, in the Estate of De Eese, due to the same management system as the starting point at Edo van Uchelen's private property where "Edo layers" are applied as explained in chapter 2.3.3. Habitat and Ecology.

The category with the most weight in the Akaike's Criteria Model was the "pool characteristics in combination with land use" which seemed to have the most influence on the occurrence on the presence of the tree frog. These two categories combined and merged into one category resulted in a 100% chance of having an influence on the population of the tree frog in Southwest Drenthe. Variables for this category, and thus the factors which have the most influence on the presence of the tree frog, included the presence of the green frog, water temperature and agriculture. The only factor having a negative impact, according to statistical results, on the presence of the tree frog when it increases was the water temperature, whereas agriculture and the presence of the green frog resulted in a positive influence on the presence tree frog.

It can be concluded that the combination of 'pool characteristics' and 'Land use with the pool vegetation' have a positive correlation with the presence of the tree frog. This means that qualitatively, the most suitable pool for the tree frog should provide a pH between 5.5 and 8, a depth of around 10-50cm and should be located in a sunny location so the water can warm up easily, desiccate during summer and there should be a cluster of pools with different surface sizes (51-13.264 m2).

The connectivity in the research area shows generally good possibilities for the tree frog to migrate to all parts of the research area. Possible taken routes for the tree frog show a high percentages of forest (>50%), which gives the conclusion that it is generally possible for the tree frog to move along areas with low cost. The high frequency of Land use changes could contribute to a better migration due to the presence of forest edges, ditches and hedges. Findings show that there is a maximum distance of 1km between the Land uses agriculture, forest and water.

6. Recommendations

Since the last tree frog location recordings in 2014 (Ravon, 2016) the tree frog population has been located in the same pools. This insinuates that the tree frog population can be classified as 'stable', even though there are no quantitative recordings from 2014.

It is advised to continue the research outside a radius of 10km. The radius for this research was taken according to literature studies and due to a limitation in time. It is advised to continue this research outside of this radius in order to see how much further the tree frogs have spread. Logically, due to seasonal factors it is only possible to do this research in the right season. From the 10km radius taken it is advised to draw another 5km radius around the formally used 10km radius. From this it can be judged where the tree frogs do and don't appear, and further radius' can be made to continue the research. Logically speaking, as tree frogs may travel up to 12km a year according to (Mattison et al., 2011). If, according to literature, tree frogs travel up to 12km per year, and now 16 years have passed since their release, is recommended to repeat this research with a 192km^2 radius as this is the maximum distance they are expected to cover over the 16 years. (This is calculated by multiplying the 12km's migrated per year by the 16 years of existence).

It has appeared that the tree frogs are very much in favor and reproduce well in the breeding pools, at the starting point, Edo van Uchelen's pools (+100 individuals). Due to this fact, it can be recommended that management of pools should adjust and adapt to the management measures used by Mr. van Uchelen (see Introduction). These measures have already been adopted by management of "De Eese". The construction of the pools here is now also called "Edo-layers". As discussed in chapter 2.3.3. Habitat and Ecology, Edo-layers are pools which are managed according to the preferences of the tree frog. In many cases, especially concerning "De Eese" it seems to pay off regarding the presence of the tree frog. It is recommended that data from this report is used to analyze which pools do and don't contain tree frogs, and according to this to analyze which pools can be adjusted. It would be realistic to start with smaller pools that desiccate during/ after the summer. This way, these pools can be reconstructed according to Edo-layers to increase the likelihood of hosting tree frogs due to their preferences and success. Over a period of two years, from 2014 to 2016 there has been no decline nor incline in the distribution of the tree frog. Therefore, within the next two years at least 10% of the pools where the tree frogs are located, Edo layers are advised to be adopted. This means that out of the 29 pools where frogs are found +-five are already being managed with Edo layers (at De Eese). Out of the remaining 24 pools, it is advised that 10% of these adopt the Edo-layer management. So two to three pools within two years should be managed by using Edo-layers by 2018.

Connectivity plays an important role for the tree frog distribution. Unfortunately, as results show, agriculture covers a large part of the research area, 55,83%. As tree frogs do not migrate across these landscapes it is important for farmers to put in effort to adjust their farmland and / or ditches according to preferences of the tree frog, which are forest edges, thicket and shrubs. It would be best if farmers allow for thicket/ trees and shrubs to grow along farmland leading from the pools across the paths and so contribute to an easier migration of the tree frog (Figure 10, page 50).

Some pools where the tree frog has been located are private pools. Many local people have little knowledge about the tree frog as a species, which became clear during the research and interviews with people. It is therefore important to educate and raise awareness for these people about the tree frog, and the possible changes they can make to enhance the possibilities for the tree frog. As well as survival and breeding, it is also of importance for the connectivity possibilities for the tree frog. Private pools which may also be smaller than the typical 'norm' may just be a 'transit pool' where the tree frogs may rest between migrations from one pool to another. For this reason, it is advised to raise awareness for example, by handing out flyers, information folders at the tourist information center and general education at primary schools. A good idea is to inform local people and tourists about this during spring time, right before the tree frogs become active after winter.

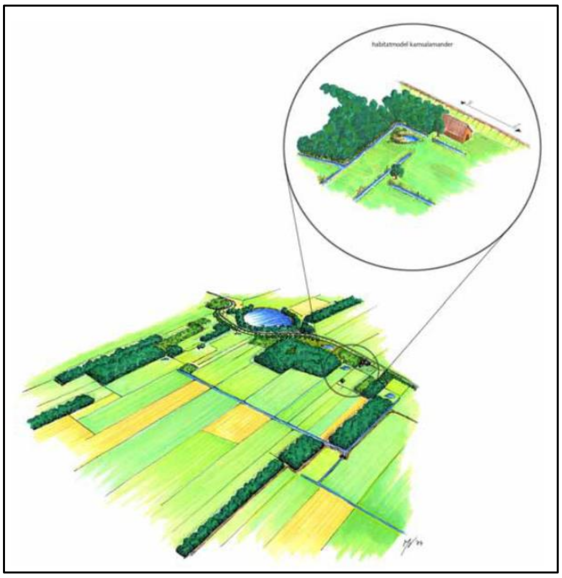


Figure 10- Habitat Model Agriculture (Smit et al., 2007).

Also the management of the Nature200 and NNN areas play a certain role when it comes to connectivity of the areas and the migration of the tree frog. All sampled pools in the research area which belongs to the Nature2000 and the NNN do not inhabit the tree frog. This area covers 14,6% of the research area and is located exclusively in the East. If the goal is to give the tree frog more opportunity to migrate across these areas, management should definitely adjust according to the "Edo-layers" close to breeding populations and so support the migration to the East. In "InstandhoudingSdoelstellingen en analyse begrenzingen Habitatrichtlijngebieden voor kamsalamander, 2005" is stated that there are too few suitable waters for the crested newt around Havelte, Dwingelerveld and Drents Friese Wold but through management of the Vledder Aa and former agriculture areas near Diever the population is supported. This can be also adjusted for the tree frog because of the similar habitat preferences as the crested newt (Ravon, 2005). Important is that the shorter ways for the tree frog to migrate over urbanization areas is cut off with small walls so there is only the possibility to walk is along safer ways (Prudon et al, 2004; NABU, 2016).

Glossary

- **Acidity**: The amount of acidy present in a solution, often expressed in terms of pH
- **Buffer**: Buffer is a great tool if we want to mark a specific area around a specific point
- **Clip**: This tool cuts out parts of the Map. This helps to reduce the data size by removing unnecessary areas.
- **Coefficient**: A measure of a specified characteristic or property of a particular phenomenon under specified conditions.
- **Connectivity**: The possibility for animals to move between interlinked nature areas
- **Convention of Bern**: binding international legal instrument in the field of nature conservation, covering most of the natural heritage of the European continent and extending to some States of Africa.
- **Desiccation**: The process of becoming completely dried
- **Dichotomous** variables: nominal variables containing two categories or levels
- **Dissolve**: This tool combines all polygon with the same attribute in a layer to one polygon
- **Destruction**: The act of destroying something
- **Ecology**: the relationships between the air, land, water, animals, plants, etc., usually of a particular area, or the scientificstudy of this
- **EHS**: All nature areas were connected offering opportunities in distribution from one area to another for many plant and animal species.
- **Fragmentation**: An alteration of habitat resulting in spatial separation of habitat units from a previous state of greater continuity.
- **Friction**: The friction gives the resistance (in form of numbers) limiting the tree frog to migrate
- **GIS**: Geographical Information Systems, a system for storing, editing, and displaying geographical information on computer
- **Habitat**: The natural environment in which a species or group of species lives
- **Habitat Directives**: The Habitats Directive ensures the conservation of a wide range of rare, threatened or endemic animal and plant species. Some 200 rare and characteristic habitat types are also targeted for conservation in their own right.
- ***Hyla arborea***: Latin species name for the tree frog
- **Invasive species**: A species not native in the area, which has been introduced to a specific location
- **Iteration tool**: With iteration in ModelBuilder, a process can be executed over and over using different settings or data in each iteration. ModelBuilder also provides flexibility in iteration, as an entire model or simply a single tool or process can be executed repeatedly.
- **IUCN**: International Union for the Conservation of nature. An international institution for nature protection
- **Logistic Regression**: a statistical analysis used for analysing a dataset in which there are one or more independent variables that determine an outcome. The outcome is measured with a dichotomous variable (in which there are only two possible outcomes).
- **Metadata**: Information given to describe or help use other information
- **Natura2000**: European network for protected nature areas where animals can live freely due to protection of habitat and biodiversity.
- **Nature Network Netherlands (NNN)**: Is a network of already existing and newly built nature areas which were formed during developments of the EHS.
- **Occupancy**: the proportion of sites occupied by the population, according to population abundance estimators.
- **pH**: A number showing how high or low (acidic or alkaline) a substance is
- **RAVON**: Reptielen Amfibieën, Vissen Onderzoek Nederland. (Reptile, amphibian and fish research in the Netherlands) Ravon is a research organization which focusses on the previously named fauna groups.
- **Reclassify**: The process of taking input cell values and replacing them with new output cell values. Reclassification is often used to simplify or change the interpretation of raster data by changing a single value to a new value, or grouping ranges of values into single values—for example, assigning a value of 1 to cells that have values of 1 to 50, 2 to cells that range from 51 to 100, and so on.

- **Select Layer by attribute**: Layers from which features can be selected in ArcGIS with the interactive selection tools. Selectable layers can be chosen using the Set Selectable Layers command in the Selection menu, or on the optional Selection tab in the Table of contents. Layers are selected according to attributes, like features. For example, selected a river according to length, label, name etc.
- **Select Layer by location**: this can select layer which location meet specific criteria like being in a certain area.
- **SNL Grant Scheme**: A grant for an agreement on nature management.

References

Ament, R., R. Callahan, M. McClure, M. Reuling, and G. Tabor (2014). Wildlife Connectivity: Fundamentals for conservation action. Center for Large Landscape Conservation: Bozeman, Montana. Retrieved on

05-04-2016

ArgGIS (2016). Cost Backlink. Retrieved on 27-04-2016 from www.desktop.arcgis.com

Bendel, R. B., & Afiffi, A. A. (1977). Comparison of stopping rules in forward regression. Journal of the American Statistical Association, 72, 46-53.

Benedick. R, E (2000). Human Population and Environmental Stresses in the Twenty-first Century

Bergmans & Zuiderwijk (1986). Atlas van de Nederlandse Amfibien en reptielen. 5de Herpetogeografisch Verslag. KNNV Hoogwoud

Biodiversiteit.nl (2016). Rond het huis en in de tuin. Retrieved on 28-6-2016 from www.biodiversiteit.nl

Blaustein, A. R., D. B. Wake, et W. P. Sousa. (1994a). Amphibian declines: Judging stability, persistence, and susceptibility of populations to local and global extinction. Conservation Biology 8:60-71

Boing (2015). Photograph of tree frog. Retrieved on 20-06-2015 from private archive

Creemers R. et al (2009). De Amphibien en reptielen van nederland, nederlandse fauna deel 9 – Nationaal natuurhistorisch Museum Naturalis, European Invertebrate survey-nederland, Leiden p.186-189

Crombaghs, B.H.J.M., H.J.M. Lenders, & Zollinger, R., (Red.) (2006). Achtergronddocument bij het beschermingsplan boomkikker. RAVON/Bureau NatuurbalansLimes Divergens, Nijmegen

Cushman et al (2010). Mapping Landscape Resistance to Identify Corridors and Barriers for Elephant Movement in Southern Africa, USA

DBU 2016. Modellhafte Revitalisierung des Gutsparks des Landgestüts Redefin unter Berücksichtigung des Naturschutzes, DBU AZ 23176. Retrieved on 30-06-2016 from www.dbu.de

De blankaart (2016). Natuurrundvlees

De Bosrand in Vledder Drenthe (2016). Vledder en omgeving. Retrieved from 12-04-2015 from www.debosrandvledder.nl

De Graaf MCC, Verbeek PJM, Cals MJR, Roelofs JGM. 1994. Effectgerichte Maatregelen Tegen Verzuring en Eutrofiëring van Matig Mineraalrijke Heide en Schraallanden. Nijmegen, the Netherlands: University of Nijmegen. Retrieved on 04-07-2016

Dorland E, Bobbink R, Messelink JH, Verhoeven JTA. 2003. Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *Journal of Applied Ecology* 40: 804–814. Retrieved on 04-07-2016

Dorp Vledder (2016). Verder naar Vledder. Retrieved on 12-04-2016 from www.dorpvledder.nl

Drenthe (2016). Zuid West Drenthe, retrieved on 12-04-2016 from www.in.drenthe.nl

EMSfilms (2015). Edo van Uchelen. Retrieved on 11-04-2016 from www.hollanddefilm.nl

Engel M. (2015). Gefeahrdung fuer Jungfisch. Retrieved on 10-04-2015 from www.deutschlandfunk.de

EPA (2016). Marshlands. Retrieved on 10-04-2016 from www.epa.gov

EU Commission (2016). Natura2000. Retrieved 01-03-2016 from www.ec.europa.eu

Esri (2015). ArcGIS. Retrieved 04-04-2016 from www.esri.nl

FFW (2016). Flora- and Fauna legislation tree frog Table 3. Retrieved 14-03-2016 from www.regelink.net

FOG (1993). Migration in the tree frog *Hyla arborea*. –In: A.H. P. Stumpel & U Tester (eds.), Ecology and conservation of the European tree frog. DLO-Instituut voor Bos- en Natuuronderzoek, Wageningen: 55-64. Retrieved on 14-04-2016.

Geldt (2004). Der Laubfrosch, ein König sucht sein Reich. Laurenti Verlag, Bielefeld

Gennip (2016). Photograph of reed habitat. Retrieved on 13-04-2016 from www.synbiosys.alterra.nl

GeoGraph (2016). Photograph of wooded bank. Retrieved on 30-04-2016 from www.geoGraph.org.uk

Geolocation (2016). PhotoGraph of gardens/parks. Retrieved on 30-04-2016 from geolocation.ws

Gilissen (2016). Photograph of the Aekingerzand. Retrieved on 12-04-2016 from www.natura2000foto.nl

Global Issues (2016). Why is biodiversity important? Who cares. Retrieved on 08-04-2016 from www.globalissues.org

Government of the Netherlands (2016). Nature and Biodiversity. Retrieved on 08-4-2016 from www.government.nl

Grosse W. (1994). der Laubfrosch. Die Neue Brehm-Bucherei, Band 615. Dortmund

Hazeu G.W et al. (2014). Landelijk Grondgebruiksbestand Nederland versie 7 (LGN7). Alterra Wageningen. Alterra-rapport 2548, 2014

Het Reestdal (2016). Photograph of Marshland. Retrieved 14-03-2016 from www.hetreestdal.nl

Holland (2016). Facts and Figures about the Netherlands. Retrieved on 10-4-2016 from www.holland.com

International Journal of Geographical Information Science (2014). An uncertainty and sensitivity analysis approach for GIS-based multicriteria landslide susceptibility Mapping. Retrieved on 1-7-2016

IUCN (2014). “Why is Biodiversity in crisis?” Retrieved on 08-04-2016 from www.iucn.org

IUCN (2016). *Hyla arborea,* retrieved 02-03-2016 from www.iucnredlist.org

Jansen (2016). Photograph of Peat in Assen. Retrieved on 13-4-2016 from www.synbiosys.alterra.nl

Johnson K.Y et al. (2007). “Evaluation of the reproducibility and accuracy of pH-determining devices used to measure urine pH in dogs”, NCBI.

Klein Zeitung (2016). PhotoGraph of roadside. Retrieved on 30-04-2016 from www.kleinezeitung.at

Kroes M.J. et al. (2011). “Structuur voor vissen. Pleidooi voor het verbeteren van vishabitats”. Onderzoek en Beheer, Vissonar. Retrieved on 05-07-2016

KNMI (2016). Uitleg over zware neerslag. Retrieved on 22.6.2016 from www.knmi.nl

Marc Mazerolle (2004). Mouvements et reproduction des amphibiens en tourbiéres perturbées, faculte de foresterie et de géomatique université laval Quebec

Marijnissen, K., (1998). Historische verspreiding van de boomkikker in Noord-Brabant. RAVON. 2 1 (2): 21-24

Mattison et al. (2011). Frogs and Toads. Natural History Museum, England 2011 p. 113.

Mazerolle, M. J. (2001). Amphibian activity, movement patterns, and body size in fragmented peat bogs. Journal of Herpatology 35 p.13-20

Meijners (2016). Owner of private terrain with the presence of the tree frog: verbal, 29-2-2016

Ministry of Economy (2016). Habitat directives. Retrieved on 01-03-2016 from www.minez.nederlandsesoorten.nl

Miniterie van Vlaamse Gemeenschap (2016). "Amfibieën onderweg, Maatregelen voor de bescherming van amfibieën op onze weg", Belgie

Mulder (2016). Private conservationist: verbal, 29-4-2016

NABU (2015). Laubfrosch Gewässer. Retrieved on 14-03-2016 from www.laubfrosch-niedersachsen.de

NAU (2016). Laubfrosch Amphibientunnel. Retrieved on 04-08-2016 from www.laubfrosch-hannover.de

National Wildlife Federation (2016). Invasive species. Retrieved on 20.6.2016 from www.nwf.org

Natura2000 (2016). Habitat types. Retrieved on 10-04-2016 from www.synbiosys.alterra.nl

Natura2000 (2008). MANAGEMENT of Natura 2000 habitats * Semi-natural dry grasslands (FestucoBrometalia) 6210, Technical Report 2008 12/24

Natuur en milieu Drenthe federatie (2016). Mooi Drenthe, retrieved on 13-04-2016 from www.nmfdrenthe.nl

Natuur-liefhebbers (2016). Nieuwe fotos van de natuur liefhebbers, retrieved on 04-03-3026 from www.natuur-liefhebbers.nl

Natuurmonumenten (2016). Drenthe. Retrieved on 14-04-2016 from www.natuurmonumenten.nl

NEM (2015). Populatietrent amfibieën Nederland, Boomkikker. Retrieved 14-03-2016 from www.netwerkecologischemonitoring.nl

NHDFL (2016). PhotoGraph of thicket. Retrieved 30-04-2016 from www.nhdfl.org

Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz – NLWKN (2011). Vollzugshinweise zum Schutz von Amphibien- und Reptilienarten in Niedersachsen. Retrieved on 29-6-2016

NSR (2015). Nederlandssortenregister, tree frog. Retrieved on 14-03-2016 from www.nederlandsesoorten.nl

Overheid (2016). Flora- and Faunawet. Retrieved on 01-03-2016 from www.wetten.overheid.nl

Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR (1996). A simulation study of the number of events per variable in logistic regression analysis. Journal of Clinical Epidemiology 49:1373-1379

Pellet, J., Hoehn, S., Perrin, N., (2004) Multiscale determinations of the tree frog (*Hyla arborea*) calling in ponds in Western Switzerland. Biodiversity and conservation in press.

Perner A. (2016). Garden service. “Tipps rund um den Teichbau“, Schifferstad (Deutschland). Retrieved on 05-07-2016

Provincie Drenthe (2016). Beheerplan Dwingelderveld Ruimte voor een groots heidelandschap, Drenthe 2016

Prudon . et al (2004). Veilig naar de overkant Een kritische kijk op constructie en onderhoud van amfibieëntunnels, Ravon 2004

Ravon (2005). InstandhoudingSdoelstellingen en analyse begrenzingen Habitatrichtlijngebieden voor kamsalamander (Triturus cristatus Laurenti, 1768), Nijmegen 2005

Ravon (2011). “Motivering Natura2000 besluiten voor poldervissen (grote modderkruiper, bittervoorn, kleine modderkruiper), beekvissen (beekprik, rivierdonderpad, beekdonderpad), rivierprik, zeeprik, kamsalamander en geelbuikvuurpad”. End report. Nijmegen, 2011

Ravon (2016). Boomkikkers. Retrieved on 02-03-2016 from www.ravon.nl

Rijksoverheid (2016). EHS. Retrieved on 01-03-2016 from www.rijksoverheid.nl

RijkSdienst voor Ondernemend Nederland (2014). Soortenstandaard KamsalamanderTriturus cristatus, Versie 2.0. 2014

Rijkswaterstaat (2005). Leidraad faunavoorzieningen bij wegen. Retrieved on 1-7-2016

Roelofs JGM, Bobbink R, Brouwer E, De Graaf MCC. 1996. Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in the Netherlands. *Acta Botanica Neerlandica.* 45: 517–541. Retrieved on 04-07-2016

Smit, G.F.J., e.a. (2007). Kansen voor de Kamsalamander. Beschermingsplan voor de kamsalamander in Noord-Brabant. Retrieved on 1-7-2016

Soortbeschermingsplan (2001). Boomkikker 2001-2005, Landbouw, Natuurbeheer en Visserij, Rapport directie Natuurbeheer Nr.42, Wageningen 2001

Sportel (2016). Photograph of surface water near Vledder. Retrieved on 05-04-2016 from private archive

Sportvisserij Nederland (2016). “Viswatertypering”. Retrieved on 05-07-2016

Stichting Internet Platform Drenthe (2016). Zuid West Drenthe. Retrieved on 01-03-2016 from www.in.drenthe.nl

Scheider H. (1971). Die Steuerung des täglichen Rufbeginns beim Laubfrosch, *Hyla arborea*, Oekologia Berlin 8 p. 310-320

Sinsch, U. (1988). Seasonal changes in the migratory behaviour of the toad *Bufo bufo*: direction and magnitude of movements. Oecologia 76:390-398

Sinsch, U. (1990). Migration and orientation in anuran amphibians. Ethology, Ecology & Evolution 2:65-79

Stumpel, A.H.P. (1986). Habitat and ecology of *Hyla arborea* in the Netherlands. – in: Z Rocek (ed.), Studies in herpetology. Charles University, Prague: 409-411

Strumpel A.H.P (2004). Reptiles and amphibians as targets for nature management- proefschrift Wageningen Universiteit. Alterra scientific contribution 13. Alterra green world research Wageningen.

Taylor, P. D. et al. (1993). Connectivity is a vital element of landscape structure. Oikos 1993 p.571-573.

Teemu Tahvanainen (2003). Seasonal variation of water chemical gradients in three boreal fens, Finnish Zoological and Botanical Publishing Board 2003, Helsinki.

Tree Nation (2016). PhotoGraph of brushwood. Retrieved on 30-04-2016 from www.tree-nation.com

Uchelen, E. (2010). Amphibien en reptielen in Drenthe, Profiel in Bedum, p76-79 Boomkikker

Uchelen (2016). Private conservationist: email contact, 12-4-2016

Uchelen (2016). Private conservationist: verbal, 29-2-2016

Dorp, D & Zoer, B. (1993). Beheersadvies voor Wildenberg-Rabbinge ten behoeve van de boomkikker. Rapport van Het Drenthse Landschap, Assen.

Vledder (2011). Vledder. Retrieved on 04-03-2016 from www.dorpvledder.nl

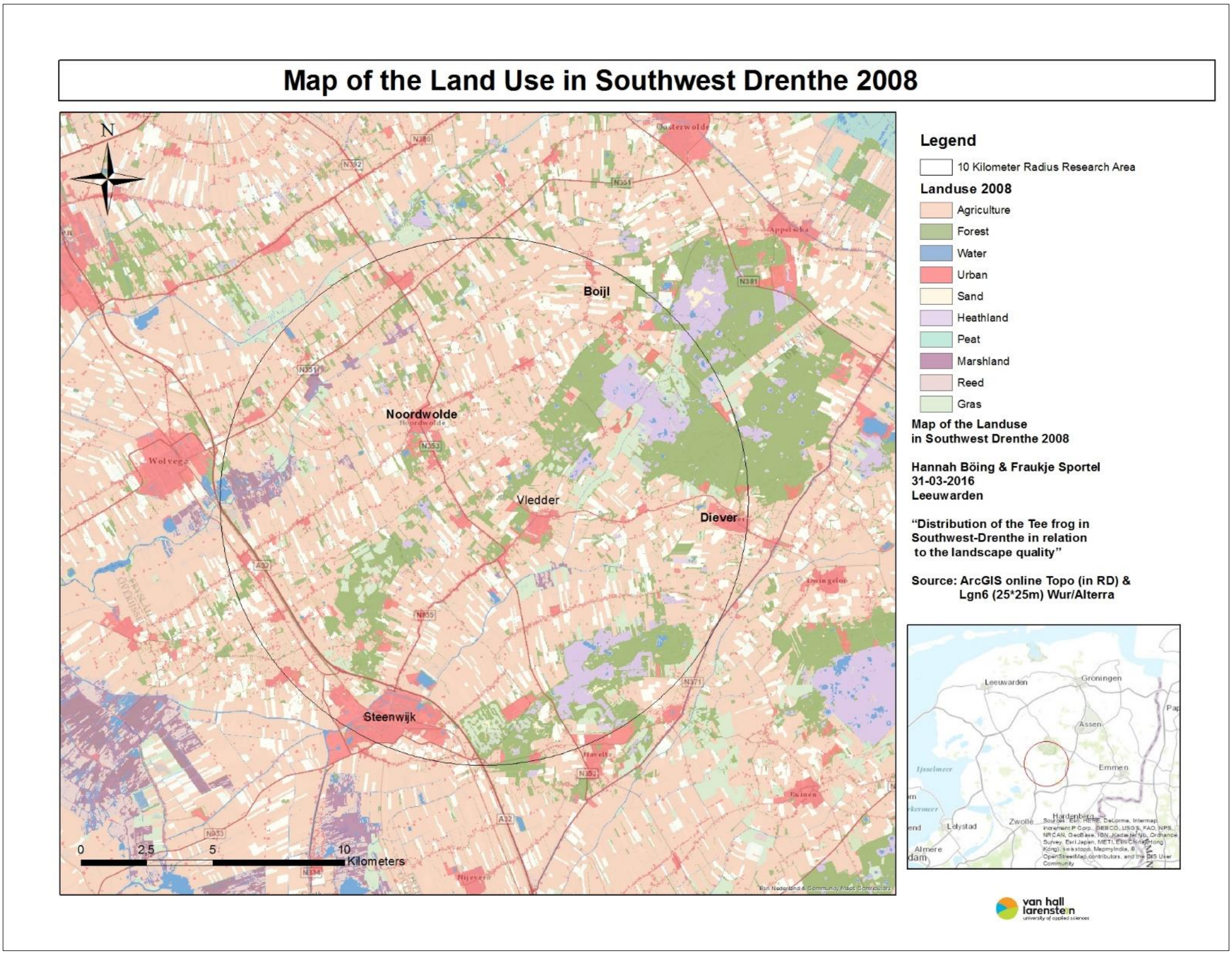
Vledderveen (2016). Vledderveld. Retrieved on 04-03-2016 from www.vledderveendrenthe.nl

Vlieger, de., W. (2016). Forester monitoring: verbal 29-06-2016.

Waarneming (2016). Boomkikker- *Hyla arborea*. Retrieved on 16-03-2016 from www.waarneming.nl

Appendices

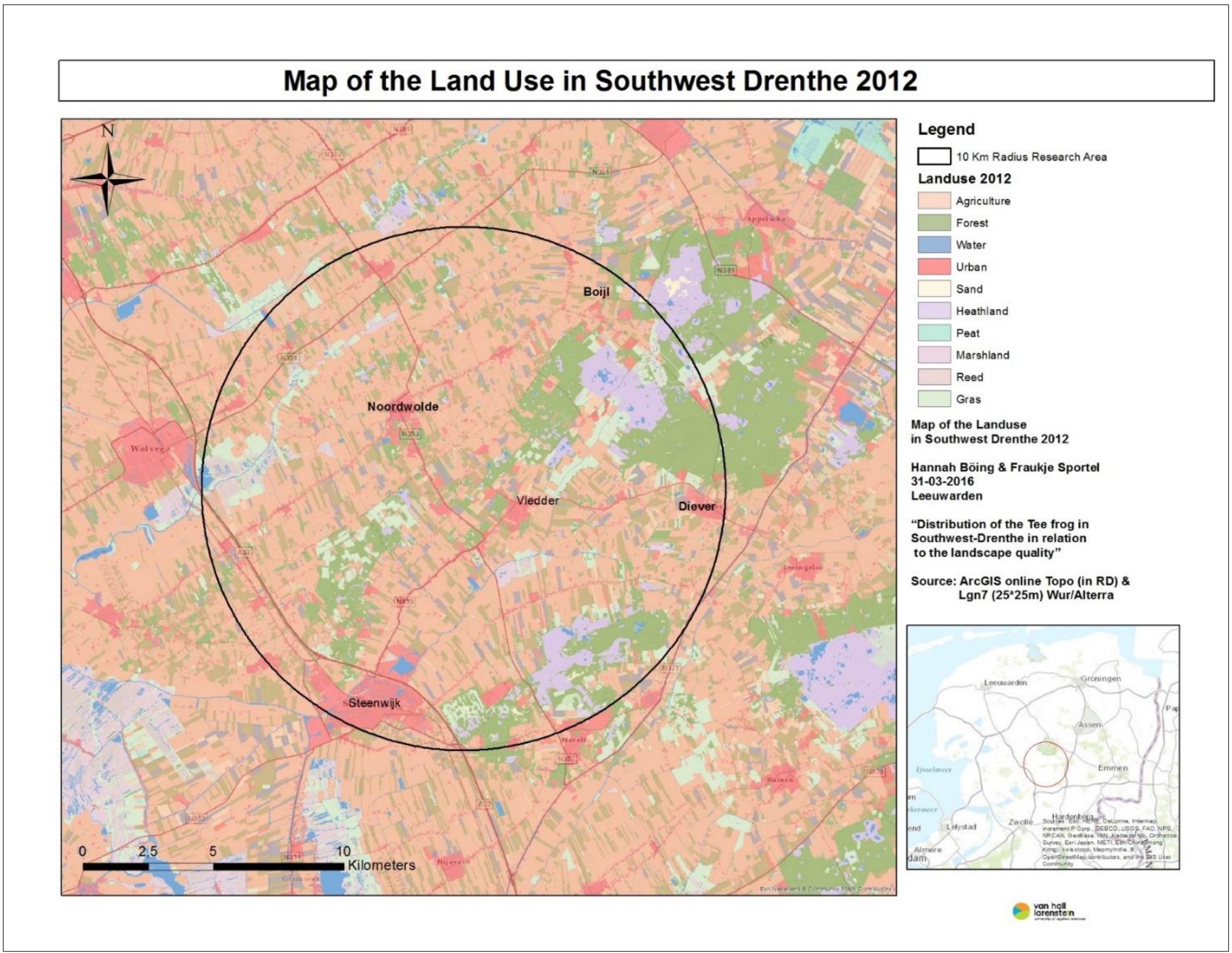
Appendix I – Land use change 2008-2012



Map 13- Map of the Land use in Southwest Drenthe 2008

Table 13- Percentage of Land use 2008

Reclassify number	Land use	Hectare	Percentage (%)
100	Agriculture	23981,9	59.43
200	Forest	6828,2	16.92
300	Water	562	1.39
400	Urban	3602,7	8.93
500	Sand	83,9	0.21
600	Heathland	2043,7	5.06
700	Peat	0	0
800	Marshland	566	1.40
900	Reed	45	0.11
1000	Grass	2637,3	6.55



Map 14- Map of the Land use in Southwest Drenthe 2012

Table 14- Percentage of Land use 2012

Reclassify number	Land use	Hectare	Percentage (%)
100	Agriculture	19931,6	55.83
200	Forest	7102,4	19.89
300	Water	501,1	1.40
400	Urban	3349,3	9.38
500	Sand	85	0.24
600	Heathland	1869,6	5.24
700	Peat	0	0
800	Marshland	360,5	1.01
900	Reed	229,8	0.64
1000	Grass	2272,5	6.37

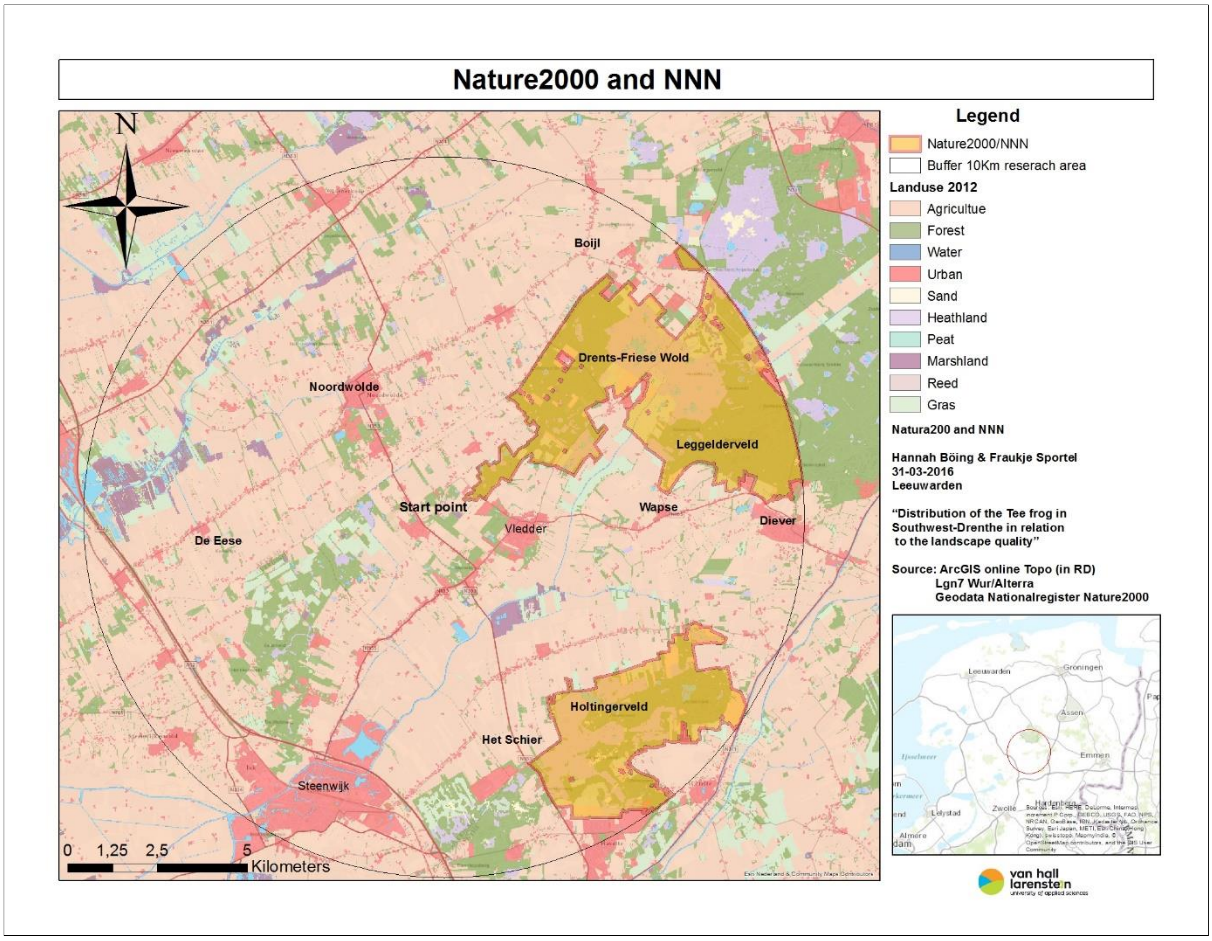
Appendix II – Habitat types

Table 15- Land use types

Land use type	Illustration	Habitat type	Illustration
Agriculture	 (Natuur en milieu Drenthe federatie, 2016)	Sand	 (Gilissen, 2016)
Forest	 (De Bosrand Vledder, 2016)	Heathland	 (Natuurmonumenten, 2016)
Urban	 (Dorp Vledder, 2016)	Peat	 (Janssen, 2016)

Marshland	 (Het Reestdal, 2016)	Grassland	 (De Blankaart, 2016)
Reed	 (Gennip, 2016)	Wooded bank/ hedges	 (GeoGraph, 2016)
Thicket	 (NHDFL, 2016)	Brushwood	 (Tree Nation, 2016)
Roadside	 (Kleinzeitung , 2016)	Surface Water	 (Sportel, 2016)
Gardens/ Park	 (Geolocation, 2016)		

Appendix III – Nature2000 / NNN areas



Map 15- Nature200/NNN

Appendix IV – Field form

Field form *Hyla arborea*

Observer: Fraukje Sportel & Hannah Böing

1. General information			
Date and Time			
Weather condition	°C	Wind:	yes/no rain
Address			
Pool number			
X-Y coordinates			
Picture number			

2. Sketch:

3. Water characteristics	
Length (in meter)	m
Width (in meter)	m
Radius	m
Water surface (in quadrat meter)	m^2
Mean depth (in centimeter)	cm
Presence of fish/other amphibians	Yes / no
pH (0-14)	
Average Temperature of the water	°C
Desiccation in summer	Yes / no

4. Vegetation characteristic at a pool	
Floating aquatic plants	%
Submerged aquatic plants	%
Emergent vegetation	%
Riparian vegetation	%

6. Presence of tree frog	
Yes	
No	

5. Landuse in a radius of 300m around a pool		
Type	**Meter**	**Percentage**
Grass/meadow		%
Forest with developed herb layer		%
Forest with little or no underground		%
Wooded bank/hedges		%
Thicket with developed herb layer		%
Thicket with little or no underground		%
Brushwood		%
Agriculture		%
Shrub or roadside strip		%
Urban (building/roads)		%
Surface water		%
Gardens/Park		%
Reed		%
Sand		%
Heathland		%
Peat		%
Marshland		%

7. Remark:

Appendix V – Example of the pools factsheet

Pool Number 3

General

Temperature	12°C
Wind strength (bft)	0
Rain	No
Address	Reeweg
Coordinates	52.859696 6.182369
Presence of tree frog	Yes (20+)

Water vegetation

Water vegetation	Percentage
Floating	0
Submerged	10
Emerged	0
Riparian	60

Water characteristics

Water surface (m2)	264,50
Mean depth	25
Fish	No
Green Frog	No
C.newt	No
pH	6.1
Av. Water temp.	12.2
Desiccation in summer	No

Land use

Land use	Percentage
Forest with little	35
Thicket with little	28,8
Agriculture	36,2

Appendix VI – Friction Table

Table 16- Friction Table

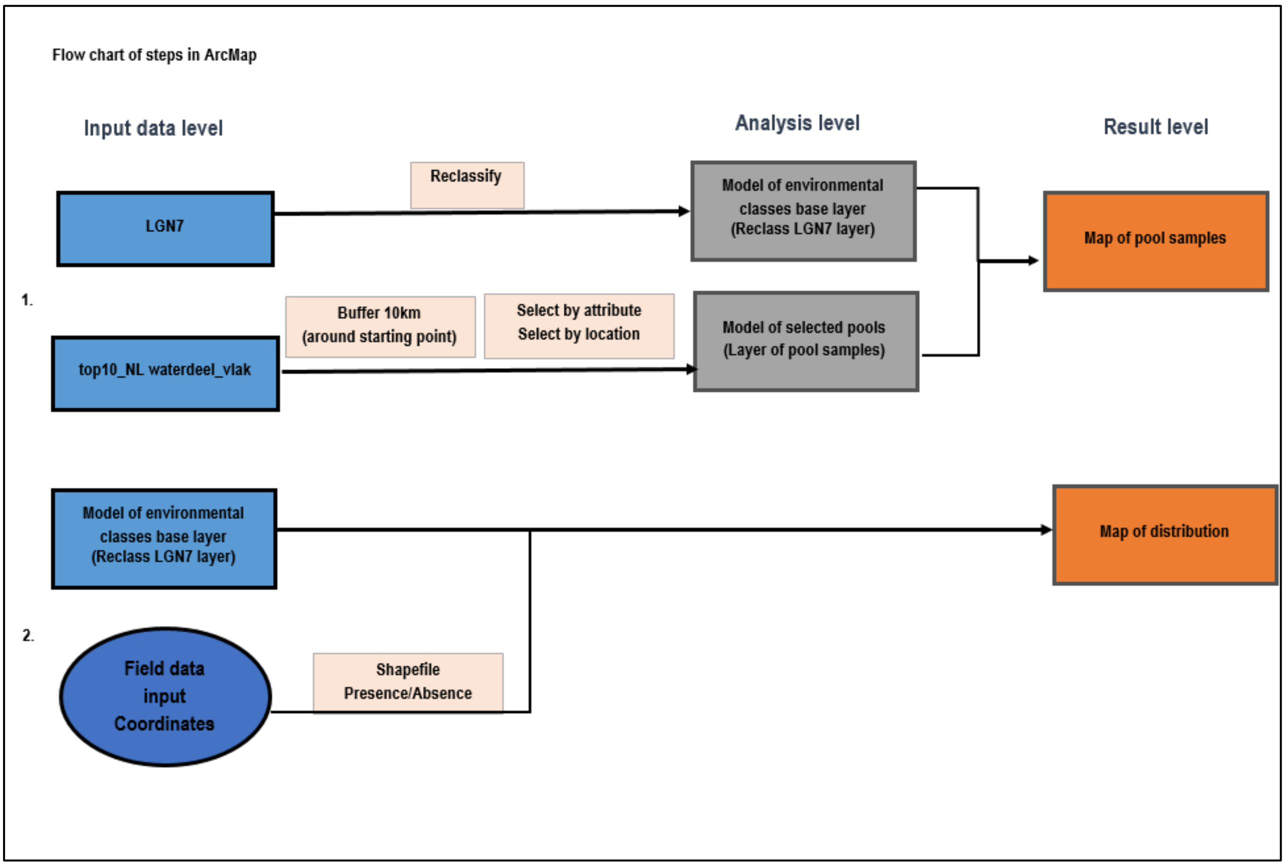
Category	Land use	Friction
100	Agriculture	5
200	Forest	1
300	Water	1
400	Urban	5
500	Sand	4
600	Heathland	3
700	Peat	-*
800	Marshland	2
900	Reed	3
1000	Grass	2

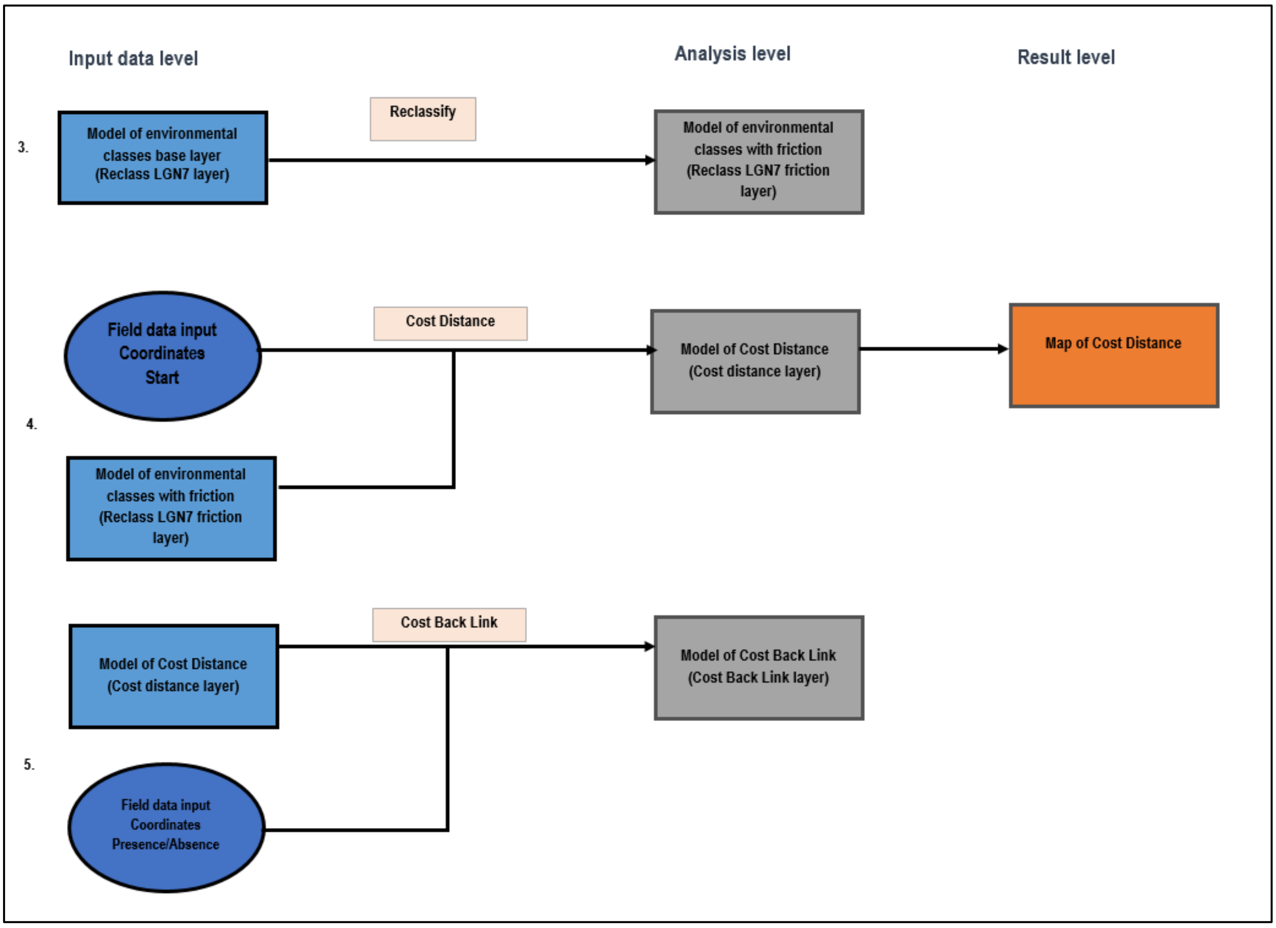
*Peat was not found in the research area so it was not taken into account during further analysis.

Appendix VII - Metadata

Table 17- Metadata

Name	Description	Data source, scale/grid size
LGN 7 (raster)	Land use 2012	Wur/Alterra (25m x 25m)
LGN 6 (raster)	Land use 2008	Wur/Alterra (25m x 25m)
Top (in RD)	TopoGraphy	Esri Nederland & Community Maps Contributors (1:10.000)
Top10NL	Water areas	Geodata Nationalregister (Polygon)
Nature2000	Nature2000 Boundaries	Geodata Nationalregister (Polygon)





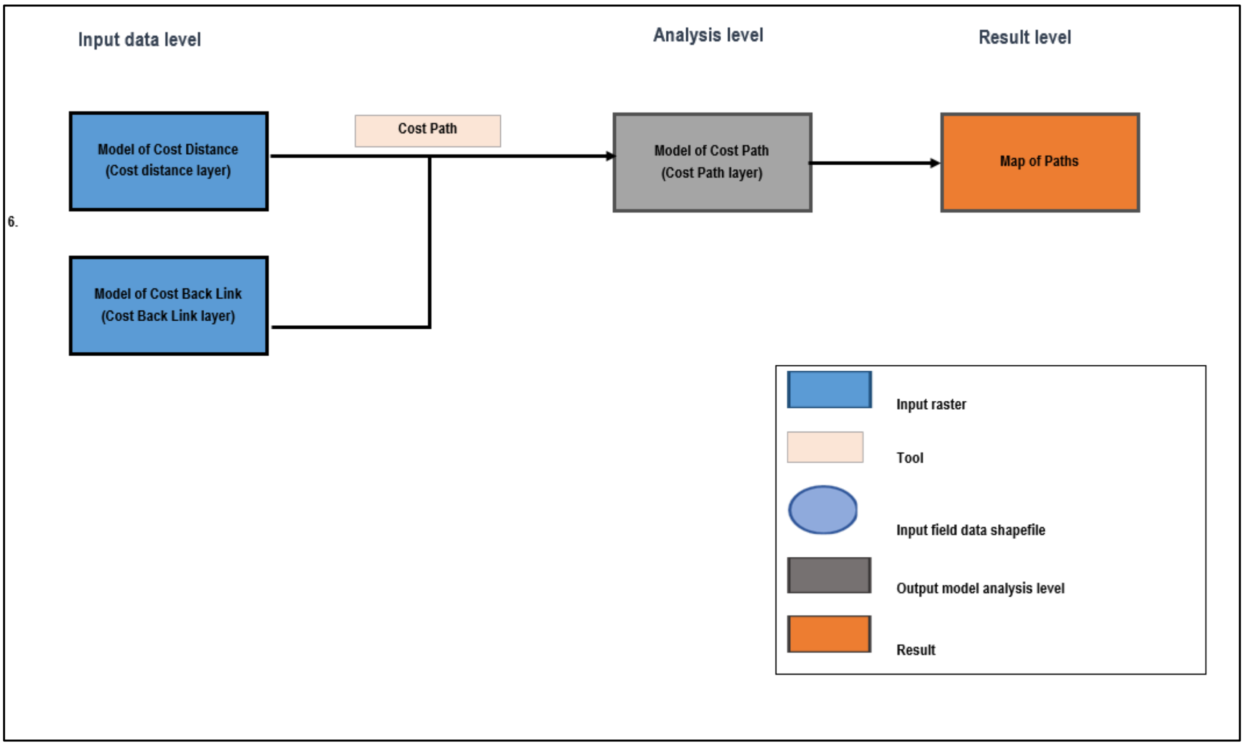


Figure 11- GIS flowchart showing procedures

In the following the steps 1 to 6 for the ArcGIS analysis are descripted.

1. A categorizing of the landscape was done using a classification (Tool “Reclassify”) in 14 groups of the Land use Map of 2012- LGN7 (agriculture, forest, urban, grass/meadow, garden/parks, sand, marshland, thicket, roadside, brushwood, peat, reed, heathland and water). This resulted in a Model of environmental classes base layer (reclass LGN7 layer).
   By adding a 10km Buffer around the starting point and characteristics of pools by choosing the tool “select by attribute” in the surface layer “top10_NL waterdeel_vlak” the preselection was done (see Map 6). This step means a selection of pools which have another function than river, fishing pools or ditches, are no main sewerages and are a water type referred to sea or lake. Furthermore, the surface size was limited between 500 and 2000m^2 which resulted in a layer of pool samples.
   Both layers, the reclass LGN7 layer and the layer of pool samples are shown together in the Map 5 “Research Area with selected Pools”.
2. To create the Map 6 “- Distribution Map of *Hyla arborea* around Southwest Drenthe 2016” the collected coordinates of the study sites (pools) were inserted in the reclass LGN7 layer as a shapefile categorized by presence and absence.
3. To get a layer for the friction of Land use the reclass LGN7 layer was again categorized with the tool “Reclassify” and the following values which resulted than in the reclass LGN7 friction layer
   Agriculture (100), Urban (400) = 5
   Sand (500) = 4
   Heathland (600), Reed (900) = 3
   Marshland (800), Grass (1000) = 2
   Forest (200), Water (100) = 1
4. To create the Map 8 “Cost Distance” the shapefile of the starting point was added to the reclass LGN7 friction layer and running the tool “cost distance”.
5. Creating the Cost back Link layer (Map 9) the coordinated of the presence and absence as shapefiles were added to the model of cost distance before running the tool “Cost back Link”.
6. Using the tool “Cost Path” together with the Cost Back Link layer and the Cost Distance layer the Map 10, page 35 was created. Therefore 3 coordinates of the presence and 3 coordinates of the absence of the tree frog were used in form of a shapefile.

Appendix VIII- Statistics

Absence pool characteristics

Summary of a set of bivariate data with the absence of the tree frog

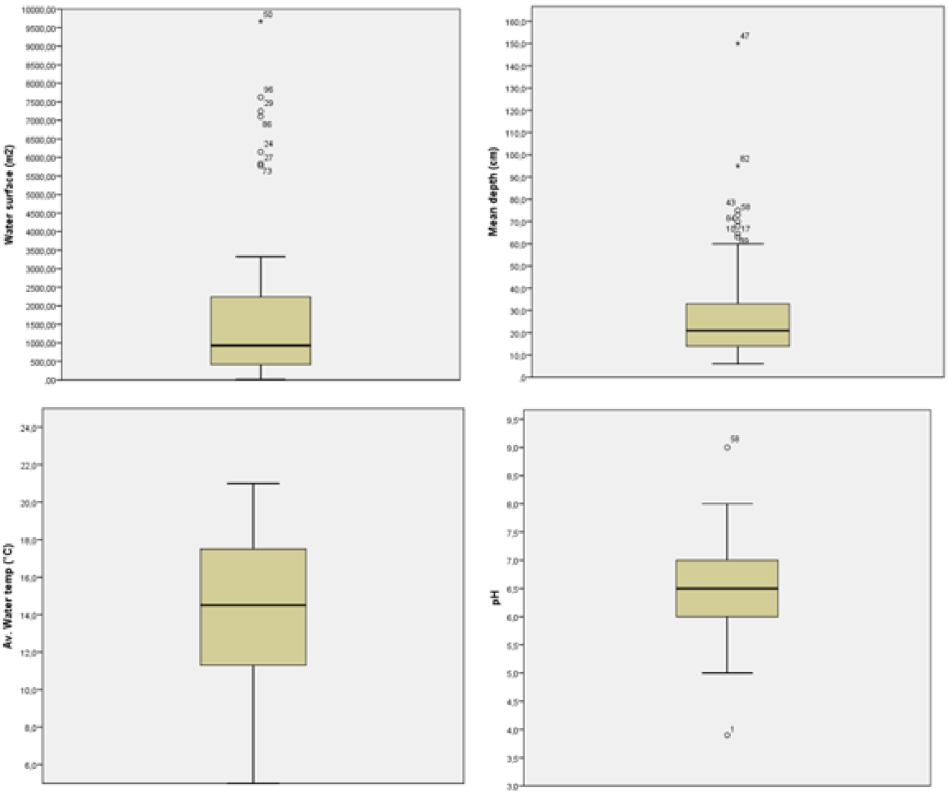


Figure 12- Graphs displaying statistic results (boxplots)

Presence general

Summary of a set of bivariate data with the presence of the tree frog

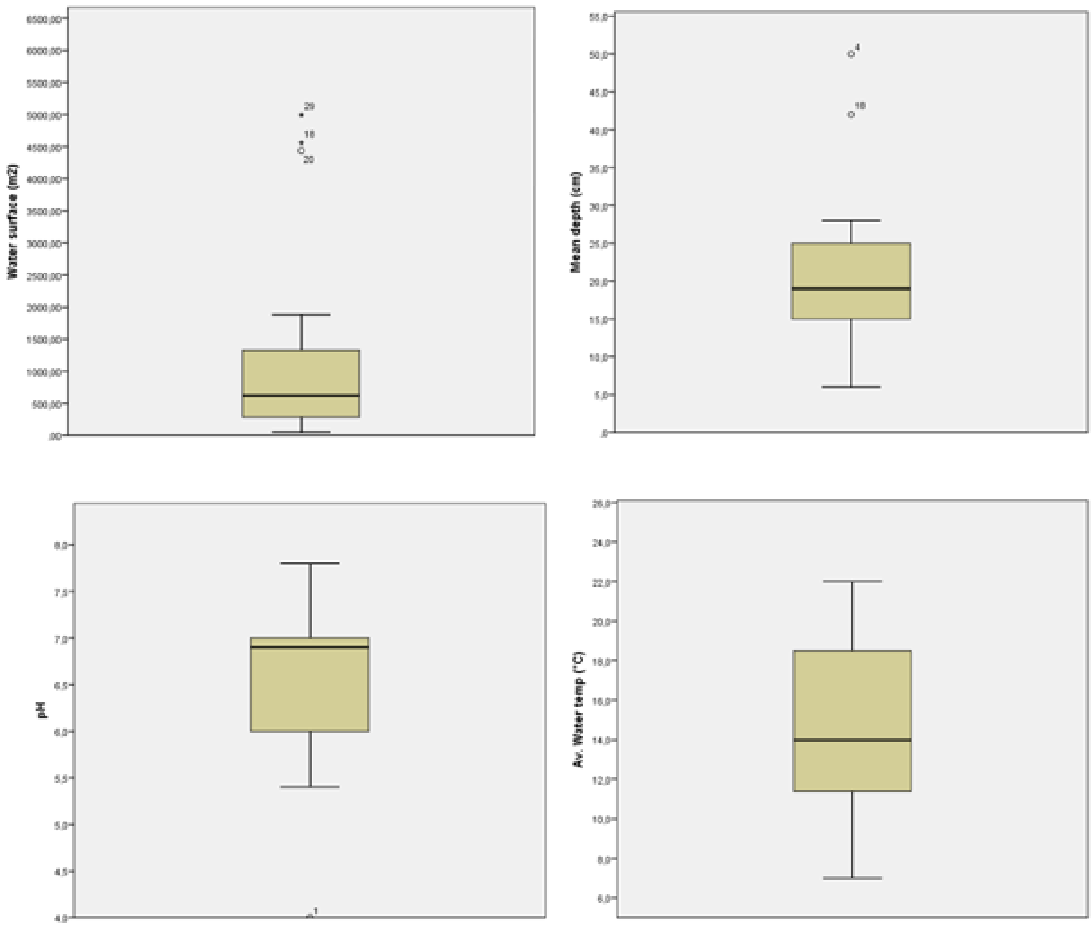
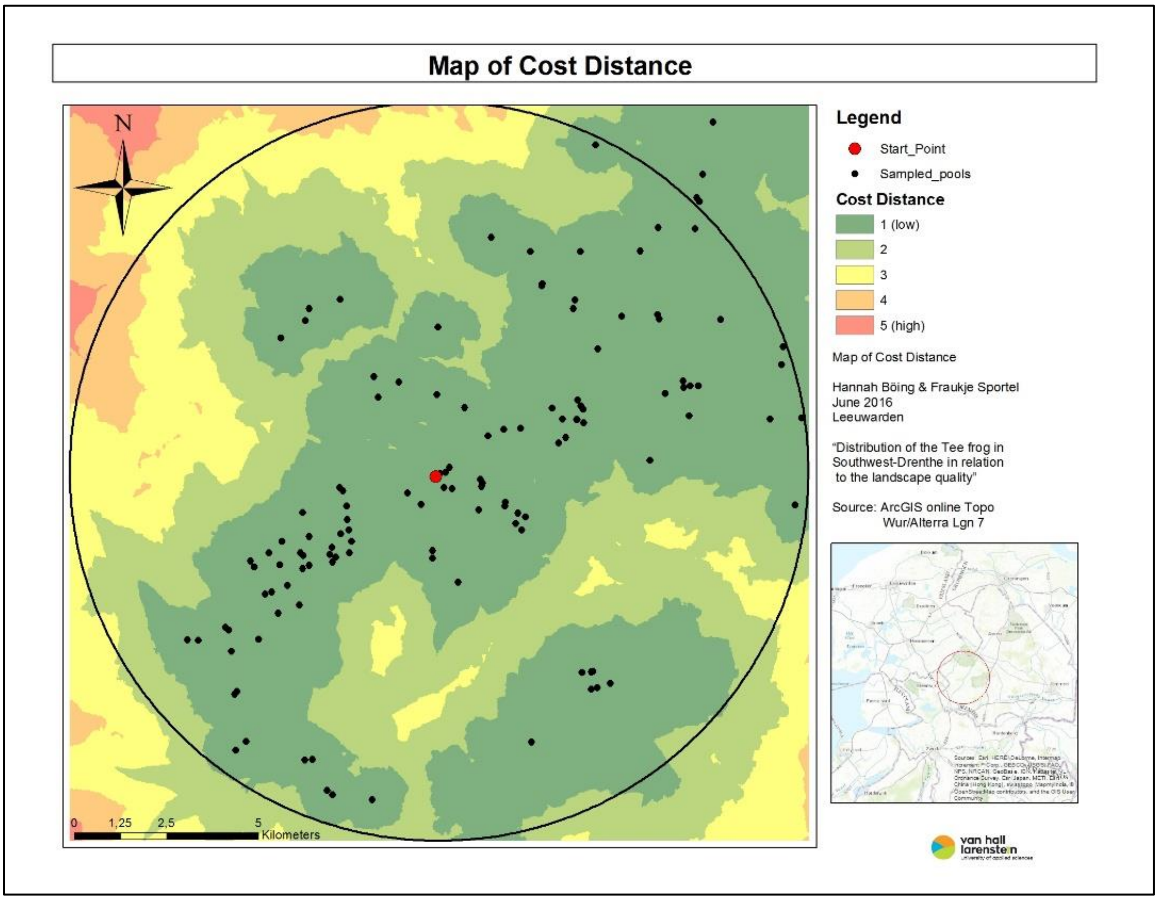


Figure 13- Graphs showing results of the statistical analysis (boxplots)

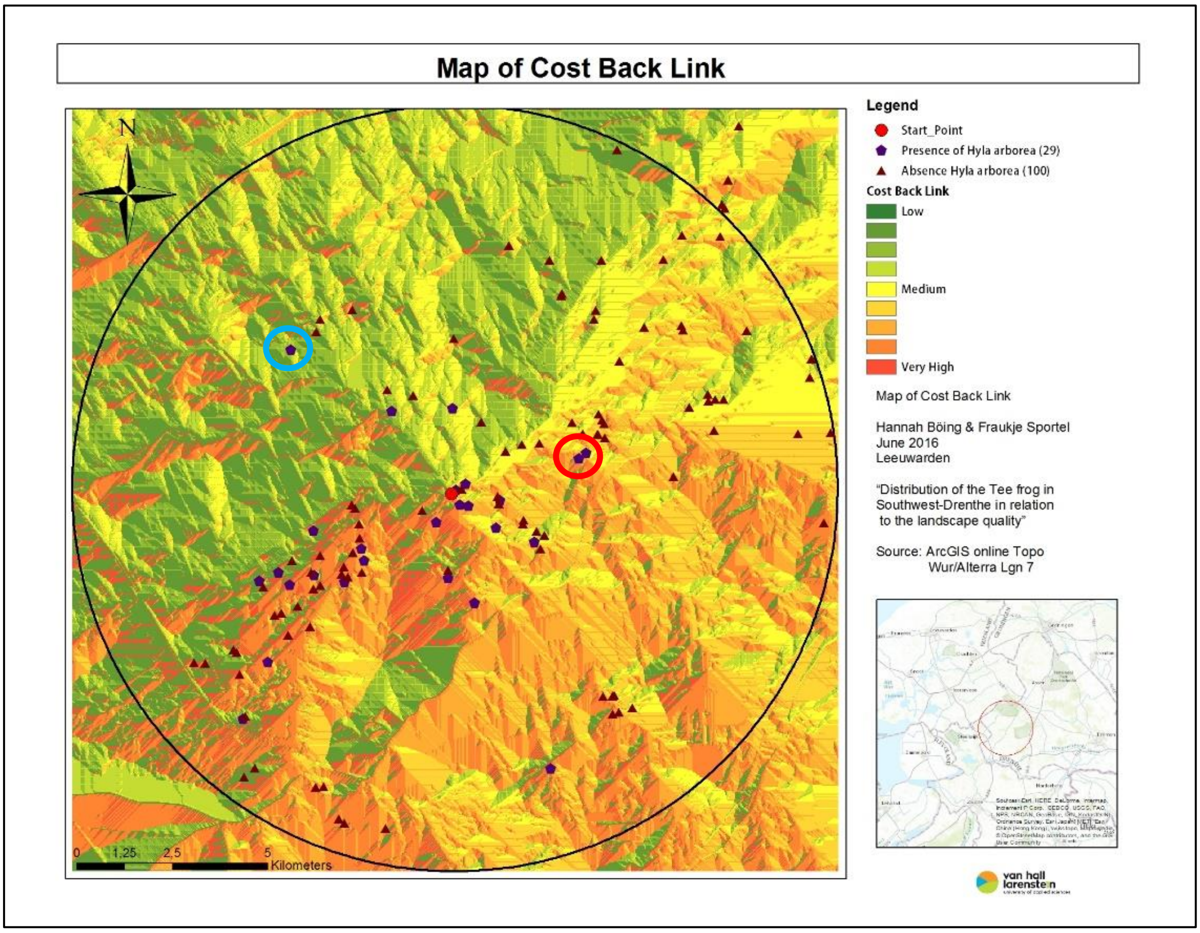
Appendix IX – Intermediate result of the ArcGIS analysis

Map 16 shows the cost of distance for the tree frog to move from one point to another by creating a range from low (green) to high cost (red). The further away from the start point, the more effort (cost) for the tree frog. Thus having a look at the starting point (red point) it seems that the tree frog only has low effort to move to other pools due to a big amount of pools (129). Outstanding are two areas in the North and in the South from the starting point which look like small islands. Here the distance is bigger and so would cost more for the tree frog to migrate to those pools. All in all, the Map shows a good connectivity between the sampled pools and a distribution possibility through the whole study area with low costs. Due to the fact that the tree frog can travel a distance of up to 12km per year and the pools are clustered, there is no high effort (cost) to reach another pool when looking at the distance (Mattison et al.,2011).



Map 16- Map of Cost Distance

Having a detailed look at the movements per 25m*25m used in ArcGIS, Map 17 shows the cost back link which defines the cost to move from one grid cell to the other.



Map 17 Map of Cost Back Link with low cost pools (blue circle) and high cost pools (red circle)

Outstanding is that the research area in the North and Northwest of the starting point (Red point) is dominated by green which means a low cost in migration.
In the South and Southeast of the starting point the cost to move is higher, displayed by the yellow and orange colors. Pools with the absence of the tree frog (red triangle) are mainly located in areas with medium to high cost and with no directly neighboring suitable areas with low cost. Looking at the pools where the tree frog is present (purple points) it stands out that they are located in low cost areas (green) but also in high cost areas (orange). This shows a clear difference compared to the areas where the tree frogs are absent, the close distance to low cost areas. The red circle in Map 17 show two opposites in terms of cost values. The red circle located in the yellow/orange area reviews two pools located in the high cost areas. The blue circle located in green shaded area shows a pool located in the low cost area.

Appendix X – Percentages of the Land uses within the routes

Table 18- Percentage of Land uses within the routes

Route / Land use	Route 1	Route 2	Route 3	Route 4	Route 5	Route 6
Agriculture (100)	11,9%	33,7%	29,6%	42,4	10,0	26,1
Forest (200)	57,3%	52,0%	44,8%	23,7	67,4	62,8
Water (300)	5,2%	7,0%	8,1%	4,1	8,6	4,1
Urban (400)	6,5%	5,5%	3,2%	4,1	4,2	3,0
Sand (500)	2,2%	1,1%	2,7%	1,0	2,2	1,1
Heath (600)	3,5%	0,4%	2,2%	3,4	3,1	1,1
Marsh (800)	3,2%	0,4%	7,1%	6,4	0,0	0,0
Reed (900)	0,2%	0,0%	1,5%	0,7	0,3	0,0
Grass (1000)	9,9%	0,0%	0,7%	14,2	4,2	1,9

Appendix XI –Linear regression after the elimination of variables

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	GrasMeadow	,014	,011	1,588	1	,208	1,014	,992	1,036
	Forestwith	,007	,010	,467	1	,494	1,007	,987	1,027
	Forestlittle	-,019	,011	2,864	1	,091	,981	,959	1,003
	Thicketwith	-,104	,085	1,498	1	,221	,901	,762	1,065
	Agriculture	,029	,014	4,547	1	,033	1,030	1,002	1,058
	Heath	-,022	,017	1,646	1	,200	,978	,945	1,012
	G.Frog1yes0no(1)	1,473	,568	6,721	1	,010	4,363	1,433	13,289
	Watersurfacem2	,000	,000	3,742	1	,053	1,000	,999	1,000
	pH	-,367	,382	,922	1	,337	,693	,328	1,465
	Av.Watertemp°C	-,305	,090	11,444	1	,001	,737	,618	,880
	Constant	5,160	2,519	4,197	1	,040	174,161		

a. Variable(s) entered on step 1: GrasMeadow, Forestwith, Forestlittle, Thicketwith, Agriculture, Heath, G.Frog1yes0no, Watersurfacem2, pH, Av.Watertemp°C.

Figure 13- Linear regression model with eliminated variables