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PREDICTING THE DENSITY OF NYMPHAL *IXODES RICINUS* TICKS (ACARI: IXODIDAE) IN THE NETHERLANDS

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Ixodes ricinus ♀

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Predicting the density of nymphal *Ixodes ricinus* ticks (Acari: Ixodidae) in the Netherlands

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Preface

This thesis is partly resulting from a decision I made at the start of my Biology Master at Wageningen University. I then decided that I wanted to combine biology and GIS/RS in a thesis, since they form a perfect combination for analysis of spatial patterns determined by biological processes. I think the tick *Ixodes ricinus* is a fascinating biological study object, because it has such a complicated life-cycle that is influenced by many factors. What's more, it also transmits various pathogens, including *Borrelia*, causing Lyme disease in humans. I enjoyed it very much to study the tick in a GIS, because I always had to come up with challenging and creative routes from the starting point to an end result.

Actually, this is a second (optional) thesis within my Master and hence only 24 credits. One of my personal goals, getting acquainted with ArcGIS and using the program in a biological context is reached. It was a new experience for me to have only 4 months for all the work and I found it difficult to stick to the research questions, since new ideas popped up rapidly during my work.
But all in all, I think I managed to perform interesting analysis on the data I got from several sources, with an end result that brought us nearer to risk modeling of Lyme disease in the Netherlands. However, the current map is not ready to use for a broad public.

All this work could never have been done without the help and input from various people. Therefore I would like to thank Ron van Lammeren and Willem Takken for their comments on the report.
All the people that were involved in collection of the ticks from the various studies are being thanked, also for allowing me to use their data in my study.
The following people from Alterra and the GRS-department are thanked for their kind help during the start-up phase; Aldo Bergsma, Gerard Hazeu, Frans Rip and Roland van Zoest.
Last but not least, Margriet Montizaan from the Royal Dutch Hunting Association is thanked for providing data on roe deer density in the Netherlands.

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Abstract

Lyme disease is an infectious disease in humans that is caused by spirochetes of the genus *Borrelia burgdorferi sensu lato*. The European sheep tick (*Ixodes ricinus* L.) is the most important vector of this bacterium and can transmit it during blood meals. The density of ticks in an area can indicate the spatial risk of obtaining Lyme disease. Therefore data from 6 different tick sampling studies, at 73 different locations broadly distributed over the Netherlands, were spatially analyzed and related to GIS and RS-derived variables. Early summer densities of the nymphal stage were found to be associated with roe deer density, land use, April NDVI and climatic variables. Using discriminant analysis, a predictive map of the nymphal density in the Netherlands was made, based on associative variables. A part of the dataset was used to test the predictive capability of the model, which turned out to be 71%. Although the current map is not ready for public use, making predictive maps of the risk to obtain infections of *Borrelia* parasites is vital because they may help people to take preventative measures at the right place and time. Therefore it is suggested that efforts should be taken in developing web-based GIS that enables spatio-temporal mapping of this risk in the Netherlands, to inform a broad public in a convenient way.

Key words: Lyme disease, *Ixodes ricinus*, risk, predictive model

1. Introduction

1.1 Lyme disease

Lyme borreliosis (Lyme disease) is an infectious disease in humans and domestic animals that is caused by the spirochete *Borrelia burgdorferi sensu lato* (Barbour, 1984; Burgdorfer *et al.*, 1982). Ticks of the genus *Ixodes* (hard ticks) are vector of this bacterium and can transmit it during blood meals.

In Europe, the primary vector of *B. burgdorferi s.l.* is the European sheep tick (*Ixodes ricinus* L.) (Rauter & Hartung, 2005). This broadly distributed tick is not only vector of *Borrelia*, but is known to transmit several other pathogenic microorganisms such as ehrlichiae and ricketsia (Wielinga *et al.*, 2006). When the *Borrelia* parasite is transmitted to humans, in ~90% of the cases an increasing red circle on the skin appears shortly after the bite, called erythema migrans (EM) (Brouqui, 2004; Parola & Raoult, 2001). This is an indication of the first stage of the three infection stages. In the final stage, occurring several months to years later, serious clinical manifestations may appear, such as skin abnormalities, intermittent attacks of joint swelling and pain, fatigue, and ocular and neurological signs (Parola & Raoult, 2001).

The prevalence of the first infection stage; EM, can be used as an indicator to gain insight in disease incidence. Hofhuis *et al.* (2006) used this approach to point out areas with an increased chance to obtain Lyme disease in the Netherlands. They reported a tri-fold increase of Lyme disease incidence in the Netherlands compared to 1994. It was assessed that the total number of patients that had visited a general practitioner (GP) because of EM, had risen from about 6000 in 1994 to 17000 in 2005. It was also assessed that more than one million people had obtained a bite from at least one tick. From this strong increase it can be concluded that Lyme disease forms an increasing threat to public health in the Netherlands.

1.2 The role and ecology of Ixodes ricinus

As mentioned before, the arthropod that causes this infection in humans is *Ixodes ricinus*. It is a seasonally active ectoparasite and uses blood as only food-source. It has a broad host-range, and feeds on three different hosts during its complicated life-cycle. In this 2-4-year life-cycle, the tick goes through three active life stages after egg-hatch; larva, nymph and adult, in which each a blood meal is necessary for development or reproduction. Sexual dimorphism and reproduction does only occur in the adult stage.

Ixodes ricinus is known for its broad host range, but larvae and nymphs mainly feed on small or medium-sized rodents or birds, while adult ticks are usually found on larger mammalian hosts such as roe deer (Randolph & Craine, 1995; Sonenshine, 1993; Tälleklint & Jaenson, 1994, 1997).

Small rodents such as bank voles (*Clethrionomys glareolus*) or *Apodemus* mice are reservoir hosts for *Borrelia burgdorferi*, which means they permit long-term survival and amplification of the infection and are significantly involved in the infection of the ticks (Gray, 1998; Randolph & Craine, 1995; Tälleklint & Jaenson, 1994, 1997). Although roe deer are considered reservoir-incompetent, these animals have an important effect on tick population density because they are one of the main hosts for the adult tick and may indirectly affect the density of infected nymphs (Gray, 1998; Tälleklint & Jaenson, 1996).

About 90% of the tick's life cycle is not spent attached to a host, but free in the habitat, where it is exposed to the local biotic and abiotic conditions (Parola & Raoult, 2001). In this habitat they are not actively dispersing in search for a host, but stay close to where eggs were laid (in case of larvae) or where they have dropped from a previous host (nymphs) (Van Buskirk & Ostfeld, 1998). Ticks that are preparing for attachment to a host perform questing behaviour. Questing is defined by Sonenshine (1993) as "behaviour expressed by the willingness of the ticks to crawl or climb to favorable locations in their environment, e.g., herbaceous or woody stems, where they may attach to transient hosts". Questing is a relatively passive host-seeking strategy, although ticks start rapidly waving their forelegs, when host cues are noticed (Sonenshine, 1993). When a tick is attached to the host, dispersal totally depends on host movements, which automatically means that ticks only may occur at locations where suitable hosts occur.

Since ticks are sensitive to desiccation, especially in the larval stage (Mejlon & Jaenson, 1997 and references therein), a suitable habitat of *I. ricinus* is beside suitable host presence, also limited to the possibility to rehydrate (Gray, 1998). This can consist of layers of decaying matter or good vegetation cover. When ticks dehydrate too much, they momentarily leave their questing site and move to the litter layer where they actively absorb water, called 'quiescence' (Perret *et al.*, 2004). Vegetation communities that do not enable this mechanism are highly unsuitable and ticks cannot survive in such a habitat during drought. Together with host density, this was also the explanation given by the authors of a recent Dutch study who found very low densities of nymphs in a heather area and higher numbers in a dune area and forest rich in blueberry (*Vaccinium corymbosum*) and oak (*Quercus*) (Wielinga *et al.*, 2006).

Ixodes ricinus density is not only spatially determined, but also temporally. The temporal abundance of ticks is primarily determined by diapause, which may be categorized in developmental and behavioural diapause (Gray, 1998). Behavioural diapause (quiescence) occurs at unsuitable times during host-seeking periods, while developmental diapause involves arrested development of an engorged stage or egg during high summer or mid winter.

1.3 Spatial analysis using GIS and RS

Because only a limited sample of the actual distribution of a vector is usually achieved in practice, epidemiologists often need to rely on spatial risk models. The combination of Remote Sensing (RS) and Geo Information Systems (GIS) provide a strong tool in risk assessment and prediction, since RS-data of the study area can usually easily be obtained by satellite images or photos taken from an airplane. And with GIS, multiple layers of spatial data can easily be obtained, stored, overlaid and displayed (Nicholson & Mather, 1996). Cartographic display capabilities inherent in GIS are also accessible and understandable to a broad public (Kitron, 2000).

GIS and RS approaches are used in entomologic risk predictions in a vastly growing frequency (Ostfeld, 2005). Most risk models are made by assessing which are possible important risk indicators and how these are spatially distributed. Subsequently it is investigated to what extent these indicators are associated with vector, pathogen or disease incidence data. Finally the strongest association(s) can be used as predictor for the risk. In this way distribution and abundance of various disease vectors such as anopheline mosquitoes, tsetse flies and ticks have already been modeled (Eisen *et al.*, 2006 and references therein).

In the United States much work has been done on mapping the distribution of ticks. There, the most important agent of tick-borne diseases is the black-legged tick *Ixodes scapularis* Say. Bunnel *et al.* (2003) related abundance of *I. scapularis* with several spatial attributes in a region in the USA. They found significant associations between adult tick numbers and land cover, distance to water bodies, distance to forest edge, elevation and soil type.

In another study to *I. scapularis*, Diuk-Wasser *et al.* (2006) compared numbers of nymphal ticks at different locations and found that peak nymphal host-seeking density occurred earlier in the southern than in the northern collection sites, suggesting indirect influence of increasing latitude. Randolph and Storey (1999) reported a measure for drying power of the atmosphere based on relative humidity (RH) and temperature, called saturation deficit (SD). In the study of Diuk-Wasser *et al.* (2006), significant negative correlations were found between mean nymphal tick numbers and SD and mean temperature.

Eisen *et al.* (2006) studied density of *Ixodes pacificus* in California in relation to environmental parameters by forward-stepwise regression modelling. They even found that a model with several GIS / RS derived parameters was more accurate in predicting nymphal density, than a model based on field-derived parameters. The observed peak nymphal density was best predicted by the July normalized difference vegetation index (NDVI), November greenness, coastal influence, May solar insolation, November hours of sunlight and dominant hydrological grouping.

Although many environmental parameters at sampling locations may directly be derived by overlaying it with other data, Kitron (1998) suggested that beside localized biotic and abiotic data, landscape structure may play a role in tick abundance as well. This was shown by several authors who considered composition or structure of the landscape surrounding focal sites (Brownstein *et al.*, 2005; Jackson *et al.*, 2006; Ostfeld, 2005). From two studies it was shown that the entomologic risk of Lyme disease (product of tick density and prevalence of *Borrelia*) was most related to fragmentation of the landscape (Allan *et al.*, 2003; Brownstein *et al.*, 2005). In another study with a different predictive variable, Jackson et al. (2006) drew similar conclusions after investigating the relation between Lyme disease incidence in humans and landscape ecology. They showed that a high interspersion between forests and herbaceous cover in the landscape was explaining the variation in disease incidence.

1.4 Risk modeling

In principle, every location where *Borrelia*-infected ticks do occur is a risk area for obtaining Lyme disease. However, the question ‘What is risk?’ is approached in many ways since data on tick abundance or infection percentage may be unavailable or an area is not open for public.

Risk is assessed in numerous ways, but in general risk assessment and prediction studies can be categorized as spatial, temporal or a combination of both (Daniel *et al.*, 2006). Apart from the categorization of studies, the sort of data on which the risk is based and the way in which the risk is expressed, also shows much variation. Generally, risk can be formulated as exposure to a chance of injury or loss. With respect to epidemiological risk one can formulate it as the chance to obtain an infection. Some studies use the disease incidence in humans to point out spatial differences in risk (Hofhuis *et al.*, 2006; Jackson *et al.*, 2006), since this data is relatively easily obtained from hospitals or GP’s.

In a more fundamental approach that is applied more often, risk is based on tick presence or tick density in the vegetation (Bunnell, 2003; Daniel *et al.*, 1998; Eisen *et al.*, 2006; Guerra *et al.*, 2002) or attached to a host (Das *et al.*, 2002). In some cases also the infection percentage of the ticks is investigated, which provides an even more precise possibility to predict the risk of getting Lyme disease (Bunnell, 2003; Das *et al.*, 2002).

Assessment of both (infected) tick density and disease incidence in humans are also reported (Brownstein *et al.*, 2005; Diuk-Wasser *et al.*, 2006; Nicholson & Mather, 1996). These studies provide possibilities of validating model outcomes.

Assuming one is susceptible to infection; the risk to get Lyme disease is based on the density of infected questing ticks and the time one spends in a habitat that supports tick populations, in absence of active preventative measures.

This indicates that humans themselves play a role in the chance of getting infected. At the one hand this shows the weakness of studies that use only disease incidence as predictor for disease risk in a certain area. Because areas that are hardly visited may still support high densities of infected ticks. Also discrepancies may occur between the site where the bite was obtained and reported (Ostfeld, 2005). At the other hand it indicates that when humans do take preventative measures at the right places, they may greatly reduce the chance to obtain a bite from an infected tick.

It would be of great use to generate public awareness about locations that have increased chances to obtain a bite from an infected tick. Maps that display the risk to obtain an infected tick-bite can help a broad public in taking preventative measures and may help developing local control strategies (Kitron, 2000). As far as my knowledge reaches, this is the first study that tries to predict spatial density of *I. ricinus* in the Netherlands. According to me, predicting the risk of exposure to infected ticks would be most correct when it is based on the spatial density of ticks and tick infection percentages and not on disease incidence in humans. In case one also wants to predict where most bites will occur, one can add human behaviour (e.g. recreation, hiking) to the predictive map as well.

1.5 Aim and research questions

In this study it is tried to see whether data on a macro scale can be used to predict processes at much smaller scale. In this approach that relies on hierarchy theory, localized factors are subsumed in macro-scale variables (Jackson *et al.*, 2006). Although the scale on which risk can be predicted may be coarser, when macro data is predictive for tick densities, risk prediction can easily be performed, since environmental data do not necessarily have to be obtained in the field.

Objectives

1. To assess the extent of association of nymphal tick densities with several spatial factors obtained on macro scale.
2. To develop a risk model for the Netherlands by predicting density of active nymphal ticks.

Research questions

1. How are sampling transects distributed over the country?
2. How and why does nymphal tick density vary between several sampling transects and sampling periods in the Netherlands?
3. To what extent are nymphal tick densities in the data-set spatially autocorrelated?
4. To what extent are nymphal tick densities at sampling transects associated with the following variables; land use, soil type, climate, host density, NDVI, distance to the coast and landscape structure?
5. Can the spatial distribution of ticks in the Netherlands be predicted by above-mentioned possibly associative variables?
6. What is the predictive capability of the model?

2. Methodology

Data on nymphs were used as input to create a map that shows predicted *I. ricinus* densities in the Netherlands. Only the nymphal stage was used in the assessment and the prediction, because of its relative importance in the transmission of Lyme disease. Nymphal density data was obtained from several tick studies conducted in different years. Tick densities as well as remote sensed data and other environmental data were stored in a GIS. Then the extent of association between tick densities and the other variables was tested with multivariate statistics and the results were displayed in a map.

2.1 Study sites and tick sampling

The tick sampling transects of the different studies were distributed over the whole of the Netherlands. The geographic coordinates of the sampling transects were only in a few cases recorded by using a GPS device. For all the other transects, the coordinates (decimal degrees) were obtained by pointing the centre of the transect in Google Earth (Google Inc.). The accuracy of the coordinates obtained by the latter method was estimated to be between 10-100 m.

All studies had the same tick collection method namely flagging. This method consists of dragging a cotton blanket (with known surface) along a transect on the ground surface. This method provides the most sensitive measure of potential contact between ticks and humans (Diuk-Wasser *et al.*, 2006 and references therein). The questing proportion of the tick population will cling to the blanket and can be counted per stage.

The combined dataset consisted of 73 sampling transects, of which the earliest study started in the year 2000 and the latest ended in 2006 (see Table 1). In all studies, ticks were collected in only one year, except in study 6 where transects were monitored in the same season for three years (one transect was monitored for six years). In study 5, some transects of 100 m^2 were located directly next to each other. Those transects were treated as one transect of 200 m^2. The dragged area of transects in study 6 was not fixed, but based on a maximum number of 50 collected ticks. In the dataset that was provided, densities were already scaled to a surface of 100 m^2. The frequency in which transects were sampled ranged from weekly to monthly.

Table 1. Specification of studies that were part of the tick density dataset

study	n sampling transects	sampling frequency	sampling surface	year (season cover)
1. Renkum	5	weekly	200 m^2	2005 (early summer)
2. Amst. Waterl. Duin	8	weekly	200 m^2	2002 (whole year)
3. De Hoge Veluwe	8	weekly	200 m^2	2002 (whole year)
4. Vector project LNV	12	weekly	100 m^2	2005 (autumn); 2006 (early summer)
5. Natuurkalender	36	monthly	100/200 m^2	2006 (autumn)
6. Animal Sciences Gr.	4	monthly	variable	2000-2002-2005 (early summer-autumn)

All three active stages of *I. ricinus* can potentially get infected with the *Borrelia* parasite, but human infection usually results from blood meals taken by nymphs, because at this stage the ticks are small and difficult to see, and their peak questing period in early summer coincides with human outdoor activity (Barbour & Fish, 1993). Beside unimodal patterns, bimodal patterns in nymphal phenology have also been reported (Perret *et al.*, 2004 and references therein), but not in the Netherlands.

Infected nymphal tick densities are directly correlated with nymphal densities (Jouda *et al.*, 2004; Tälleklint & Jaenson, 1996) so densities of ticks may serve as an indicator for risk of exposure to infected ticks. Besides, in an ongoing study in the Netherlands, no *Borrelia*-negative sites were found up till now when ticks were present (Takken, unpublished data).

Therefore in the analysis maximum nymphal density in overlapping months of the studies was used as the predictive variable. The periods in which the studies overlapped, did not cover the whole period of nymphal questing activity (early summer till autumn), but it was assumed that densities in early summer and autumn could separately be used as indicator for those periods. Therefore early summer was defined as the period between May and June, whereas autumn was defined as September till October. Unfortunately, the number of transects that was sampled during the known nymphal peak period (early summer) (Tälleklint & Jaenson, 1997) was rather low (n = 37). All recorded densities were scaled to 200 m^2 and densities of early summer and autumn were separately used to obtain and validate both predictions. Because ticks of three transects in study 6 were sampled for three subsequent years and one transect even 6 years, densities in this study were averaged per month and the maximum average nymphal density of a early summer or autumn was used in analysis.

2.2 Input data and processing

Before the RS and GIS data could be used as variables in the statistical analysis, they were processed according to the data-action model shown in Figure 1, to obtain environmental data for each sampling transect in both seasons. The steps in the action model were performed using the computer program ArcGIS 9.1 (ESRI Inc.). In this paragraph, the processing is explained in more detail for each possible risk variable.

The output consisted of a dBase table containing the maximum nymphal *I. ricinus* density in early summer and autumn and the values of the environmental variables at each transect.

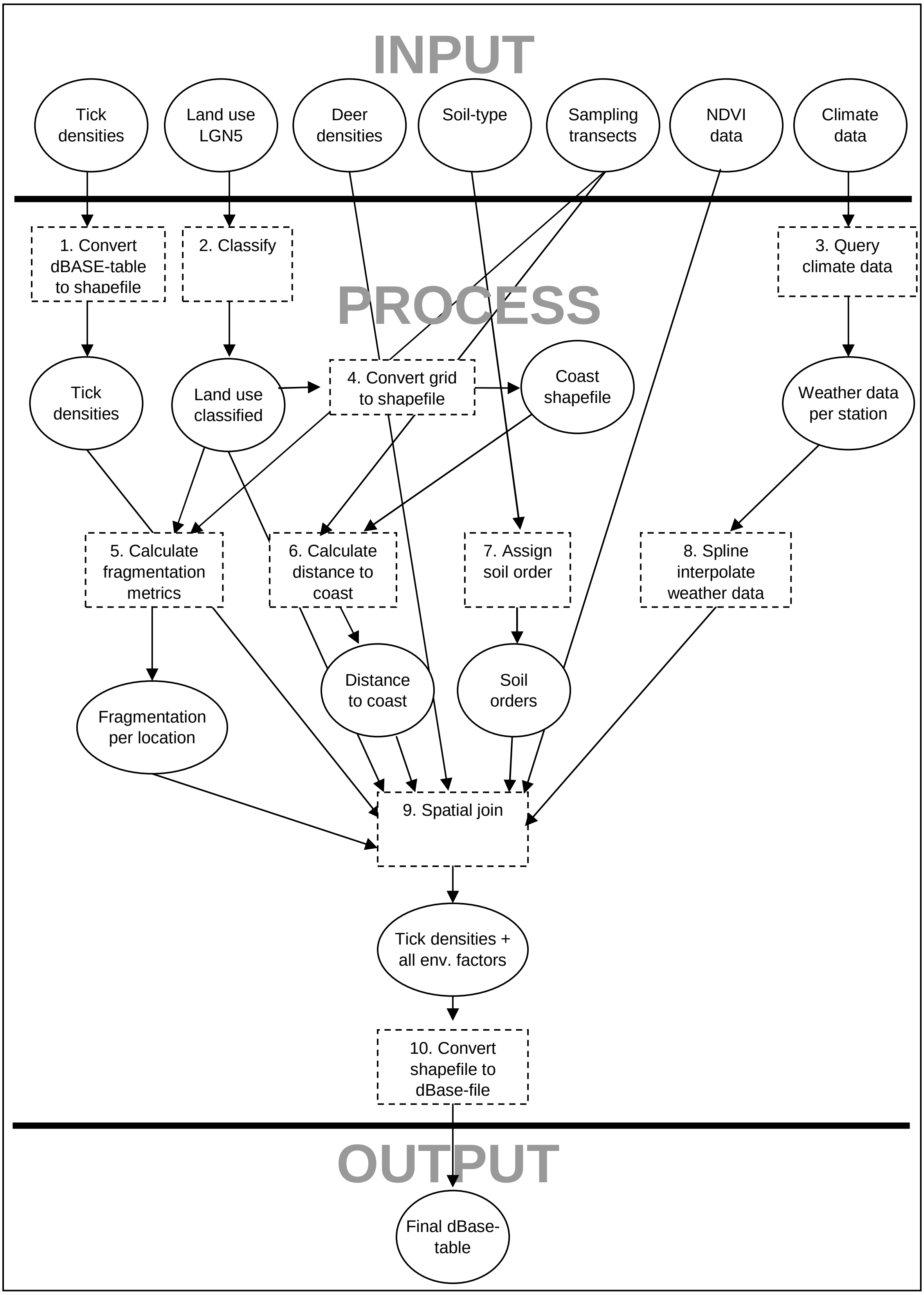

Figure 1. **Data action model showing 7 input datasets, 10 numbered actions and 1 output table containing nymphal densities and environmental variable values at each of the transects, to be used for analysis of association in both seasons.**

2.2.1 Land use

Land use of the Netherlands (LGN5) was obtained from Geodesk (Alterra, WUR). The file consists of 39 land use classes based on satellite images from Landsat TM5 taken in 2003 and 2004. The original 39 classes were reclassified into six categories (action 2) according to a table presented in Appendix Ia; 1. water 2. not vegetated ground, roads and buildings, 3. grasslands and agriculture, 4. open vegetation, 5. coniferous forest, 6. deciduous forest.

The file was in grid format with a spatial resolution of 25 m (map is presented in Appendix IIa).

2.2.2 Soil type

Data on soil types of the Netherlands was also obtained from Geodesk (Alterra, WUR). The file consisted of 9 soil classes which were reclassified into four categories (action 7) according to a table (Appendix Ib). The classification was based on water retaining capabilities of different soils: 1. sand, 2. silt, 3. clay and loam, 4. peat (Bunnell, 2003; Guerra *et al.*, 2002). A relatively coarse classification was used since the distribution of sampling transects over all soil categories was rather unbalanced. The file was in shape format (map is presented in Appendix IIb).

2.2.3 Climate

Macrometeorological data were obtained from 46 weather stations (Meteoconsult, Wageningen, The Netherlands) located in the Netherlands, Germany and Belgium. Seasonal averages were calculated on hourly observations for temperature and dew point temperature. Data from three of the six sampling years were available; 2003, 2004 and 2006 and were stored in an Access (Microsoft Inc.) database. Before climatic parameters were calculated, the database was queried to obtain annual averages and an overall average for three years (action 3.).

Climatic parameters that are generally used in tick studies were not directly available, but had to be calculated from the available parameters. Therefore average actual vapor pressure (AVP, equation 1) and the average saturated vapour pressure (SVP, equation 2) were calculated using the average temperature (T) and the average dew point temperature (DT). Average relative humidity was then calculated according formula 3 (equation 1, 2, 3 obtained from D. Van Kralingen via A. Bergsma, personal communication).

An integrated measure for ‘drying power’ of the atmosphere; saturation deficit, was calculated using equation 4 (Randolph & Storey, 1999).

$$AVP = 6.10588 * e^{(17.32491 * T / (T + 238.102))} \quad \textbf{(Equation 1)}$$

$$SVP = 6.10588 * e^{(17.32491 * DT / (DT + 238.102))} \quad \textbf{(Equation 2)}$$

$$RH = (AVP / SVP) * 100 \quad \textbf{(Equation 3)}$$

$$SD = (1 - RH / 100) * 4.9464\, e^{(0.0621*T)} \quad \textbf{(Equation 4)}$$

Average parameter values were calculated for each of the weather stations for both early summer and autumn. The climatic parameters that were included in the analysis were average temperature, average RH and average SD. For both seasons and the three parameters, the surface between the weather stations was interpolated using Spline tension interpolation (action 8) with cell size 2000 m (maps are presented in Appendices IIc-IIh). On the maps clear effects of the buffering effect of seawater is shown on all three parameters.

2.2.4 Roe deer density

Data on the density of roe deer (*Capreolus Capreolus* L.) were obtained from the Royal Dutch Hunting Association (Dutch: Koninklijke Nederlandse Jager Vereniging, KNJV). This organization has divided the country in management units with varying size (Dutch: Wild beheerseenheden) and the number of observed roe deer is counted per management unit. However, this number is an underestimate, because not all animals show themselves at the moment of the counting. Therefore this number is corrected to an estimated number of present roe deer according to a standard procedure (M. Montizaan, KNJV, personal communication). The data that were provided were from a roe deer counting that was performed in spring of 2003. Before the analysis these roe deer numbers were divided by the surface area of each management unit to calculate the density per area unit. The file was in shape format (map is presented in Appendix IIj).

2.2.5 NDVI

NDVI values were obtained via G. Hazeu (Alterra, WUR) who processed Landsat TM5 images of the following dates; 31-05-2003, 11-08-2003, 14-04-2004 and 05-09-2004. NDVI combines the red and near-infrared bands [NDVI = (band 4 - band 3) / (band 4 - band 3)] and is related to the fraction of photosynthetically active radiation.
Unfortunately, the images did not cover all the sampling transects; therefore the two images with maximum cover (95% of the sampling transects) were included in the model: 14-04-2004 and 05-09-2004. The Landsat TM images had 30 m spatial resolution.

2.2.6 Distance to coast

The earlier mentioned land use file was classified into two classes; salt water and other (action 2). The salt water was then transformed into a polygon (action 4). This polygon was smoothened with a 1000 m. buffer. Subsequently this layer was spatially joined with the point layer containing the sampling transects (action 6). In this way the shortest distance from each of the sampling transects to the coast polygon was calculated (map is presented in Appendix IIi). This variable was chosen since distance to coast may reflect the possible influence on ticks due to indirectly affecting climate or vegetation (Eisen *et al.*, 2006).

2.2.7 Landscape structure

Metrics of landscape structure were calculated using Fragstats 3.3 (McGarigal *et al.*, 2002) that enables calculation of landscape fragmentation metrics. Therefore the land use file was classified in three categories: background, forest and vegetation (action 2). Background consisted of water, not vegetated ground, roads and buildings; forest was a combination of deciduous and coniferous forest and vegetation consisted of grasslands, agriculture and other vegetation.

Many metrics to describe landscape structure are available (McGarigal & Marks, 1995), but two metrics describing the level of forest fragmentation were used; mean patch area and total edge length (see Table 2 for definitions). Only total edge-length was corrected for effects of roads and buildings, but average forest patch size was not corrected.

As shown by Brownstein *et al.* (2005), Allan *et al.* (2003) and Jackson *et al.* (2006), forest fragmentation defines the local scale heterogeneity of tick densities, through influencing mammalian host density and species composition. The two selected metrics in this study, may indicate habitat suitability for roe deer, since they have a preference for fragmented habitats with high adjacency of forest and other vegetation (Said & Servanty, 2005). Fragmentation might also indicate habitat suitability for rodents (Jackson *et al.*, 2006 and references therein), however no studies were found explicitly describing this for rodent hosts of *I. ricinus.* The Fragstatsbatch extension (Mitchell, 2005) for ArcGIS 9 was used to run Fragstats at each sampling transect within a user-defined radius around the sampling point (action 5). The radius was was set at 250 m, which represents the average home-range size of roe deer in the Netherlands (Lange *et al.*, 1994).

Table 2. Definition landscape structure metrics

Acronym	Fragstats metric	Unit	Description
AREA_MN	Mean Patch Area	m^2	Mean surface area of forest patches
TE	Total Edge	m	Total edge length between forest and agriculture / open vegetation

2.3 Spatial statistics

Before predicting nymphal density, the degree of spatial clustering was measured. Therefore spatial autocorrelation was calculated using Moran's *I* statistics (Cliff & Ord, 1973). This index measures the relationship among values of a variable according to the spatial arrangement of the values (Cliff and Ord, 1973). Moran's *I* was calculated using the model builder in ArcGIS version 9.1 (ESRI Inc.). Index values range from -1 to 1 with a tendency around 0, of which the latter indicates that values are distributed according to a random pattern.

In order to perform statistical analysis, the point shapefile containing the nymphal densities and the environmental variables at each transect was transformed into a dBase table. This table was then imported in the statistical software package SPSS 12.0 (SPSS, Inc.).

Since the number of cases did not substantially exceed the number of predictor variables in both early summer (3:1) and autumn (7:1), multiple regression could not be used to test the extent of association. Furthermore, not all variables were normally distributed and two were having an ordinal scale. Therefore Spearman rank correlation was performed using the density categories and the possible risk variables as input. This was separately done for both early summer and autumn.

The significantly associated variables were then subjected to discriminant analysis (DA) to obtain functions that discriminated between the density categories. To obtain the discriminating functions, 80% percent of the cases were randomly selected. To test the discriminative power of the functions, discriminant functions were applied on both the 80% and the other 20% of the cases and original and predicted category membership were compared.

The significantly discriminating functions were also used to generate a nymphal density prediction map, by applying the functions at points regularly dispersed over the country. This was done according the scheme displayed in Figure 2. Hawth's Analysis tools for ArcGIS (Beyer, 2004), was used to generate a point layer of regularly distributed points separated by 1000 m. in x and y direction (action 11). This point layer was intersected with all layers that had shown significant association with nymphal density (action 12). SPSS was then used to predict category membership of each point in the DA (action 13). These categories were subsequently displayed in a map after being converted to raster format with 1000 m resolution (action 14).

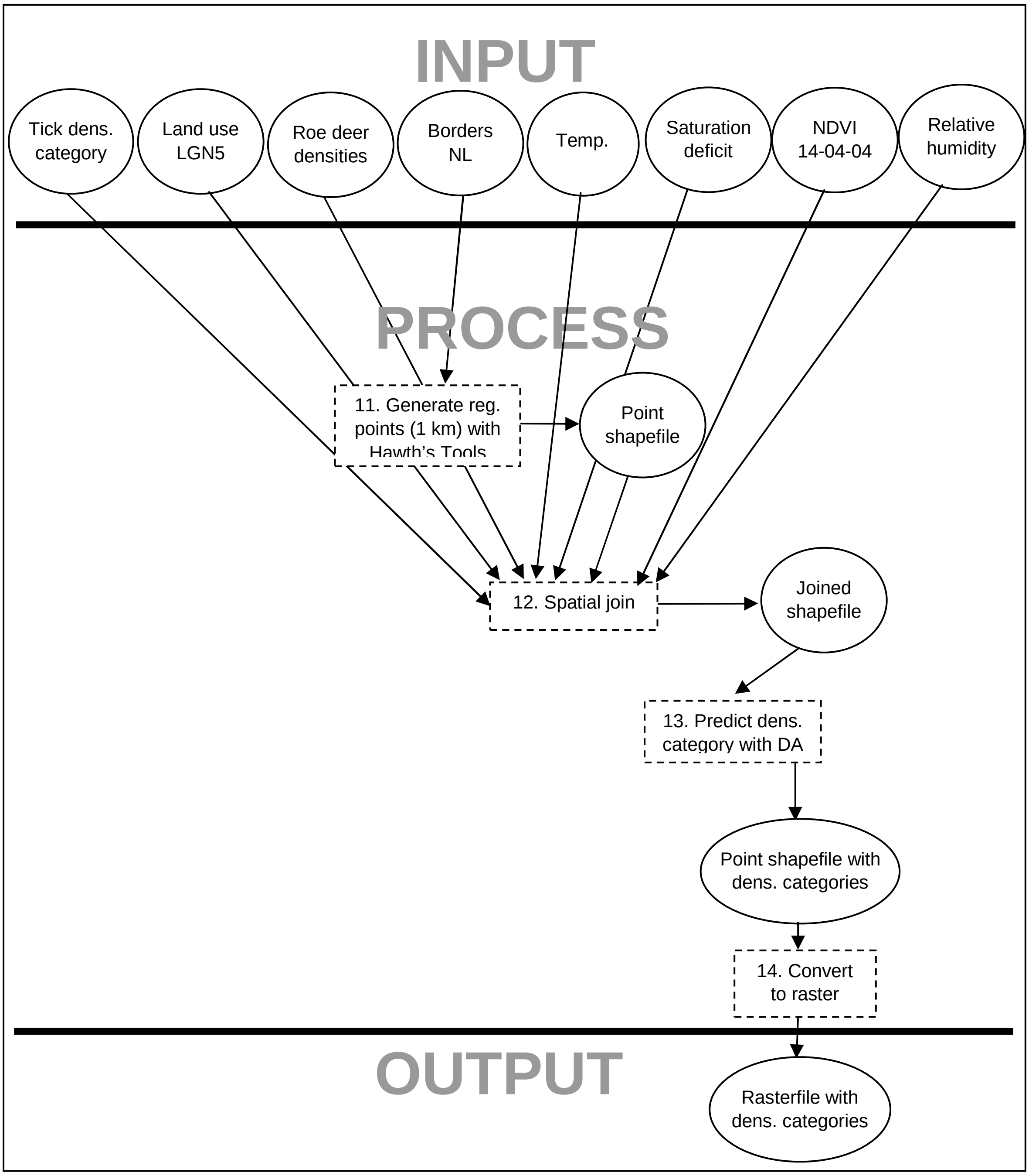

Figure 2. **Data-action model with 8 input datasets, 4 numbered actions and 1 output map displaying nymphal density categories in the Netherlands in early summer.**

3. Results

3.1 Nymphal densities

A number of 37 transects were sampled in the period May till June. The maximum density of questing nymphs in this period, defined as early summer, ranged between 0 and 490 (Mean, 104; SD, 121). The dates on which maximum densities were observed varied between locations and years and ranged from begin May till the end of June. Densities were skewed to the left. The nymphal densities were classified in three density categories according to percentiles; 1. low (0–33rd percentile), 2. moderate (34–66th percentile), and 3. high (66–100th percentile) (see Figure 3A). This was a relatively coarse classification, but necessary to maximize the number of cases in each density category. The highest nymphal densities were observed in forested areas along the coast while zero and lowest densities were found in grasslands and floodplains of the rivers.

The densities of the nymphs were tested for spatial autocorrelation using Moran's I statistics and significant clustering was found (Moran's $I = 0.41$; $Z(I)$ 2.89; $P < 0.01$).

Sixty transects were sampled in the period from September till October. Of these 60 transects, 24 were also sampled in early summer. The maximum density of questing nymphs in this autumn period was lower than in early summer and ranged between 0 and 186 (Mean, 25; SD, 43). The dates on which maximum densities were observed here also varied between locations and years and ranged from begin September till half of October. With respect to the 24 transects that were sampled in both periods, maximum densities were significantly lower in autumn compared to spring ($t = 4.20$; $P < 0.01$; Paired sample t-test).

The autumn nymphal densities were also classified in three categories according to percentiles. The distribution of nymphal densities in this season was left-skewed as well. Highest nymphal densities were again found along the coast, and also in De Hoge Veluwe area. Zero and lowest densities did not seem to cluster in specific areas of the country.

Sampling transects in autumn were distributed over a greater area compared to early summer (Figure 3) and Moran's I statistics did not find significant clustering (Moran's $I = 0.09$; $Z(I)$ 0.61; $P = 0.27$).

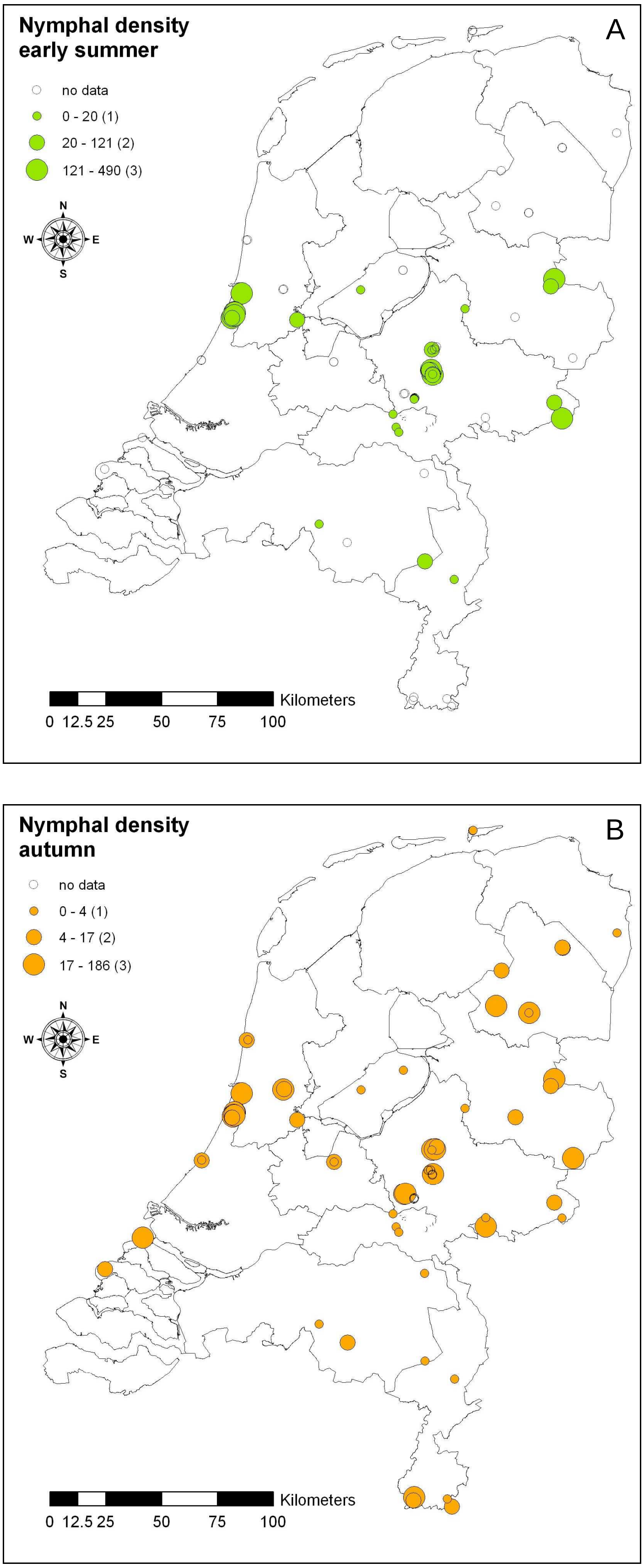

Figure 3. Maximum density of host-seeking nymphal *Ixodes ricinus* in early summer (A) and autumn (B) at each transect (scaled to 200 m^2). Number of sampling transects at which data was collected is 37 (A) and 60 (B). Density categories are indicated by filled symbols; 1. zero-low, 2. moderate and 3. high. Not sampled transects are indicated by open symbols.

3.2 Association with environmental variables

The density categories of both seasons were separately tested for correlation with the environmental variables. The variables that significantly correlated with density in both early summer and autumn were roe deer density (r = 0.76 and 0.49, respectively; P < 0.01) and April NDVI (r = -0.73 and -0.33, respectively; P < 0.01). Other variables that significantly correlated in early summer were land use (describing vegetation categories) (r = 0.39; P = 0.02) and mean relative humidity (r = 0.56; P < 0.01), mean saturation deficit (r = -0.59; P < 0.01) and mean temperature (r = -0.58; P < 0.01).

In autumn, similar but also different variables correlated with density, namely; soil order (r = -0.27; P = 0.04), mean patch size (r = 0.31; P < 0.01), mean relative humidity (r = -0.26; P < 0.05) and mean saturation deficit (r = 0.26; P < 0.05).

Table 3. Spearman rank correlations of nymphal density categories with environmental variables in early summer and autumn. The number of transects varies, since not all variables were available for each transect.

	Early summer			**Autumn**		
	Spearman's *r*	*P*-value	*n*	Spearman's *r*	*P*-value	*n*
Roe deer density	0.76	< 0.01	33	0.49	< 0.01	54
Soil order	-0.20	0.23	36	-0.27	0.04	59
Land use	0.39	0.02	37	0.12	0.35	59
NDVI 05-09-2004	-0.23	0.19	35	-0.18	0.19	56
NDVI 14-04-2004	-0.73	< 0.01	35	-0.33	0.01	56
Edge length	0.29	0.08	37	-0.07	0.62	60
Mean patch size	0.30	0.07	37	0.31	0.01	60
Distance to coast	-0.30	0.07	37	-0.11	0.42	60
Mean relative humidity	0.56	< 0.01	37	-0.26	0.05	58
Mean saturation deficit	-0.59	< 0.01	37	0.26	0.05	58
Mean temperature	-0.58	< 0.01	37	0.17	0.21	58

3.3 Prediction density categories

After using only the significantly correlating variables, the discriminant analysis yielded two functions for both seasons with coefficients as shown in Table 4 and Table 5. The two discriminant functions did enable discrimination between the three density categories for selected cases.

The functions for early summer were significantly better discriminating between density categories than chance (p < 0.01 and 0.05). In early summer the first function accounted for 80% of the total among-groups variability and the second for 20%. In autumn the first of the two functions almost explained all variation (93 vs. 7 %) although not significantly (p = 0.19 and 0.92).

Cases in which at least one discriminating variable was missing could not be used, since the functions could not be applied to variables without value. This caused that in early summer 6 transects were not selected in the DA and in autumn 12.

Table 4. Standardized canonical discriminant function coefficients and Wilks' Lambda in early summer.

	F(x) 1	**F(x) 2**
Roe deer density	0.72	-0.07
Land use	0.07	0.76
NDVI 14-04-2004	-0.37	-0.08
Relative humidity	0.84	0.92
Saturation Deficit	1.41	2.02
Temperature	-0.81	-0.43
% of Variance	80.2	19.8
Wilks' Lambda	0.13	0.56
P-value	< 0.01	0.05

Table 5. Standardized canonical discriminant function coefficients and Wilks' Lamda in autumn.

	F(x) 1	**F(x) 2**
Roe deer density	0.45	-0.54
Soil order	0.31	0.76
NDVI 14-04-2004	-0.57	-0.32
Mean patch size	0.50	0.46
Relative humidity	1.02	0.60
Saturation Deficit	1.34	1.06
% of Variance	92.7	7.3
Wilks' Lambda	0.61	0.96
P-value	0.19	0.92

From the early summer period significant discriminating functions could be obtained, therefore only from this season a predictive map was generated (Figure 4). The majority of the points was classified to have zero to low density in early summer (81%), 4% was classified as supporting moderate density and 15% high density of host seeking nymphs. Important risk areas that were indicated were: three of the Wadden islands, De Hoge Veluwe, De Amsterdamse Waterleidingduinen and the Drenthe province.

Missing NDVI data in the outer west and east of the country resulted in the lack of a density prediction. Furthermore, other white areas on the map were caused by missing data on roe deer densities, or presence of water, buildings or infrastructure.

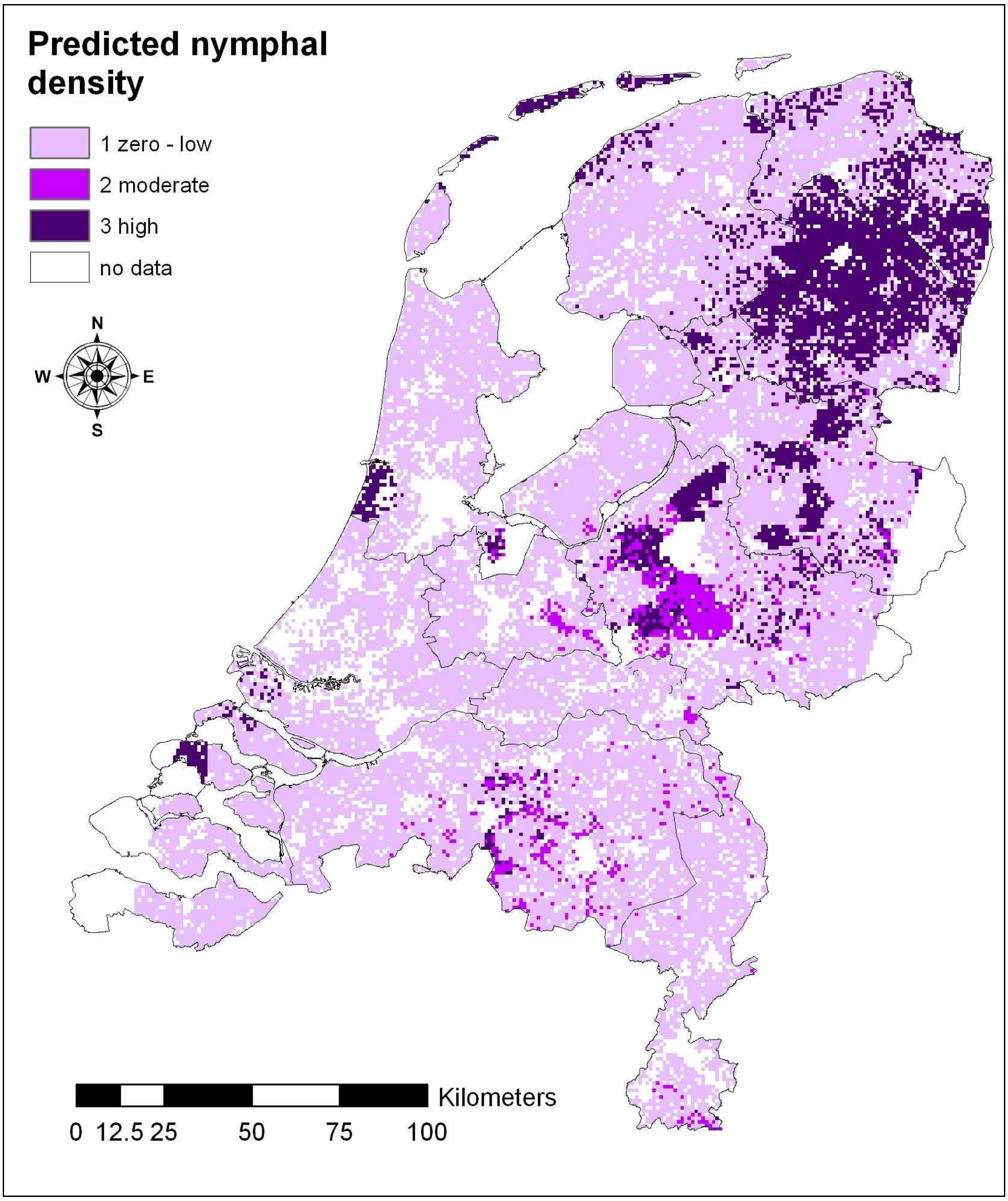

Figure 4. Predictive map of early summer densities of host-seeking *Ixodes ricinus* nymphs. Density classes correspond with classes as defined in Figure 3A.

3.4 Predictive capability

The predictive capability of the discriminant functions was tested by applying them to the build set on which the discriminant functions were based, and a validate set. The first consisted of randomly selected 80% of the samples and the latter of the other 20%. For early summer, the model classified 83% of the build data correctly and 71% of the validate data (Table 6). The classification efficacy in autumn was worse; 66% of the build set and 20% of the cases in the validate set were correctly classified (Table 7). As a visual support of these results, category scatter plots are shown, depicting both the build and the validation cases (Figure 5 and 6). These plots indicate to which category cases were originally belonging to and what discriminant scores they got on both functions in the analysis. Cases are classified in the category with the least 'Mahalanobis distance' to the category centroid. This distance is expressed in standard deviations of the function scores and takes in account the correlation between the variables. As already indicated by the significance of the discriminating capability of the functions, density classes in early summer are better separated (Figure 5) compared to autumn (Figure 6).

Table 6. Early summer classification results after application of the discriminant functions. The functions were applied on the build set and a validate set. Highlighted numbers are correctly classified densities.

		Predicted				
	Class	1	2	3	*n*	Correctly classified
Build set original	1	**9**	0	0	9	83.3%
	2	1	**5**	0	6	
	3	1	2	**6**	9	
				total	24	
Validate set original	1	**2**	0	0	2	71.4%
	2	1	**1**	1	3	
	3	0	0	**2**	2	
				total	7	

Table 7. Autumn classification results after application of the discriminant functions. For explanation see description Table 6.

		Predicted				
	Class	1	2	3	*n*	Correctly classified
Build set original	1	**9**	2	1	12	65.8%
	2	2	**6**	3	11	
	3	3	2	**10**	15	
				total	38	
Validate set original	1	**2**	3	0	5	20.0%
	2	3	**0**	0	3	
	3	1	1	**0**	2	
				total	10	

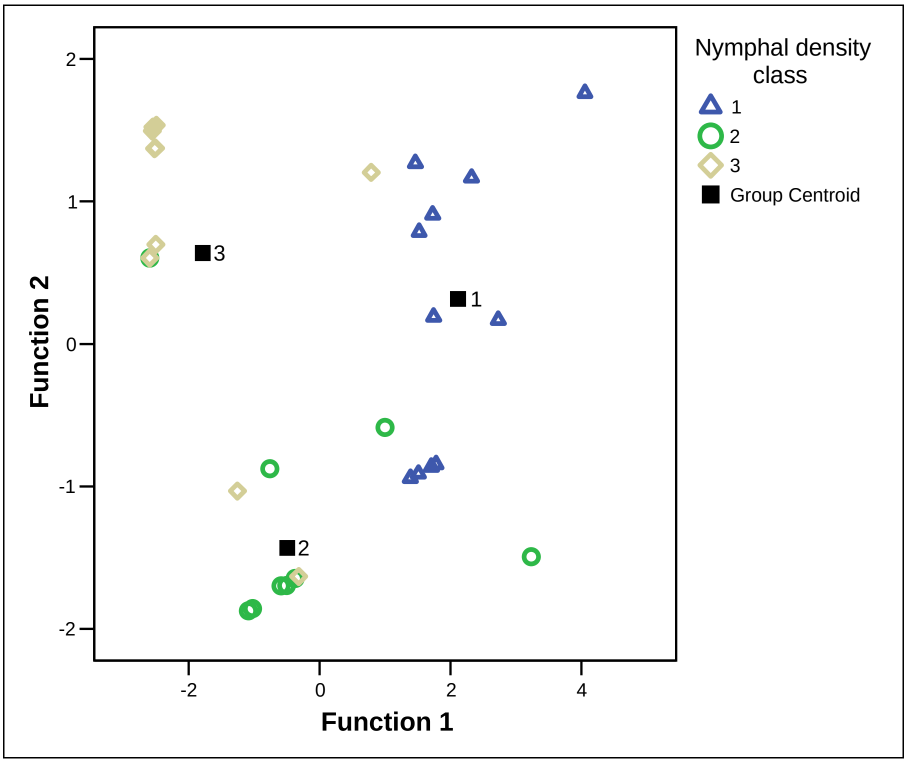

Figure 5. Category classification scatter plot early summer (n = 31).

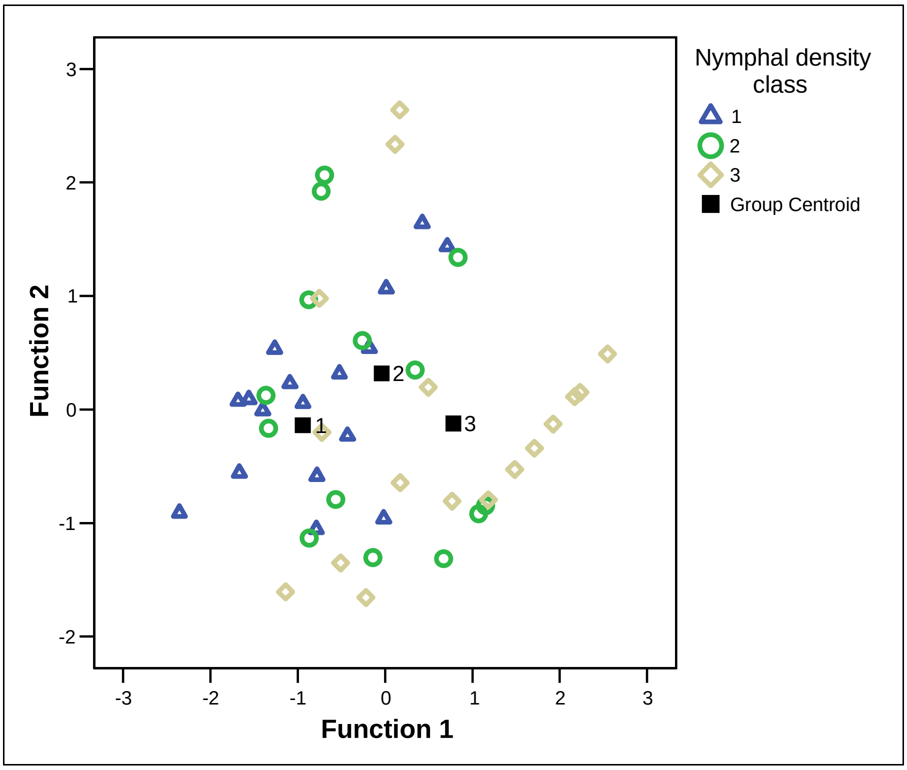

Figure 6. Category classification scatter plot autumn (n = 48).

4. Discussion

As far as my knowledge reaches; this is the first study predicting densities of questing *I. ricinus* nymphs at different locations in the Netherlands, based on RS and GIS-derived data on the environment. Environmental variables may individually or combined, determine survival of ticks and hence indicate risk of obtaining Lyme disease. Variables that were tested for association with nymphal densities were obtained without actually visiting the tick sampling transects. Some variables were indirectly derived from RS-images (NDVI, landscape structure, land use and distance to coast) others by geo-referenced field observations in larger areas (climate, roe deer density and soil type). Several factors showed association with nymphal densities. The discriminant analysis allowed distinguishing density categories and testing of the predictive capability of the discriminant functions. Prediction efficacy is bad in autumn and fairly good in early summer, although predictions could be validated with only few cases.

4.1 Variation in nymphal densities

By dragging a blanket through the vegetation, only the questing proportion of the ticks will be collected. These form a representation of the overall abundance of ticks in the near environment. Much variation was observed in the nymphal densities collected at the different transects and different seasons. It has to be noted that no direct comparison between all early summer and autumn densities can be made. But the 24 transects that were sampled in both seasons however, showed that early summer densities were significantly higher. This is because the density of questing nymphs reaches its highest annual peak in early summer with a gradual decline towards autumn (Perret *et al.*, 2004). Differences between nymphal densities in suitable and unsuitable habitats may thus become more explicit in early summer. Another explanation for the lower densities in autumn may be that data collected in the ‘Natuurkalender project’, which forms more than one third of the cases in that period, are collected after an extreme hot and dry July month in 2006. The high temperatures and the drought may possibly have resulted in high mortality among ticks at locations that form suitable habitat during ‘normal’ summers, but not under these extreme desiccating circumstances. This can be seen in transects situated in deciduous forest probably having a bigger buffering capacity against dehydration compared to coniferous forests, although both forest types are known to support tick populations (Lindström & Jaenson, 2003).

The variation in dates on which the highest density was reached in early summer can be explained by the annual and local influence of temperature on the tick activity (Gray, 1998) and the sampling frequency. Ticks were collected in multiple years and at multiple locations with varying frequency. When average spring temperature is higher at a certain location, a density peak earlier in the season can be expected because ticks get activated sooner (Diuk-Wasser *et al.*, 2006; Jouda *et al.*, 2004). Also a weekly sampling frequency may result in that a peak is noticed earlier, compared to a monthly sampling frequency.

Densities in early summer were autocorrelated, while autumn densities showed a random pattern. Since data from sampling locations in close proximity to each other tend to be more similar to each other than data from sites farther apart (Cliff & Ord, 1973), the better distribution of transects over the country in autumn can explain this outcome. Transects in early summer were situated only in a band from east to west, but not in the north and south like in autumn. Furthermore, some transects were only a few hundred meters separated from each other, and the number of transects in early summer was lower than in autumn.

4.2 Extent of association with variables

In both seasons densities were correlated with roe deer density and April NDVI. Although correlations in early summer were stronger, in both seasons nymphal density correlated strongly with these variables. In several studies deer density has been shown to be associated with *I. ricinus* densities (Gray *et al.*, 1992; Pichon *et al.*, 1999; Rizzoli *et al.*, 2002). A high roe deer density may probably facilitate higher tick densities due to their necessity for adult tick reproduction and feeding of female ticks (Gray *et al.*, 1992). With an increased roe deer density, the density of nymphs may indirectly be affected due to more frequent production of larger egg batches (Pichon *et al.*, 1999).

The relation between NDVI and tick occurrence or density has also been reported by other authors (Eisen *et al.*, 2006; Estrada-Pena, 1998, 2002). Data on monthly NDVI was also used by Estrada-Pena (2006) to predict habitat suitability for *I. ricinus* in several western Palaearctic ecoregions. In the current study, only the April NDVI was significantly negatively correlated with the densities and not the September NDVI. This is due to the difference in photosynthetic activity of forests compared to other vegetation in each particular time of the year. Grasslands and vegetated ground may have an increased photosynthetic activity earlier in the year compared to forests. Since forests with a leaf litter layer or undergrowth of grasses form suitable habitats for *I. ricinus* (Estrada-Pena, 2001; Gray, 1998; Lindström & Jaenson, 2003), higher densities may be expected in areas with a low photosynthetic activity in April.

NDVI may be a crucial predictive factor in future risk studies in the Netherlands, since it enables classification of different forest types. As Eisen (2005) showed, it is possible to distinguish forest types with or without grass ground layer, as well as forests with or without leaf litter layer, and relate this to tick density. In contrast, the assessment of tick distribution on the basis of direct botanical units in the field is highly time-consuming and difficult to apply to large areas according to Daniel *et al.* (1998). Furthermore, the comparison of habitats composed of different syntaxonomic units but with the same suitability for tick survival is not straightforward (Estrada-Pena, 2001). In the current study, different forest categories could not be distinguished based on the NDVI, because satellite images of only two days were available, while one needs images of multiple dates for such a classification. It is possible that this may also become a problem in the previously idea suggested, since the Netherlands are ~85% of the time cloud covered, limiting the view of the satellite.

In this study, a relatively coarse land use classification was used as variable describing vegetation cover. The classification distinguished grasslands from other vegetation and coniferous from deciduous forests. This land use variable was positively associated with the density categories in early summer, confirming that forested areas are generally more suitable habitats for *I. ricinus* than open vegetated ground (Lindström & Jaenson, 2003).

All three climate parameters were significantly correlated with nymphal density in early summer. Since climate is determining off-host tick survival during questing as well as quiescence periods, it can be used in both predicting habitat suitability (Brownstein *et al.*, 2003) and daily activity rates (Daniel *et al.*, 2006; Perret *et al.*, 2004).

Mean early summer temperature and saturation deficit are negatively correlated with density, and relative humidity positively. Higher temperatures and SD cause the tick to dehydrate, which limits its questing activity, while a higher RH enables rehydration. This relation was previously reported in other studies (Diuk-Wasser *et al.*, 2006; Hubalek *et al.*, 2003; Perret *et al.*, 2000; Perret *et al.*, 2004).

The weaker correlation in autumn, where RH was negatively correlated and SD positively correlated with density of the nymphs, can be explained by the relatively low maximum SD, namely: 3.5 (see Appendix IIh). Since density of the questing proportion of ticks decreases when the SD reaches values above 4.4 mmHg (Perret *et al.*, 2000), a positive correlation may occur, because the mean atmospheric drying power was always below the ticks equilibrium. The regional influence of the seawater temperature on air temperature in autumn (see Appendix IIf) did not show direct correlation with tick questing activity. This also applied for the variable ‘distance to coast’. However, in a study of Eisen *et al.* (2006) coastal woodlands showed to support higher densities of nymphal *I. pacificus* nymphs than inland woodlands. The difference with the current study may be due to a better adaptation of *I. pacificus* to coastal influence, while *I. ricinus* population density may be less affected by this factor in the Netherlands. Although data presented in the thesis of (Holtkamp, 2003) suggests that phenology of *I. ricinus* is affected by coastal influence through the effect of the sea on climate. Ticks at the coast were found questing earlier in the season than ticks more inland.

Weather variables are indispensable in a temporal predictive model with tick densities, since there is no risk when ticks are not questing, irrespective of the actual tick density. Daily activity rates of *I. ricinus* are well predictable based on climatic models with few parameters (Daniel *et al.*, 2006). In the current study however, spatial phenology could not yet be predicted with the climate data, because ticks were sampled in multiple years and mostly not in a continuous season.

Soil type and landscape structure were not, or slightly (in autumn) correlated with nymphal densities. Although soil type has a direct and indirect influence on habitat suitability of ticks due to moisture content and suitability of supporting plant and host populations (Bunnell, 2003), densities could not be explained by this factor. This may be due to the unbalanced distribution of cases over the four soil types or the stronger influence of another variable. Landscape structure was also not associated with densities; this may be due to the radius-size (250 m) in which metrics were calculated. When different radius-sizes are used, they will reflect fragmentation on different orders of spatial magnitude, which may affect the meaning and explanatory power of the parameter. Another reason for finding no association may be the effect of roads on fragmentation. The average forest patch size may be smaller because of roads, although habitat suitability for mammalian hosts of the tick will not automatically be better. In other studies it has been shown that fragmentation does positively affect tick densities (Allan *et al.*, 2003; Brownstein *et al.*, 2005), however in these studies the sampling was stratified by deciduous forest density.

4.3 Predictive capability

Using the associative variables from the Spearman correlation in the discriminant analysis, two predictions were made; nymphal density in early summer and in autumn. Although the early summer densities had fewer cases and showed autocorrelation, density predictions in this season showed a better fit to the validation data (71% vs. 20% correctly classified). This is probably due to the lack of discrimination between low and high densities in autumn. Since the nymphal density range was smaller and more skewed in the latter season, it is likely that both discrimination and prediction of categories is worse compared to early summer, because cases are not well separated. But it may also be caused by the earlier-mentioned extreme climatic circumstances in the summer of 2006, resulting in misclassification of potential suitable habitats.

The predictive map of early summer predicted a relatively large proportion of the points to support high nymphal densities. Indeed the highest density predictions in the Waterleidingduinen, De Hoge Veluwe and Drenthe correspond with the large numbers of collected nymphs at those locations in early summer. The prediction also corresponds to certain extent with maps published in the Dutch Bulletin of Infectious Diseases (see Appendix V) (Hofhuis *et al.*, 2006). This paper showed the prevalence of erythema migrans per municipality in three different years. Although the maps should not directly be compared, the map of 2005 showing EM-prevalence and the current map show a similar pattern.

Differences are probably caused by the coarse classification of density categories in the current study, resulting in that few locations were classified as moderate density. Also not many cases were used to build the model, likely resulting in a not very well balanced prediction. Besides, the risk map of Hofhuis *et al.* (2006) is based on reports of erythema migrans and this does not automatically has to represent the location where the tick bite was obtained (Ostfeld, 2005). This indicates the weakness of using EM-prevalence as risk indicator, but the strength of the current study.

With respect to the different geographical scales of the input data, the results highlight the strength but at the same time the limitation of the GIS/RS approach. Predictions can relatively easy be made when one uses data on macro scale, but it is undoubted that heterogeneity in a sampling area is overlooked. The current study predicts the density of nymphs based on data obtained on multiple scales; ticks were collected on the scale of a transect (200m^2), while NDVI was calculated for cells of 25 m resolution (625 m^2) and roe deer were counted per management unit with a varying size of several ha. The issue of scale should therefore be considered in interpretation of the risk model outcomes (Kitron, 1998, 2000).

At some locations only one transect was sampled, while it is known that distribution of questing ticks is usually clumped at micro geographical scale (Estrada-Pena, 2001; Tälleklint & Jaenson, 1997 and references therein). Therefore it would be good to perform future sampling at multiple transects at each location as in Diuk-Wasser *et al.* (2006) to control for heterogeneity.

The predictive map also shows much resemblance with the map on roe deer densities per management unit (Appendix IIj). This is not surprising, because strong correlations were found between tick and roe deer density. But since this factor is measured on a relatively coarse scale, the proportion of points with high nymphal densities are probably overestimated, since management units do not give accurate information where the roe deer were observed. For instance, high densities were predicted at ‘middle Schouwen Duiveland’, while this area is not known to support high tick densities. It is likely that the boundaries of the management units are the cause for this overestimation. It is possible to control for this in the future by assigning the roe deer density only to the surface-area of land use types that are likely to support roe deer populations. In this way the estimations may already become more accurate.

The classification of nymphal density categories is somewhat conservative, but the used tick data did not allow more categories, because time-span was in some cases too short to create a ‘zero density category’. Therefore the low density also comprises zero densities. The spatial resolution on which predictions were done (1000 m) also needs refinement in future studies to get a better indication of localized risks.

It cannot be excluded that the sampling method has had possible effect on the chance that a tick gets into contact with the blanket, as mentioned in (Wielinga *et al.*, 2006). Differently (seasonally) vegetated undergrounds may have had effects on how good the blankets touched the underlying vegetation.

4.4 Implications

Using GIS and RS enables us to assign areas with increased risk of obtaining a tick bite. Predicting density of *I. ricinus* nymphs is a useful approach to reduce the risk to get bitten because it enables strategic promotion of preventative measures by humans. However the current predictions are limited and not yet suitable for informing a broad public. The predictive quality is moderate and the current study predicts the spatial density of questing nymphs on a national scale with low resolution. The usability may improve when risk is predicted on multiple geographical scales (national, regional and local).

Uninfected nymphs cannot transmit a disease, and only result in biting nuisance. The map may therefore also become more useful when actual infection prevalence of the nymphs is incorporated in the analysis. These data were not available at the start of this study, but analysis that is currently undertaken as part of the ‘Natuurkalender project’ may provide data for that in the future. However, up till now no *Borrelia*-negative sites were found in that project when ticks were present at a sampling location (Takken *et al.*, unpublished data). This indicates that nymphal density itself may already display the actual risk, and infection data may only slightly refine this. Preliminary results of the ‘Natuurkalender project’ show alarming high infection percentages and indicate that chances of human infection are high when a tick bite is obtained (Takken *et al.*, unpublished data).

The current predictive map lacks a temporal component, because the autumn-data resulted in very low predictive capability. Since *I. ricinus* ticks are known to have distinct seasonality in their activity patterns (Gray, 1998) it is undoubted that adding a temporal component to the map would greatly improve its usability. The next step from there on would be a web-based GIS that uses environmental data as spatial predictor and up-to-date climatologic data as temporal predictor. The internet provides a very effective platform to communicate the message of spatio-temporal risk to a broad public and may greatly contribute in reducing the number of tick bites and Lyme disease patients in our country.

5. Conclusions

- It is possible to predict early summer nymphal density in the Netherlands based on environmental data on macro scale. Although the quality of the current predictive map is limited, it gives good indication of locations with increased risk of obtaining Lyme disease. To improve the risk predictions, density categories need refinement and temporal aspects and *Borrelia*-infection prevalence in ticks need to be incorporated.
- The relatively low number of cases compared to the number of predictor variables and the climatic circumstances in July 2006, are likely the reason for the bad predictive capability in autumn.
- Since contrast between densities is strongest in early summer, it seems most effective to use nymphal densities as dependent variable in spatial predictive models with nymphal *I. ricinus*. It may even be sufficient for spatial risk predictions to only use early summer samplings as a base..
- Visiting forested areas with high roe deer density without taking preventative measures positively affects the risk of obtaining Lyme disease in early summer.
- Predicting nymphal density on different geographical scales would greatly improve the usability of the predictive maps. Therefore other sampling strategies than the current are needed.

6. Recommendations

In order to gain more insight in the spatial risk of obtaining Lyme disease in the Netherlands I would like to recommend the following for future studies:

- To prevent effects of spatial autocorrelation, sampling transects should be selected in a stratified random design way using GIS and RS on forehand. Maximum distance between sampling locations can then be used as selection criterion.
  Using RS on forehand also enables the division of forests in different categories based on NDVI data. Also it will help to distribute sampling locations evenly over the different values / categories within variables.
- To reduce influence of heterogeneity in the densities at sampling locations (Tälleklint & Jaenson, 1997 and references therein), multiple randomly selected transects should be monitored at each sampling location as in Diuk-Wasser *et al.* (2006). This also allows analysis on multiple geographical scales.
- Tick population structure categories (Guerra *et al.*, 2002) or tick absence / presence may also be interesting dependent variables to relate to environmental variables, because they gives a more holistic view on the tick population than nymphs alone. This can be tested simultaneously with maximum nymphal density.
- Using GPS devices on forehand instead of Google Earth afterwards will help mapping the sampling transects more precisely, and provide means for cross-validation of GIS data by field visits.
- The data that is currently collected by the Dutch public who report tick bites on the website of 'Natuurkalender' can be used as validation of model outcomes, when these reports are corrected for numbers of visits.
- Possibilities of web-based spatio-temporal risk modeling should be tested using historical climatic data to build the model and actual weather data to inform a broad public with up-to-data risk maps.

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Appendices

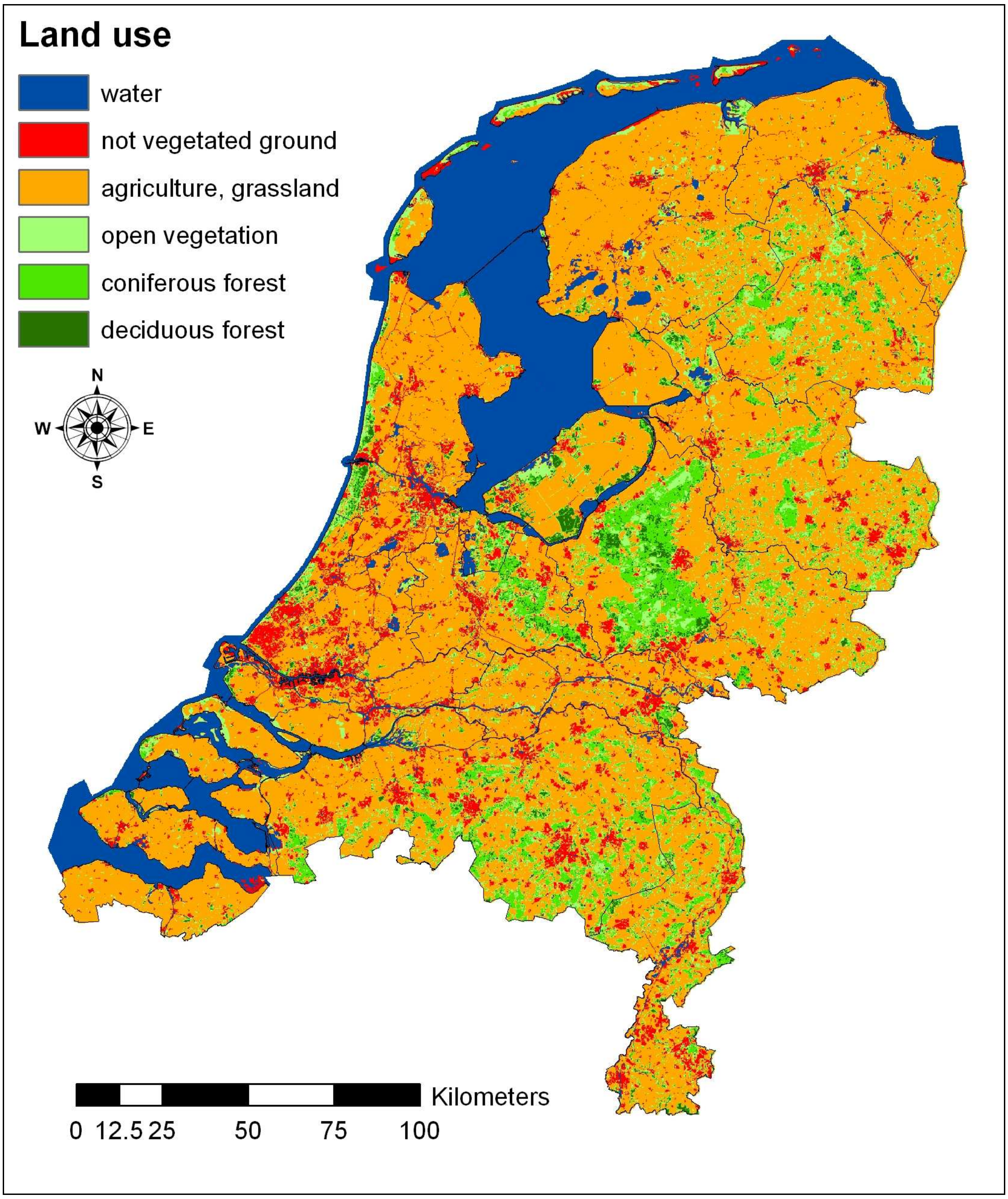
Appendix Ia Table reclassification land use

Code	Hoofdklasse	Klasse origineel	New class	New description
1	Agrarische gebied	gras	1	agriculture
2		mais	1	agriculture
3		aardappelen	1	agriculture
4		bieten	1	agriculture
5		granen	1	agriculture
6		overige landbouwgewassen	1	agriculture
8		glastuinbouw	0	not vegetated
9		boomgaard	1	agriculture
10		bollen	1	agriculture
11	Bos	loofbos	4	dec. forest
12		naaldbos	3	con. forest
16	Water	zoet water	-1	water
17		zout water	-1	water
18	Bebouwd gebied	stedelijk bebouwd gebied	0	not vegetated
19		bebouwing in buitengebied	0	not vegetated
20		loofbos in bebouwd gebied	4	dec. forest
21		naaldbos in bebouwd gebied	3	con. forest
22		bos met dichte bebouwing	3	con. forest
23		gras in bebouwd gebied	1	agriculture
24		kale grond in bebouwd buitengebied	0	not vegetated
25	Infrastructuur	hoofdwegen en spoorwegen	0	not vegetated
26	Agrarisch gebied	bebouwing in agrarisch gebied	0	not vegetated
30	Natuur	Kwelders	0	not vegetated
31		Open zand in kustgebied	0	not vegetated
32		Open duinvegetatie	2	open vegetation
33		Gesloten duinvegetatie	2	open vegetation
34		Duinheide	2	open vegetation
35		Open stuifzand	0	not vegetated
36		Heide	2	open vegetation
37		Matig vergraste heide	2	open vegetation
38		Sterk vergraste heide	2	open vegetation
39		Hoogveen	2	open vegetation
40		Bos in hoogveengebied	4	dec. forest
41		Overige moerasvegetatie	2	open vegetation
42		Rietvegetatie	2	open vegetation
43		Bos in moerasgebied	4	dec. forest
44		Veenweidegebied	1	agriculture
45		Overig open begroeid natuurgebied	2	open vegetation
46		Kale grond in natuurgebied	0	not vegetated

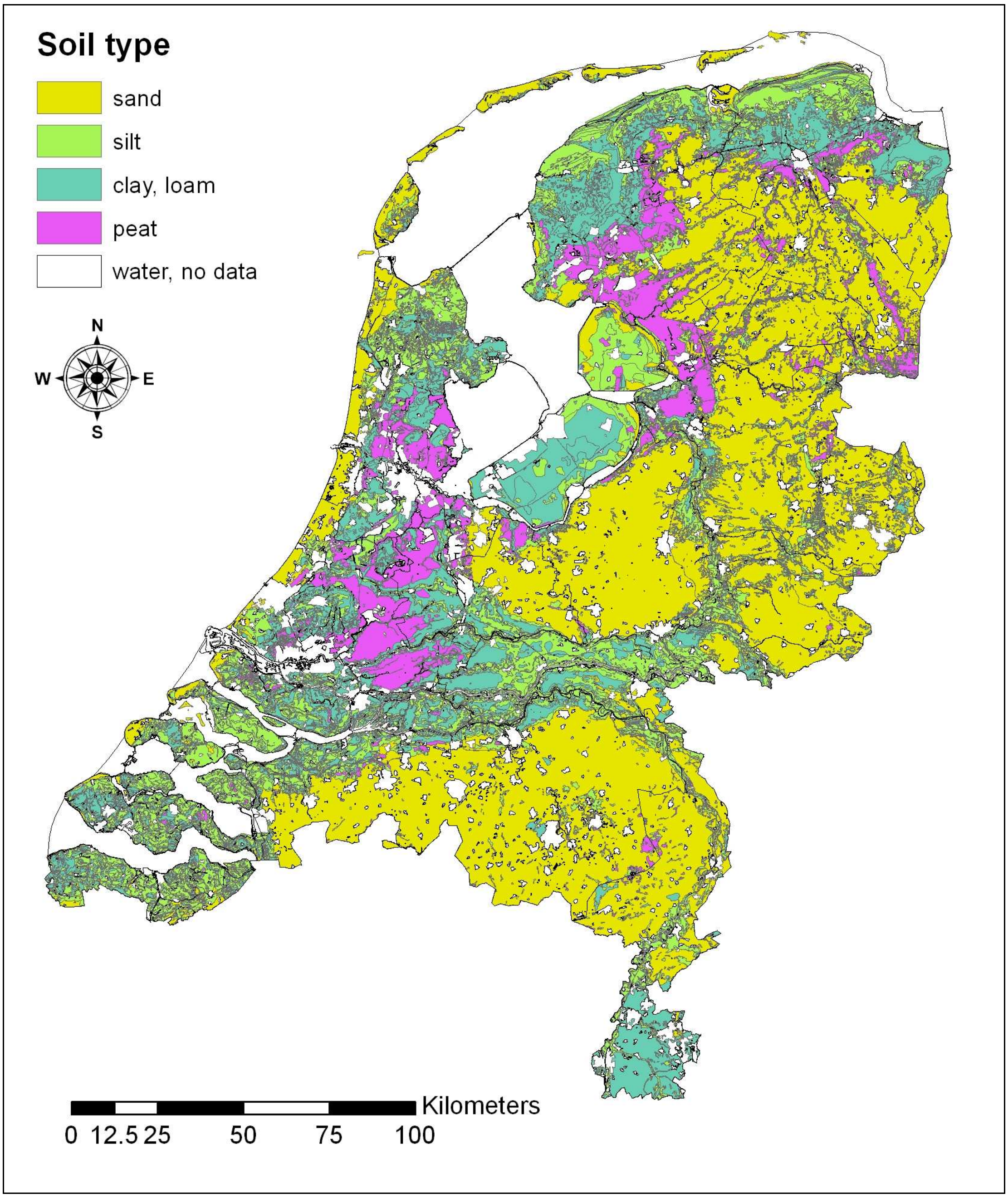
Appendix Ib Table reclassification soil types

Code	Beschrijving origineel	New class	New description
10	Veen	4	peat
20	Zand	1	sand
21	Moerig op zand	2	silt
30	Lichte zavel	2	silt
40	Zware zavel	2	silt
50	Lichte klei	3	clay, loam
60	Zware klei	3	clay, loam
70	Leem	3	clay, loam
98	Bebouwing	-9999	
99	Water	-9999	

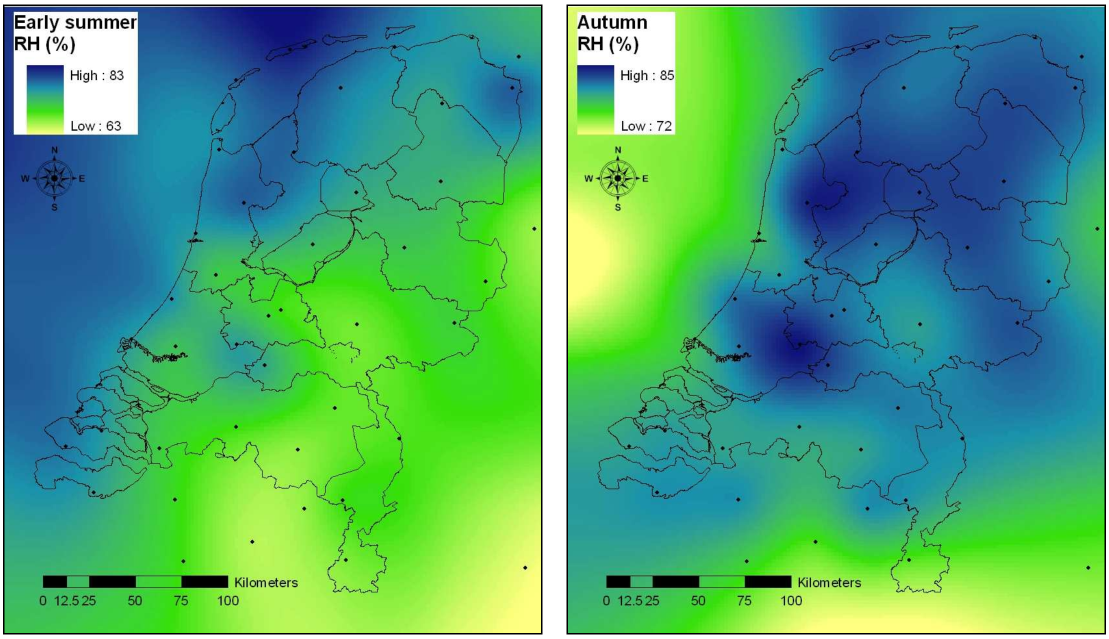
Appendix IIa Map landuse reclassified



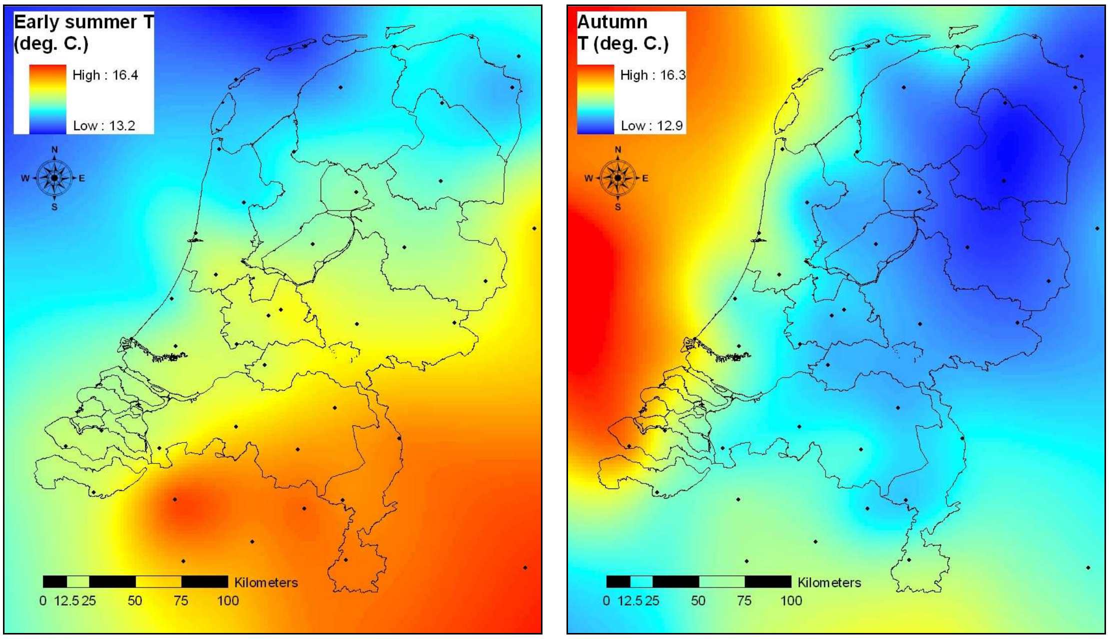
Appendix IIb Map soil type reclassified



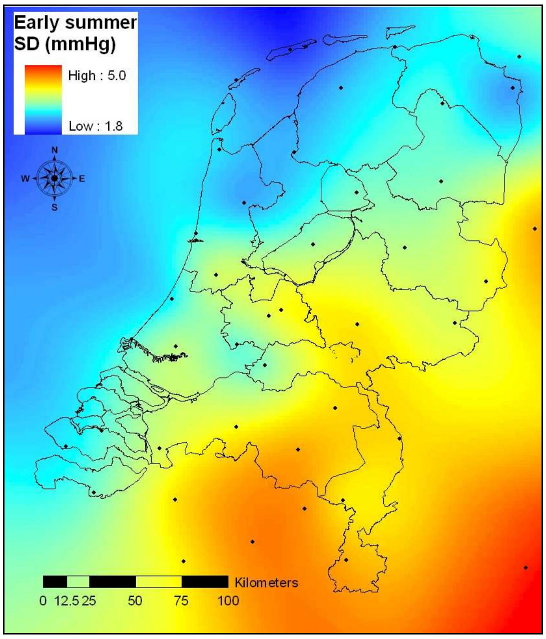
Appendix IIc,d Maps average relative humidity

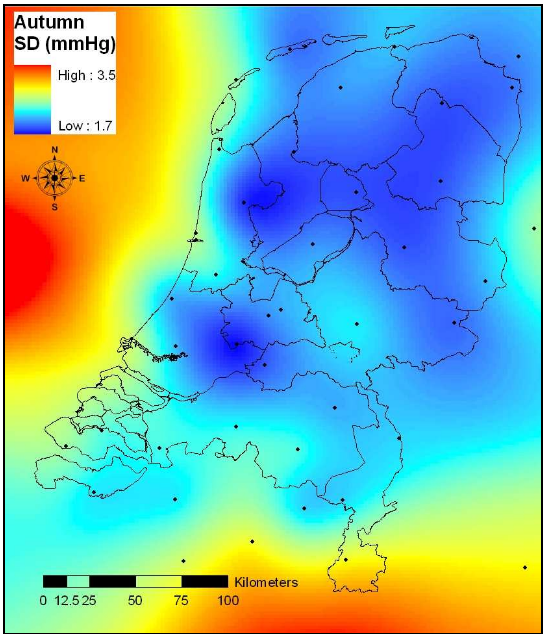


Appendix IIe,f Maps average temperature



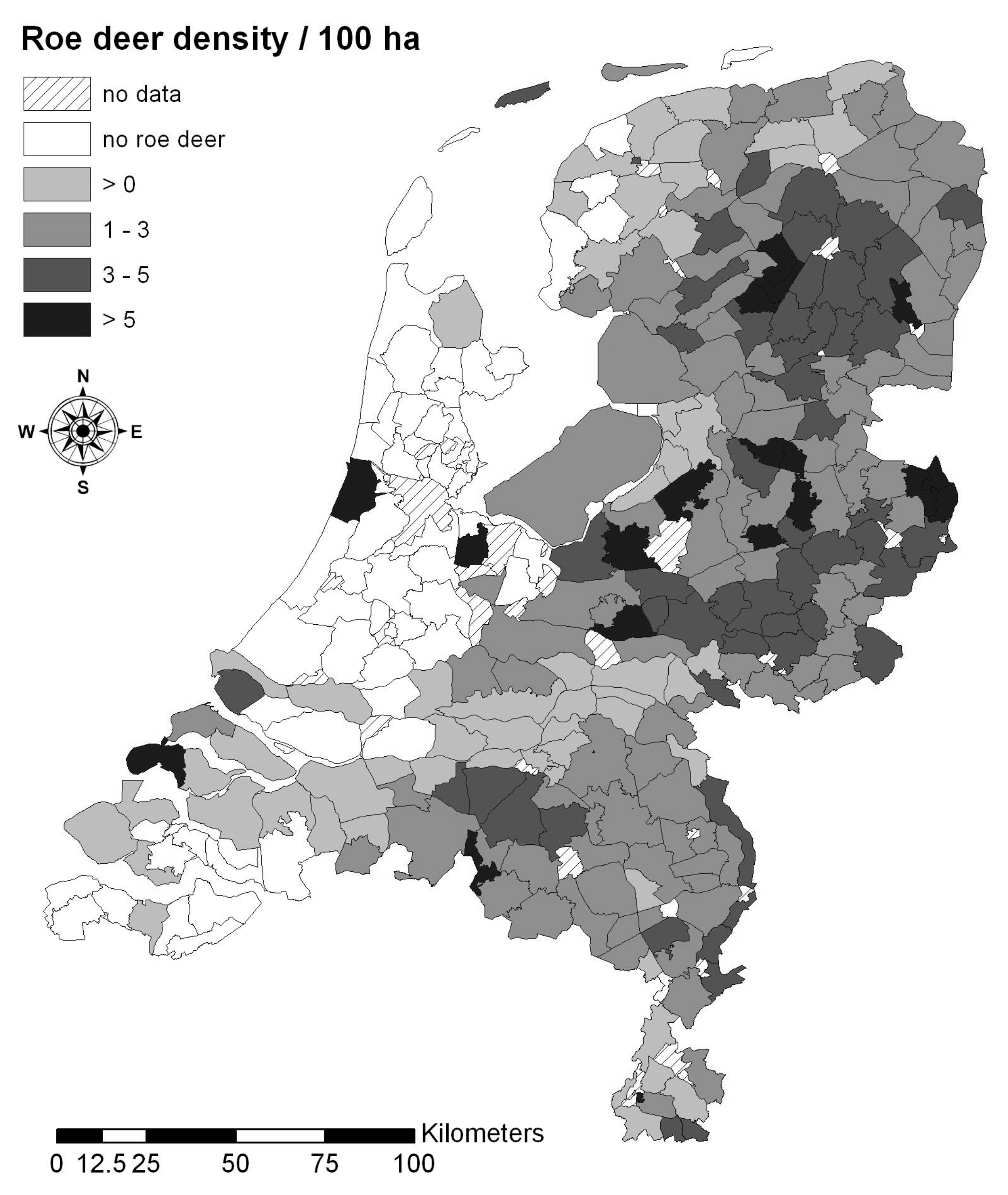
Appendix IIg,h Maps average saturation deficit



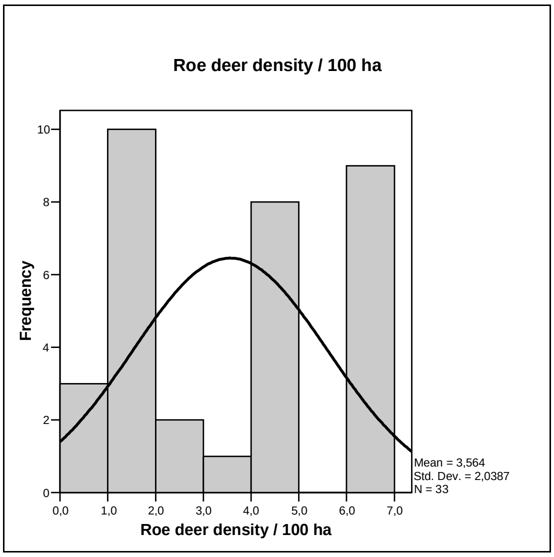


Appendix IIi Map coastline

Appendix IIj Map Roe deer density

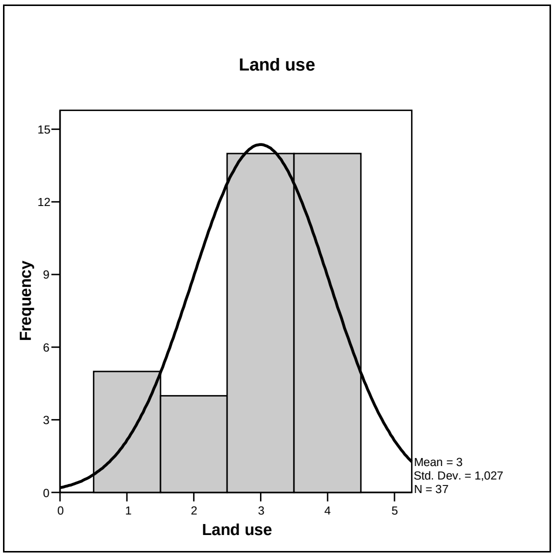


Appendix III Histograms early summer

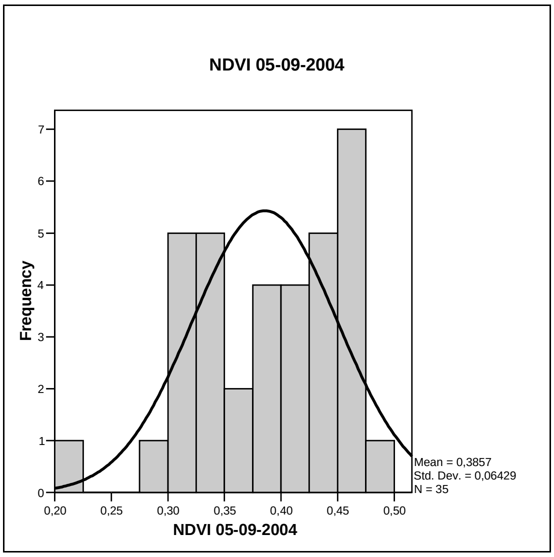


Roe deer density / 100 ha

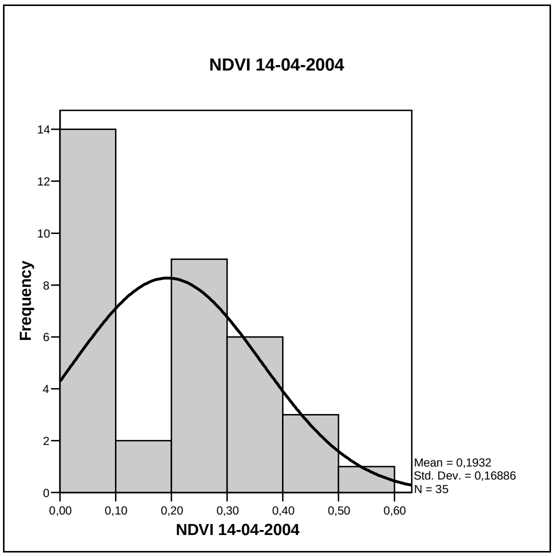
Soil order



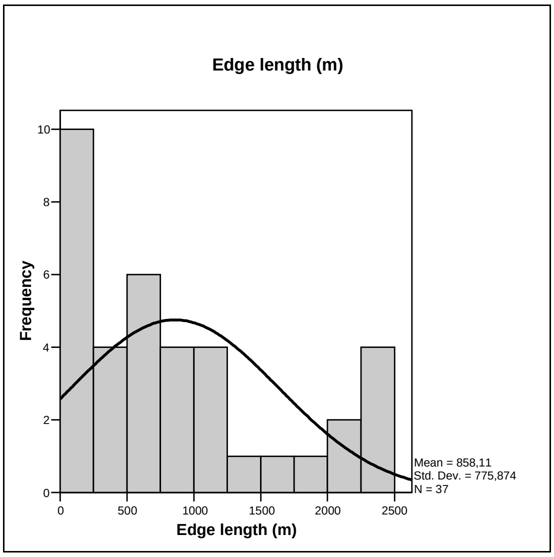
Land use



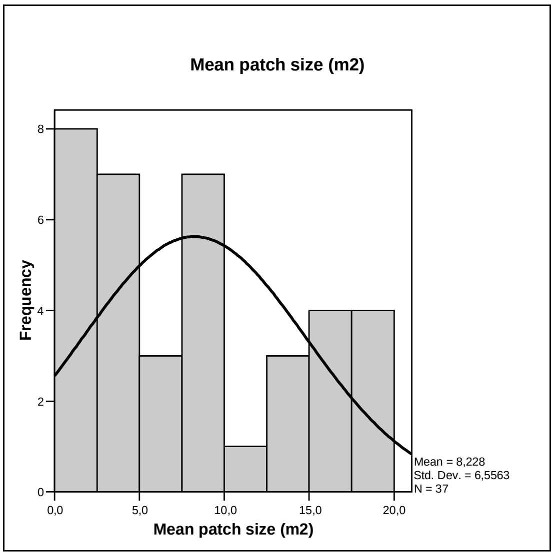
NDVI 05-09-2004

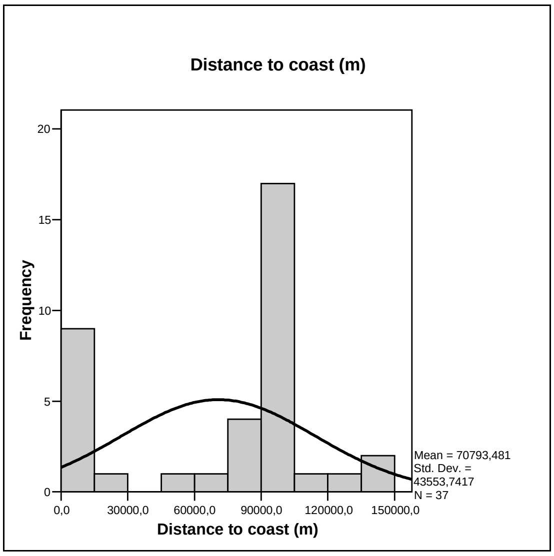


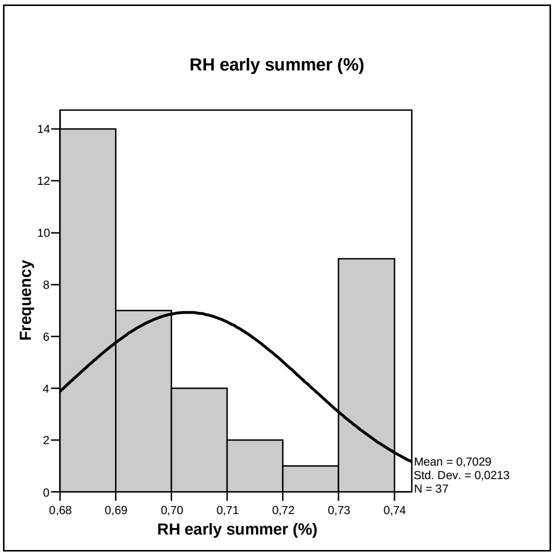
NDVI 14-04-2004

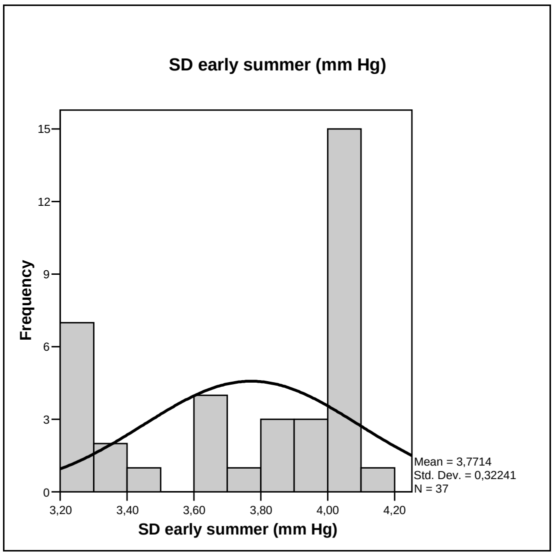


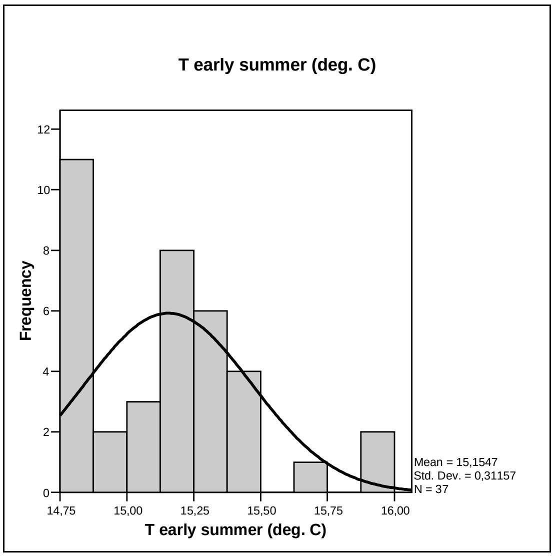
Edge length (m)


Mean patch size (m2)
Frequency
0
2
4
6
8
0,0
5,0
10,0
15,0
20,0
Mean patch size (m2)
Mean = 8,228
Std. Dev. = 6,5563
N = 37

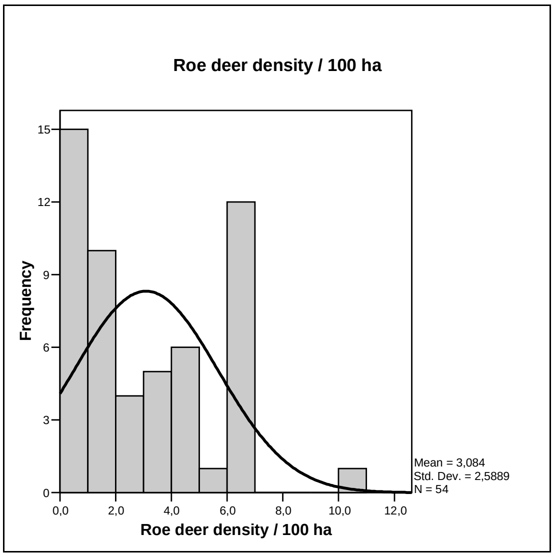

Distance to coast (m)
Frequency
0
5
10
15
20
0,0
30000,0
60000,0
90000,0
120000,0
150000,0
Distance to coast (m)
Mean = 70793,481
Std. Dev. = 43553,7417
N = 37

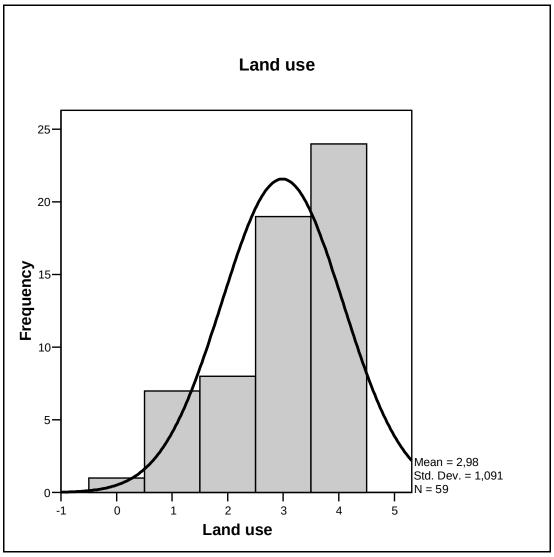

RH early summer (%)
Frequency
0
2
4
6
8
10
12
14
0,68
0,69
0,70
0,71
0,72
0,73
0,74
RH early summer (%)
Mean = 0,7029
Std. Dev. = 0,0213
N = 37

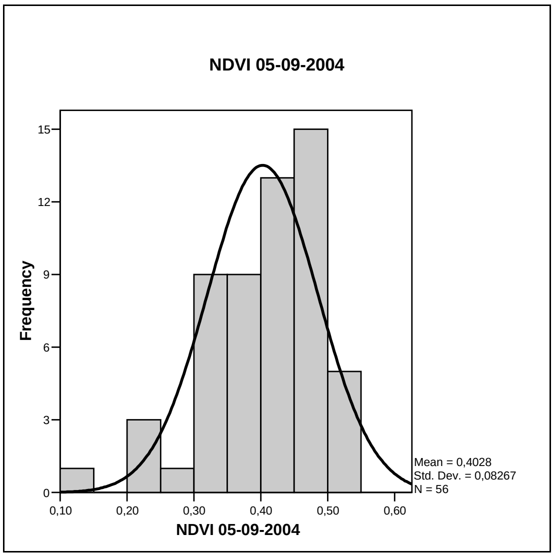

SD early summer (mm Hg)
Frequency
0
3
6
9
12
15
3,20
3,40
3,60
3,80
4,00
4,20
SD early summer (mm Hg)
Mean = 3,7714
Std. Dev. = 0,32241
N = 37

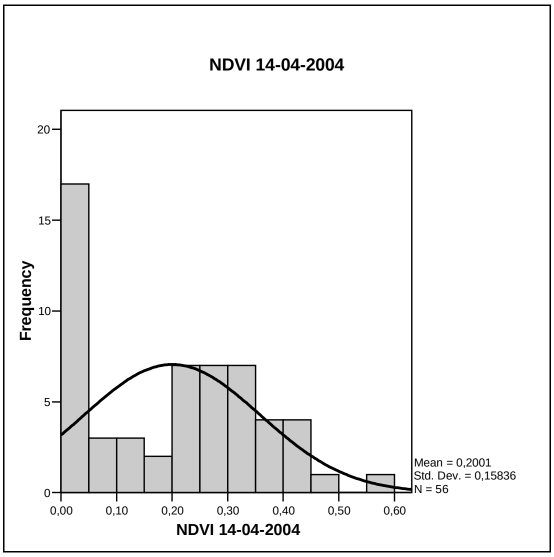

T early summer (deg. C)
Frequency
0
2
4
6
8
10
12
14,75
15,00
15,25
15,50
15,75
16,00
T early summer (deg. C)
Mean = 15,1547
Std. Dev. = 0,31157
N = 37

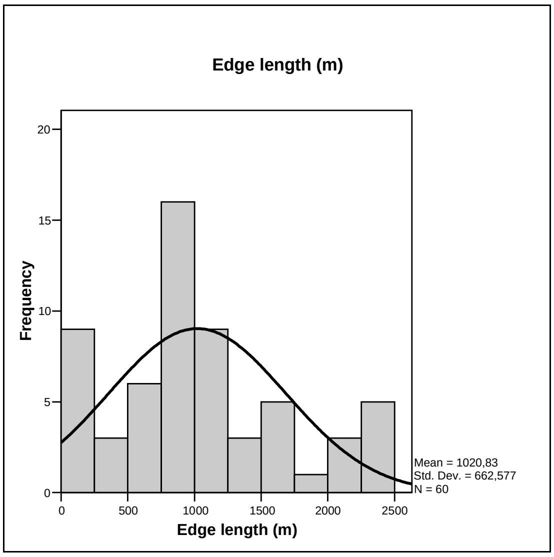
Appendix IV Histograms autumn

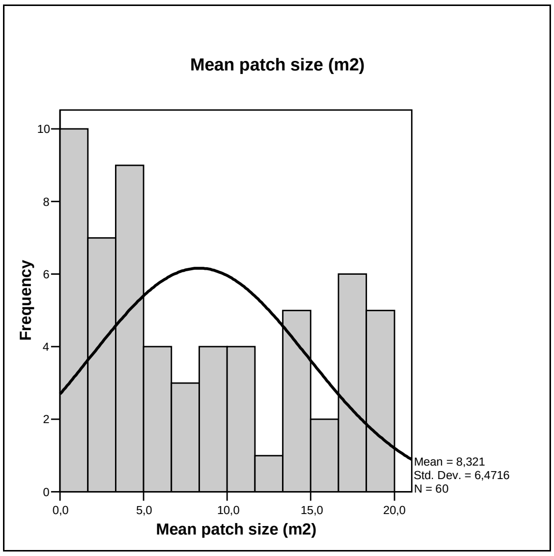


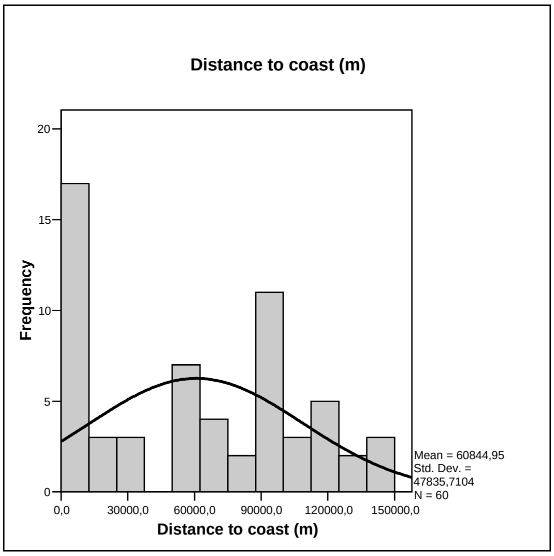


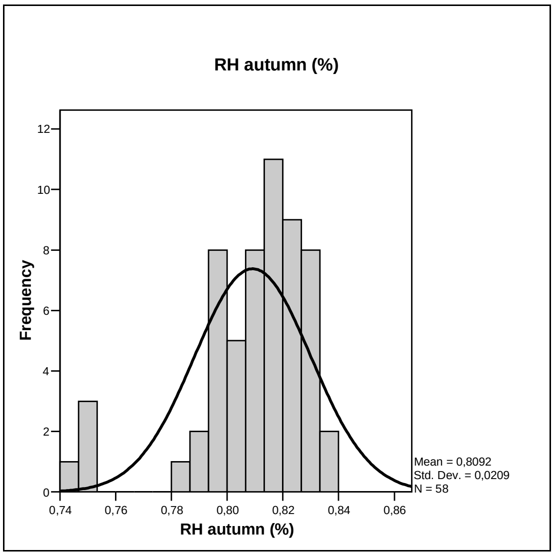


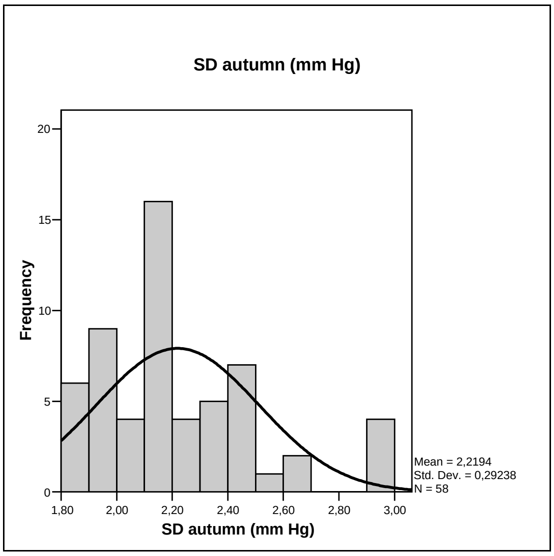


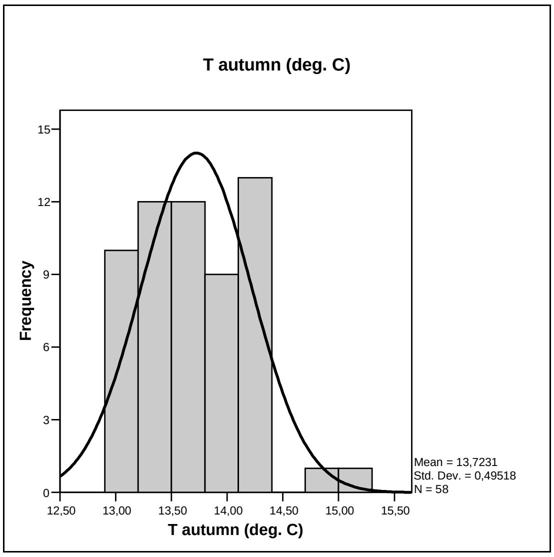



Mean patch size (m2)
Frequency
0
2
4
6
8
10
0,0
5,0
10,0
15,0
20,0
Mean patch size (m2)
Mean = 8,321
Std. Dev. = 6,4716
N = 60

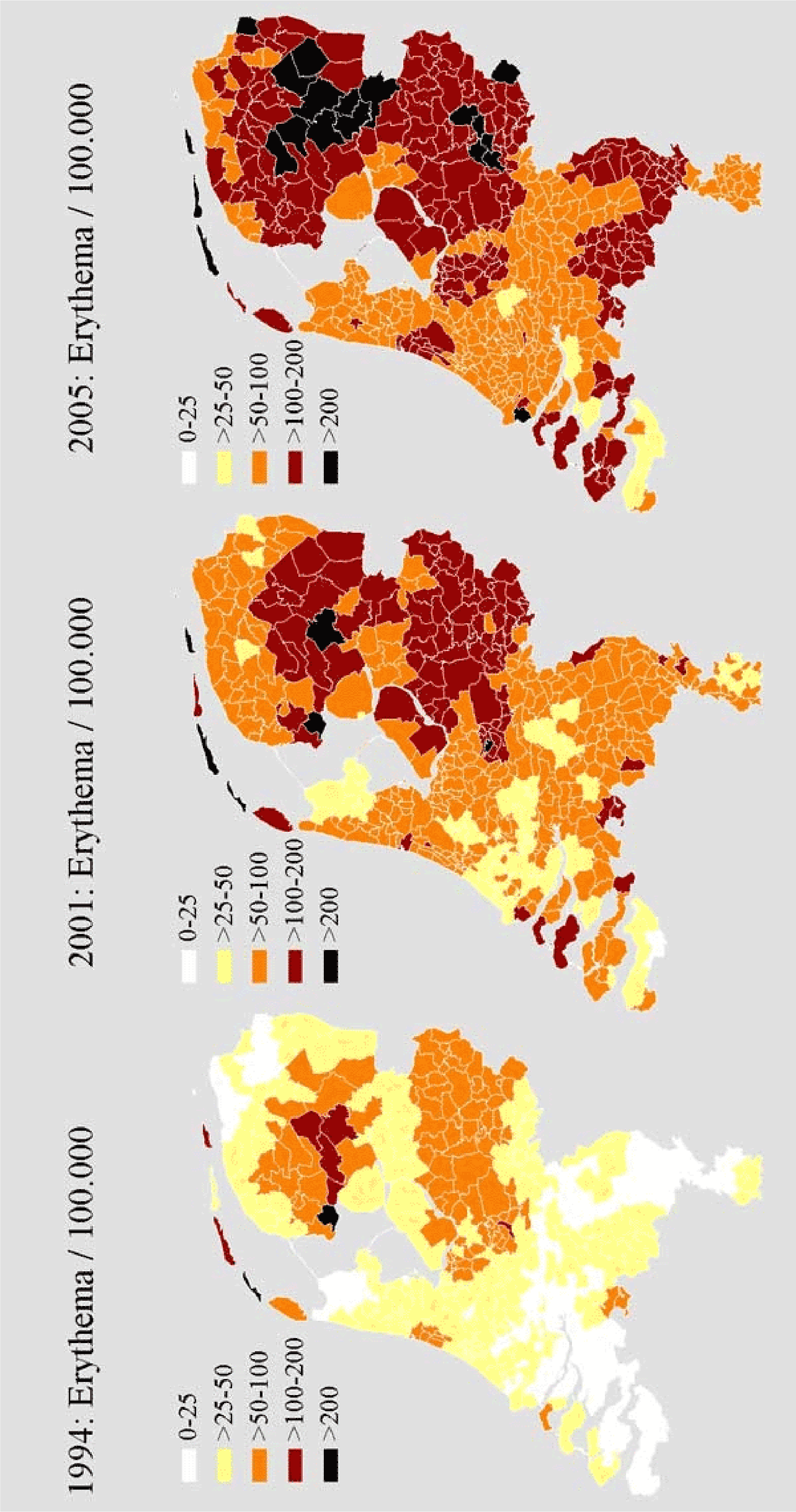

Distance to coast (m)
Frequency
0
5
10
15
20
0,0
30000,0
60000,0
90000,0
120000,0
150000,0
Distance to coast (m)
Mean = 60844,95
Std. Dev. = 47835,7104
N = 60


RH autumn (%)
Frequency
0
2
4
6
8
10
12
0,74
0,76
0,78
0,80
0,82
0,84
0,86
RH autumn (%)
Mean = 0,8092
Std. Dev. = 0,0209
N = 58


SD autumn (mm Hg)
Frequency
0
5
10
15
20
1,80
2,00
2,20
2,40
2,60
2,80
3,00
SD autumn (mm Hg)
Mean = 2,2194
Std. Dev. = 0,29238
N = 58


T autumn (deg. C)
Frequency
0
3
6
9
12
15
12,50
13,00
13,50
14,00
14,50
15,00
15,50
T autumn (deg. C)
Mean = 13,7231
Std. Dev. = 0,49518
N = 58

Appendix V Erythema migrans, source: (Hofhuis et al., 2006)



Source: (Hofhuis *et al.*, 2006)

Appendix VI Coordinates and nymphal densities at the sampling transects

PLOT_ID	LAT	LONG	PLOT_NAME	LOCATION	DENS_ES_200	DENS_A_200
0	52.00544	5.753294	E	Renkum	0	
1	52.00685	5.754361	C	Renkum	4	
2	52.00824	5.753819	D	Renkum	6	
3	52.01031	5.758367	B	Renkum	11	
4	52.01067	5.756144	A	Renkum	20	
5	52.33198	4.557314	AB	AWD	100	11
6	52.33269	4.554761	AA	AWD	197	12
7	52.33803	4.562006	VA	AWD	490	26
8	52.33927	4.56145	VB	AWD	182	30
9	52.34265	4.570817	DB	AWD	163	18
10	52.34291	4.567725	DA	AWD	227	40
11	52.35425	4.574081	WA	AWD	207	105
12	52.35485	4.5711	WB	AWD	250	91
13	52.12487	5.866086	OD	HV	142	
14	52.12484	5.864575	OE	HV	77	
15	52.12352	5.8476	SD	HV	100	
16	52.12428	5.851514	SE	HV	76	
17	52.10729	5.87605	HBE	HV	59	
18	52.10658	5.877466	HBD	HV	89	
19	52.10474	5.876462	HED	HV	189	
20	52.10463	5.875005	HEE	HV	44	
21	52.45548	6.661169	EZ	LNV1	74	10
22	52.48459	6.684336	EN	LNV2	348	46
23	52.37081	6.091647	DW	LNV3	2	0
24	52.44804	5.405061	OP	LNV4	6	0
25	51.92028	6.718567	B	LNV5	134	4
26	51.98446	6.668565	KV	LNV6	54	8
27	51.89258	5.635661	AW	LNV7	0	0
28	51.94489	5.614832	BK	LNV8	0	0
29	51.49998	5.134822	VH	LNV9	2	0
30	51.27394	6.006489	W	LNV10	0	0
31	51.34988	5.818328	GP	LNV11	26	2
32	51.87107	5.652383	R	LNV12	0	0
33	53.07012	7.121705	Bwedde	NK1		2
34	53.49275	6.163133	Schier1	NK2		4
35	53.49517	6.165367	Schier2	NK2		4
36	52.92604	6.34585	Oosterwolde	NK3		6
37	53.01502	6.755297	Gieten1	NK4		16
38	53.01569	6.753561	Gieten2	NK4		14
39	52.78195	6.304547	Ruinen	NK5		19
40	52.75222	6.523961	Hveen1	NK6		172
41	52.75324	6.522492	Hveen2	NK6		0
42	52.33217	6.423564	Hdoorn	NK7		16
43	52.16411	6.799019	Hbergen	NK8		23
44	51.89214	6.219018	Mland1	NK9		58
45	51.92785	6.221432	Mland2	NK9		2
46	52.20771	5.892073	Adoorn1	NK10		18
47	52.21587	5.904998	Adoorn2	NK10		8
48	52.02798	5.696896	Ede1	NK11		76

49	52.02663	5.689195	Ede2	NK11		104
50	52.10591	5.876736	Hveluwe	NK12		179
51	52.15757	5.228372	DeBilt1	NK13		2
52	52.15686	5.228556	DeBilt2	NK13		10
53	52.527	5.686175	Dronten1	NK14		0
54	52.52565	5.686589	Dronten2	NK14		0
55	52.64511	4.647208	Kland1	NK16		6
56	52.64696	4.655067	Kland2	NK16		0
57	52.44972	4.897389	Twiske1	NK17		10
58	52.4475	4.893472	Twiske2	NK17		18
59	51.84027	3.983692	Goeree	NK18		25
60	52.15889	4.361631	Wnaar	NK19		14
61	52.1597	4.359269	Wnaar	NK19		0
62	51.70979	3.742019	Schouwen	NK20		5
63	51.42477	5.319864	Bergeijk	NK22		16
64	51.70397	5.818581	Cuijk	NK23		2
65	50.78928	5.738111	Eijsden1	NK24		10
66	50.80185	5.743216	Eijsden2	NK24		20
67	50.79409	5.955741	Baarlo1	NK25		0
68	50.76321	5.983129	Baarlo2	NK25		14
69	52.43131	4.619662	DK	ASG	406	186
70	52.32626	4.986552	BW	ASG	38	12
71	52.2063	5.872901	KH-B	ASG	115	44
72	52.20451	5.871418	KH-H	ASG	2	2

Appendix VII Complete tick density datasets of the six studies (see CD-ROM)

Appendix VIII SPSS outputs (see CD-ROM)

Appendix IX Environmental data (see CD-ROM)

Appendix X Prediction data (see CD-ROM)