

AVIAN INFLUENZA AND MIGRATORY BIRDS:  
A SPATIAL-ECOLOGICAL PERSPECTIVE

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**UNIVERSITY OF TWENTE.**

**ITC**

FACULTY OF GEO-INFORMATION SCIENCE AND EARTH OBSERVATION

# AVIAN INFLUENZA AND MIGRATORY BIRDS: A SPATIAL-ECOLOGICAL PERSPECTIVE

DISSERTATION

to obtain  
the degree of doctor at the University of Twente,  
on the authority of the Rector Magnificus,  
prof.dr. H. Brinksma,  
on account of the decision of the graduation committee,  
to be publicly defended  
on Thursday 16th of June 2011 at 14:45 hrs

by

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born on 15 September 1982

in Hubei, China

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To my parents  
Jiang Caiting  
Si Hongye



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# **Chapter 1**

## **General introduction**

## **AVIAN INFLUENZA AND MIGRATORY BIRDS**

The avian influenza virus occurs naturally in many species of bird and is maintained in wild populations (Muzaffar et al. 2006). Waterfowl, including ducks, geese, swans, gulls, terns and shorebirds, are considered to be the main reservoir (Olsen et al. 2006; Webster et al. 1992). The avian influenza virus is subtyped according to characteristic hemagglutinin (HA) and neuraminidase (NA) glycoproteins, located on the outer surface of the viral envelope. Sixteen HA and nine NA subtypes have been identified (Swayne and Halvorson 2003; Swayne and King 2003). Avian influenza viruses can also be classified based on their pathogenicity in chickens. Low pathogenic avian influenza (LPAI) viruses mainly cause respiratory illnesses in poultry but generally low mortality. Highly pathogenic avian influenza (HPAI) viruses cause systemic disease, often resulting in high mortality in turkeys and chickens (Swayne and Halvorson 2003; Swayne and King 2003), and can also infect humans and other species of animal (Cardona et al. 2009). The H5 and H7 subtypes of avian influenza can be either low or highly pathogenic, all other known HA subtypes are low pathogenic (Yee et al. 2009).

The recent outbreak of HPAI H5N1 has received considerable attention as it poses a significant risk to human and animal health (Cardona et al. 2009). The virus was first shown to cause human mortality in Hong Kong in 1997 (Subbarao and Katz 2000). Millions of domestic birds have been killed by HPAI H5N1 infection and more than 230 million domestic birds have been culled to constrain the spread of the virus (Whitworth et al. 2007). Furthermore, 507 human HPAI H5N1 cases have been documented up to 2010, of which 302 were fatal (WHO 2010a). Until spring 2005, the occurrence of HPAI H5N1 was restricted to East and Southeast Asia (WHO 2010b). However, during the period from May to July 2005, a panzootic resulted in the deaths of more than 3000 bar-headed geese (*Anser indicus*) at Qinghai Lake in China (Chen et al. 2006). The occurrence of HPAI H5N1 infection in migratory waterfowl indicates that this virus has the potential to be a global threat (Liu et al. 2005). By early 2006, the infection had been detected widely across South Asia, Europe, and Africa (WHO 2010b). A global HPAI H5N1 panzootic had become a reality.

The basic elements of the avian influenza transmission cycle are well understood (Fig. 1). In general, the virus is transmitted from bird to bird without intermediate vectors, but it can remain dormant in bodies of water for some period of time. Mammals, including humans, are occasionally infected. Mammal-to-mammal transmission,

including human-to-human, appears to be rare. Essentially all known human cases occurred after direct contact with birds, usually poultry, suggesting a clear connection to the bird-driven transmission cycle. Some indications of mammal-to-mammal transmission do exist (Thanawongnuwech et al. 2005), but these transmission chains are not self-sustaining.

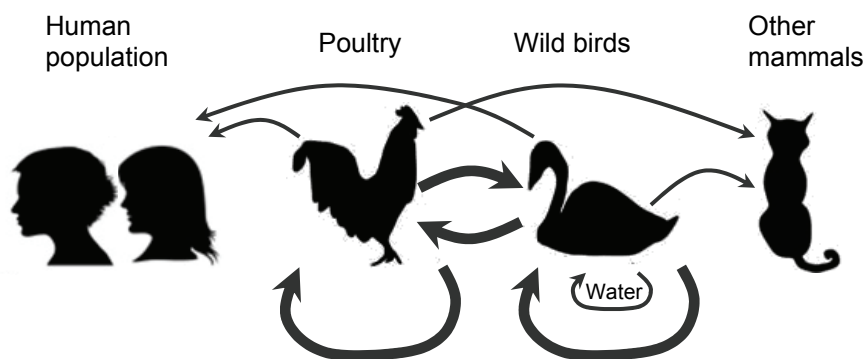


Fig. 1: Diagrammatic summary of the transmission routes of avian influenza viruses. Major transmission routes are shown with thick arrows and minor routes with thin arrows (adapted from Peterson and Williams 2008)

## PROBLEM STATEMENT

Our understanding of avian influenza mainly comes from virology, veterinary science, and medical science. Most studies have been conducted from the perspective of either domestic animal or human health (Muzaffar et al. 2006). The increasing problems with HPAI H5N1 outbreaks over large geographical areas, emphasize a significant gap in our knowledge of the ecology of HPAI H5N1 viruses, especially in wild birds (Munster et al. 2007). An ecological way of thinking is required for a better understanding of HPAI H5N1 spread due to the wide range of environmental factors involved. Ecological processes occur in a spatial and temporal context but this is often not considered when examining infectious diseases. Ecological thinking, combined with spatio-temporal consideration, is changing our understanding of the processes that drive the spread of diseases (Elliott and Wartenberg 2004). For example, the HPAI H5N1 risk in Southeast Asia has been successfully predicted based on rice cropping intensity, as free-grazing ducks use rice paddies for feeding (Gilbert et al. 2007). However, the ecology of HPAI H5N1 occurrence in wild birds has not yet been sufficiently examined. The interactions among HPAI H5N1 occurrence, waterfowl distribution and environmental factors require further study.

Although the elements of the transmission cycle of avian influenza are well understood, their relative roles are the topic of considerable debate, especially the role of migratory waterfowl (Peterson and Williams 2008). Some researchers are of the opinion that the entire system is driven by movements of poultry and poultry products, and that wild birds are merely incidental hosts, with little importance in overall HPAI H5N1 transmission (Feare and Yasué 2006; Goutard et al. 2007; Peterson and Williams 2008). Others suggest migratory birds play a significant role in HPAI H5N1 transmission as well (Chen et al. 2005; Gilbert et al. 2006b; Kilpatrick et al. 2006; Olsen et al. 2006). More effort is needed to understand the role of migratory waterfowl in the spread of the HPAI H5N1 virus, especially in long-distance transmission. HPAI H5N1 outbreaks in poultry and wild birds were observed to be unevenly distributed across geographical regions, as wild bird infections were mainly concentrated in Western Europe. Previous studies primarily focused on the interactions between environmental features and disease outbreaks in poultry, particularly in Southeast Asia (Gilbert et al. 2006a; Gilbert et al. 2008). The influence of environmental factors on the HPAI H5N1 virus in wild birds in Europe has not yet been investigated. The ecology of migratory waterfowl and their interaction with the environment have been linked to ecological principles, such as the foraging maturation hypothesis and the green wave hypothesis. However, previous studies were mainly conducted at a field experimental level. The distribution of waterfowl has not yet been quantified using these ecological principles. The quantification of waterfowl distribution and migration patterns requires up-to-date, accurate measures of environmental features, especially concerning food resources, extending over large geographical regions. Currently only surrogates of food resources, such as vegetation indices, are used in epidemiological and ecological studies, and an accurate estimation of forage properties across the landscape is as yet not available.

## **RESEARCH OBJECTIVE AND QUESTIONS**

The objective of this thesis was to investigate the interactions among highly pathogenic avian influenza (HPAI) H5N1 occurrence, the distribution of its potential spreading agent migratory waterfowl, and environmental factors, from a spatial-ecological perspective. The following research questions were formulated to achieve the research objective of this thesis.

- Do migratory birds play a role in the global spread of HPAI H5N1?

- What environmental factors determine the occurrence of HPAI H5N1 in wild birds in Europe, and do wild birds play a prime role in it?
- How do environmental factors influence the distribution of migratory waterfowl?
- Can spatio-temporal variation of forage quantity and quality be accurately mapped at a landscape scale?

## **METHODS**

The development of ecological thinking in a spatio-temporal context benefits greatly from the advances in tools and technologies such as geographic information systems (GIS), remote sensing (RS), spatial statistics, and geostatistics (O'Neill et al. 1999; Ward 2008). GIS is a computer-based system for automating, storing, manipulating, and displaying mapped information (Burrough 1986). RS is defined as the measurement of properties of an object of interest by a sensor that is not in direct physical contact with that object (Jensen et al. 1989). Spatial statistics and geostatistics are a set of tools developed to describe, explain, extrapolate, and predict the distribution of objects and processes in space (Anselin and Getis 1992; Kitron 1998). Combined approaches integrating these technologies have been utilized in this thesis to understand the interactions among HPAI H5N1 occurrence, migratory waterfowl distribution, and environmental factors. Field measurements were conducted to facilitate the application of these approaches.

## **STUDY AREA**

This research was conducted at four different scales (Fig. 2). At a global level, it covers Eurasia and Africa, where all outbreaks of the HPAI H5N1 virus has been reported. At a continental level, it looks at Europe, where most HPAI H5N1 occurrence in wild birds has been recorded. At a regional level, it focuses on the northern part of the Netherlands, in the provinces of Friesland and Groningen. At a local (habitat) level, it includes the Lauwersmeer area (in the northern part of the Netherlands).

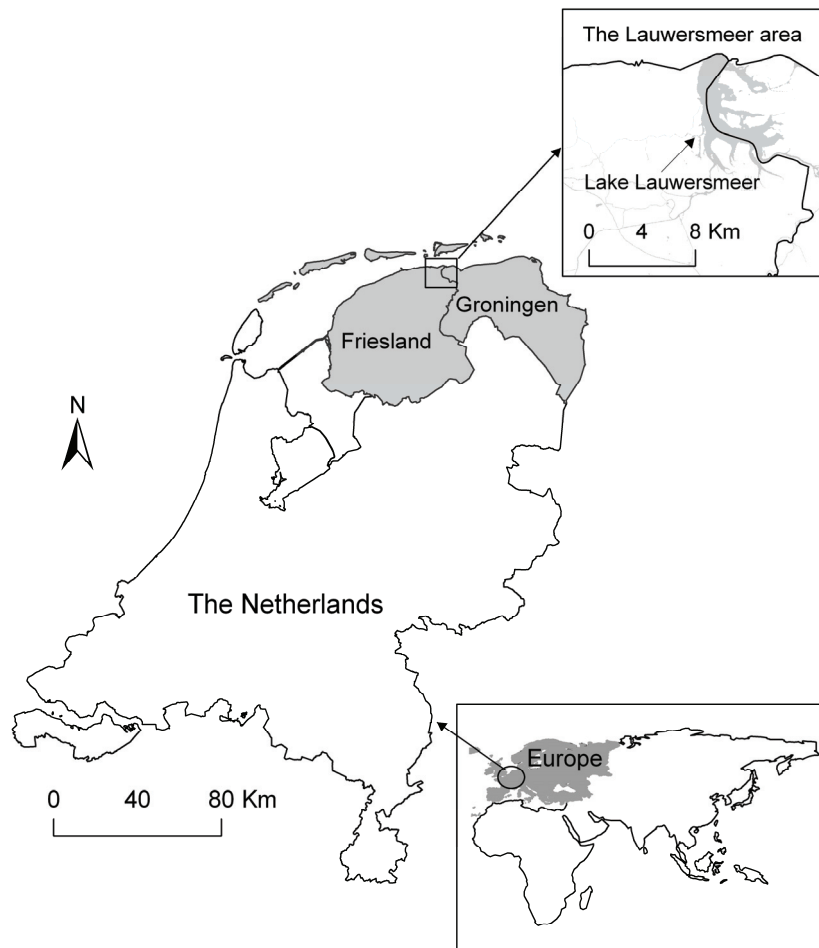


Fig. 2: Location of study areas at the global level (Eurasia and Africa), the continental level (Europe), the regional level (the provinces of Friesland and Groningen in the northern part of the Netherlands), and the local level (the Lauwersmeer area)

The Netherlands is a core overwintering and spring staging site for migratory waterfowl (Bos et al. 2008; van Eerden et al. 2005). Two types of foraging areas are represented in this study area: agricultural and semi-natural grasslands. The agricultural grassland is managed by local farmers with regular fertilization, mowing, and cattle grazing. The semi-natural grassland is managed as natural reserves, and in some of these naturalized cattle is allowed to graze year-round. The plant community in agricultural fields is dominated by *Lolium perenne* and *Poa pratensis* (Prins and Ydenberg 1985), while in semi-natural areas it is dominated by *Festuca rubra*,



*Puccinellia maritima*, *Elymus repens*, *Trifolium repens*, *Plantago maritima*, and *Triglochin maritima* (Esselink 2000).

## **OUTLINE OF THE THESIS**

This thesis consists of several coherent chapters which contribute to the understanding of the interactions among HPAI H5N1 occurrence, migratory waterfowl distribution and environmental factors.

Chapter 1 presents a brief research background, the statement of problem, research objective and questions, methods, description of the study area, and as well as the outline of the thesis.

Chapter 2 investigates the linkage between the spatio-temporal dynamics of global HPAI H5N1 outbreaks and waterfowl migration patterns. The hypothesis tested is that spatio-temporal dynamics of global HPAI H5N1 outbreaks coincide with waterfowl migration patterns.

Chapter 3 examines the influence of physical and anthropogenic environmental factors on the occurrence of HPAI H5N1 in wild birds in Europe. The hypothesis tested is that migratory waterfowl is the primarily agent in the spread of HPAI H5N1 in wild birds in Europe, and HPAI H5N1 infections in wild birds occur under consistent environmental circumstances.

Chapter 4 assesses the effect of physical and anthropogenic environmental factors on the distribution of migratory waterfowl at the habitat level. The hypothesis tested is that the distribution of migratory waterfowl at the habitat level is related to food resources, the distance to roosts, and the distribution of refuges.

Chapter 5 estimates the spatio-temporal variation of forage quantity and quality at a regional scale, using the PROSPECT+SAIL model and MERIS imagery. The quantified forage properties can be used to further enhance the accuracy of waterfowl modelling and HPAI H5N1 risk prediction.

Chapter 6 ties the previous chapters together. The scientific gaps that have been filled by our key findings and the implications our study has for the surveillance and control of HPAI H5N1 incidents are discussed. Ultimately, suggestions are made for the further studies.

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## **Chapter 2**

### **Spatio-temporal dynamics of global H5N1 outbreaks match bird migration patterns**

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This chapter is based on: Yali Si, Andrew K. Skidmore, Tiejun Wang, Willem F. de Boer, Pravesh Debba, Albert G. Toxopeus, Lin Li, and Herbert H.T. Prins, 2009. Spatio-temporal dynamics of global H5N1 outbreaks match bird migration patterns, *Geospatial Health*, 4(1): 65-78.

## **ABSTRACT**

The global spread of highly pathogenic avian influenza H5N1 in poultry, wild birds and humans, poses a significant pandemic threat and a serious public health risk. An efficient surveillance and disease control system relies on the understanding of the dispersion patterns and spreading mechanisms of the virus. A space-time cluster analysis of H5N1 outbreaks was used to identify spatio-temporal patterns at a global scale and over an extended period of time. Potential mechanisms explaining the spread of the H5N1 virus, and the role of wild birds, were analyzed. Between December 2003 and December 2006, three global epidemic phases of H5N1 influenza were identified. These H5N1 outbreaks showed a clear seasonal pattern, with a high density of outbreaks in winter and early spring (i.e., October to March). In phase I and II only the East Asia Australian flyway was affected. During phase III, the H5N1 viruses started to appear in four other flyways: the Central Asian flyway, the Black Sea Mediterranean flyway, the East Atlantic flyway and the East Africa West Asian flyway, respectively. Six disease cluster patterns along these flyways were found to be associated with the seasonal migration of wild birds. The spread of the H5N1 virus, as demonstrated by the space-time clusters, was associated with the patterns of migration of wild birds. Wild birds may therefore play an important role in the spread of H5N1 over long distances. Disease clusters were also detected at sites where wild birds are known to overwinter and at times when migratory birds were present. This leads to the suggestion that wild birds may also be involved in spreading the H5N1 virus over short distances.

## **BACKGROUND**

The HPAI H5N1 (hereafter H5N1) virus is a highly pathogenic strain of the influenza A virus, which can cause systemic disease, resulting in high mortality in bird populations (Swayne and Halvorson 2003; Swayne and King 2003), and which can also infect humans and many other animal species (Cardona et al. 2009). The virus was detected for the first time in farmed geese in southern China in 1996 (Xu et al. 1999). The first case of a human becoming infected with the H5N1 virus was documented in Hong Kong in 1997 (Subbarao and Katz 2000). The present outbreak of H5N1 began in December 2003, when South Korea identified the virus in poultry populations (Lee et al. 2005). The virus circulated in east and southeast Asia during 2003 and 2004. In May 2005, the first H5N1 outbreak in migratory waterfowl was detected at the Qinghai Lake in western China (Chen et al. 2006; Chen et al. 2005; Liu et al. 2005). During the July of that same year, the virus was detected in Russia, and it arrived in Romania in October (Gilbert et al. 2006b). Africa reported its first emergence of H5N1 in Nigeria during February 2006 (Ducatez et al. 2006). This global H5N1 epidemic continues to date and has raised many questions. It is important to find out what the dispersion patterns of the virus are. Subsequently, which mechanisms are responsible for the observed patterns, and more specifically, do migratory birds play a role in the global H5N1 transmission?

A number of studies have been undertaken to answer these questions. The worldwide avian influenza activity has been summarized (Alexander 2007; Yee et al. 2009). Descriptive methods have been used to analyse the spread of H5N1 outbreaks (Gilbert et al. 2006b; Tiensin et al. 2005). Phylogenetic analyses of the lineage relationship were executed on the virus strains isolated in eastern Asia (Li et al. 2004), South Korea (Lee et al. 2008), Nigeria (Ducatez et al. 2006) and western Africa (Ducatez et al. 2007). Local level space-time cluster analyses of H5N1 outbreaks over short time periods have been carried out in China (Oyana et al. 2006), Vietnam (Pfeiffer et al. 2007), Romania (Ward et al. 2008). The purpose of these studies was to identify spatio-temporal patterns of the spread of the disease and to ascertain possible underlying mechanisms. A study, covering an extended period of time on a global scale, has not been undertaken to date, though this is important if knowledge is to be gained on the worldwide spread of the H5N1 virus. Previous studies (Gilbert et al. 2008; Kilpatrick et al. 2006) suggested, that the movements of wild birds and domestic poultry were the suspected agents for spreading the virus. Poultry transportation and the wild bird trade were also suspected agents. Their role in

spreading the H5N1 virus is relatively easy to track and detect, by analysing trade data (Kilpatrick et al. 2006). However, to what extent migratory waterbirds can be regarded as the cause of the virus spread is still under investigation (Feare and Yasué 2006; Normile 2006).

Some studies have questioned the role of wild birds in spreading the H5N1 virus. Weber and Stilianakis (2007) indicated that it is implausible for an infected bird to migrate over long distances, as migration leads to immunosuppression and is negatively affected by virus infection. Feare and Yasué (2006) questioned an experiment of asymptomatic infection of wild birds with H5N1, due to challenges of sample species identification and capture methods. Also, even though large numbers of birds migrate through the East Atlantic flyway and East Asian Australian flyway, the disease currently only circulates in Eurasia and Africa, without further spread to America and Australia. This suggests that migratory birds may not be responsible for the long-distance spread of the H5N1 virus.

A number of studies, however, do suggest wild birds may spread the H5N1 virus. Chen et al. (2004) indicated that ducks can carry the H5N1 virus asymptotically. Gilbert et al. (2006b) detected, that the directions in which the disease spread, were consistent with the major bird migration routes in the western Palaearctic. Brown et al. (2008) observed that swans and geese can shed H5N1 virus before and after the onset of clinical signs, on the basis of an experimental infection of H5N1 virus. Keawcharoen et al. (2008) found that some wild duck species showed abundant virus excretion without clinical or pathologic evidence of debilitating disease, and therefore could potentially be long-distance vectors of H5N1.

If H5N1 is spread by wild birds, either over long or over short distances, this might be deduced from spatial-temporal patterns of the disease outbreaks (Onozuka and Hagihara 2008; Ward et al. 2008). Wild birds utilize large number of over-staging sites along the flyways to forage during the migration, and settle in breeding and wintering sites during the non-migration seasons. This behaviour determines a potential role of wild birds in H5N1 spread over long and short distances, which requires both regional and local disease patterns to be generated. Due to intercontinental bird migration and the international trade of fowl, the role of wild birds as disease vectors should be viewed on a global scale (Kilpatrick et al. 2006). This study was designed to throw light on the role of wild birds in spreading H5N1 viruses. The objectives of this study were to identify the spatio-temporal patterns and dynamics of H5N1 outbreaks on a



global scale, and to further deduce the role of wild birds in the worldwide transmission of H5N1 over long and short distances.

## **MATERIALS AND METHODS**

The main reservoirs of avian influenza are considered to be waterbirds, such as duck, geese, swan, gulls, terns and shorebirds (Alexander 2000; Olsen et al. 2006; Suarez 2000; Webster et al. 1992). Therefore, the possible role of migratory birds in spreading the H5N1 virus was examined by comparing the spatio-temporal dynamics of the disease clusters with the timing, location and direction of the major waterbird migration flyways. A flyway is the entire range a migratory bird species (or groups of related species or distinct populations of a single species), uses when moving on an annual basis from their breeding grounds to non-breeding areas and back, including intermediate resting and feeding places (Boere and Stroud 2006). Figure 1 depicts the eight broadly grouped flyways of waterbirds, adapted from information collected and analyzed by the International Wader Study Group (Asia-Pacific Migratory Waterbird Conservation Committee 2001; Stroud et al. 2004). In each flyway, the spring migration passes in a northward direction and the autumn migration passes in a southward direction. The migratory direction is defined based on the eight waterbird flyways, omitting the complexity of the migration strategies and systems of individual waterbird species (Boere and Stroud 2006). Such attempts to simplify the main migration routes may lose information, for example, an important component of east-west migration in Eurasia is excluded in the above flyways (Boere and Stroud 2006; Scott and Rose 1996). These limitations are considered when interpreting the results. On the other hand, strong relationships between the spread of avian influenza and the major bird migratory routes would suggest a role.

### **Data**

The time-location series of H5N1 outbreaks were extracted from official reports provided by the World Organization for Animal Health (OIE; <http://www.oie.int>). Each report contained the following attributes: province, district, sub-district, type of epidemiological unit, location, latitude, longitude, start time, end time, affected species, as well as the number of susceptible cases, deaths, destroyed, and slaughtered animals. However, from December 2003 to December 2005, most southeast Asian reports (mainly in Thailand and Vietnam) lacked latitude and longitude. In this study, the locations of these outbreaks were geocoded on the basis of the information provided in the reports, using the centre of the specific administrative region involved. The average area of the largest administrative division

(province) in Thailand and Vietnam is 6716 km<sup>2</sup>, equal to an area with a radius of approximately 46 km. Hence, some of the clusters detected (radius < 46 km) in Southeast Asia during this stage, may suffer from a bias in geocoding, as the original location of the outbreaks may have been located somewhere else (maximum 46 km error) than the centroid of the administration division.

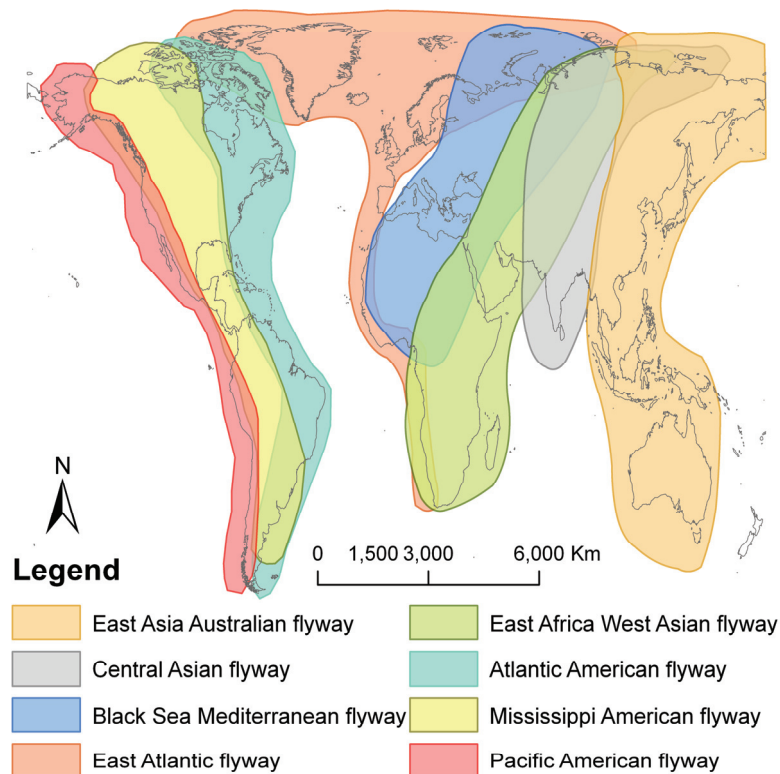


Fig. 1: Eight broad flyways of migratory waterbirds (source: International Wader Study Group, 2004)

Three years of H5N1 outbreak data were used, from December 2003 to December 2006. Both Australian and American continents were omitted from the map because of no report of HPAI H5N1 outbreaks to date (Pei et al. 2009; Yee et al. 2009). Figure 2a shows the locations of the outbreaks. The outbreaks were all concentrated in south and southeast Asia during 2003 and 2004. In 2005, outbreaks continued to be reported in Asia, but also started occurring in western Russia and Europe. In 2006, the disease became pandemic around the Black Sea region, the Mediterranean region, western Europe and eastern Africa. Figure 2b shows the locations of two different types of outbreaks (i.e., in wild birds and in poultry). Wild bird outbreaks were

concentrated in western Europe, with sporadic outbreaks being observed in southern Europe and central Asia. Poultry outbreaks, however, were concentrated in southeast Asia (together with wild bird outbreaks and a few mixed outbreaks), western Russia, the Black Sea region, Arabia and Africa. As figure 2 is displayed for visualization, some specific outbreaks could be invisible because of overlap with other outbreaks, such as the wild bird H5N1 outbreak occurrence in Egypt in February 2006.

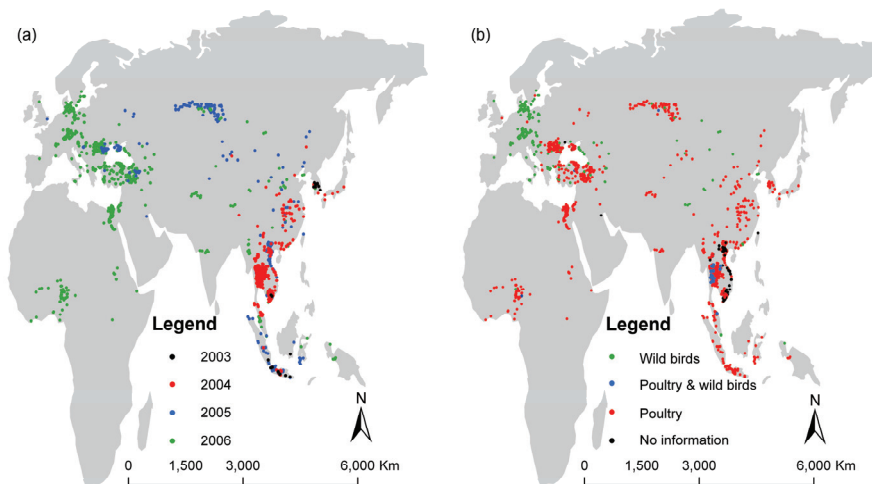


Fig. 2: Distribution of H5N1 outbreaks displayed by year (a) and by type of population (poultry or wild birds) (b) worldwide from December 2003 to December 2006.

### Epidemic curves

To display the outbreaks' magnitude and trend over time, epidemic curves were constructed by counting weekly numbers of outbreaks from 2003 to 2006 in Asia, Europe and Africa. Because the incubation period (i.e., the length of time between exposure and onset of symptoms) for avian influenza is about 21 days (OIE 2005), the time unit on the x-axis was defined as 7 days, equalling one-third of the incubation period (CDC 2008). The incubation period can be shorter, depending on the species and exposure conditions (WHO 2006).

### Space-time permutation model

The space-time permutation model in scan statistics has been used to test for spatio-temporal clusters, and to identify their approximate location and timing (Kulldorff et al. 2005). Space-time clusters occur when an excess number of H5N1 outbreaks are observed, within a user defined spatial and temporal range. Disease spread is strongly influenced by the spatial and temporal behaviour of the population at

risk. The H5N1 outbreaks are of concern to multiple populations (i.e., poultry, wild bird and human), which do not always have a clearly defined distribution, like wild birds. The advantage of the space-time permutation model is, that it only requires actual H5N1 outbreak data (Kulldorff et al. 2005). The space-time permutation model does require the population at risk to be constant, so short analysis periods were selected in order to respect this requirement (Kulldorff 2006). The disease patterns of the different epidemic phases, were therefore, analysed separately, using the space-time permutation model and SaTScan software (<http://www.satscan.org>). The populations involved (i.e., the number of bird flocks, farms and villages) could then be assumed to be constant for each epidemic phase.

The space-time permutation model analyzes clusters of H5N1 outbreaks both spatially and temporally, by testing whether outbreaks that are close in space are also close in time (Kulldorff 2006). In the permutation model, the scanning window forms a cylinder with the base representing space and the height representing time. The cylinder begins as a single point, gradually increasing both in diameter and in height, from zero to some maximum value, defined by the user. The expected number of outbreaks was calculated on the basis of the null hypothesis, assuming complete spatial randomness, which is synonymous with assuming that the observed outbreaks were approximately independent Poisson random variables with a constant mean (Diggle 2003). Based on this approximation, a likelihood ratio was measured to determine whether the cylinder contained a cluster or not. Of all the cylinders evaluated, the one with the maximum likelihood is considered to be the primary candidate for a true cluster (Kulldorff et al. 2005). Statistical significance of detected clusters was evaluated using a Monte Carlo simulation (Dwass 1957). The test  $p$ -value was estimated by comparing the rank of the maximum likelihood from the real data set, with the maximum likelihood from the random data sets, defined as  $p = \text{rank} / (1 + \text{number of simulations})$  (Dwass 1957; Kulldorff 2006). A detailed description and application of space-time permutation scan statistics can be found in other publications (Cooper et al. 2008; Kulldorff et al. 2005; Pearl et al. 2006).

### **Input parameters**

The maximum spatial scanning window should not exceed 50% of all outbreaks, because otherwise an extremely low outbreak rate outside the scanning window may not be detected by the algorithm (Kulldorff 2006). Two maximum spatial scanning windows were chosen for this analysis. One for detecting local clusters, with a 10 km radius, similar

to the surveillance area adopted in the European Union (Pittman and Laddomada 2008) and Asian (Buranathai et al. 2007) surveillance procedures (i.e., using a 10 km radius zone around infected premises or flocks). Secondly, a window was chosen with a radius equivalent to an area covering 10% of all outbreaks for detecting regional clusters (Norstrom et al. 2000). Even though poultry movement was supposedly strictly controlled to remain within the surveillance areas, the virus could have been transmitted outside the surveillance zones by wild birds. Given the size and shape of the study area, the maximum spatial window covering 10% of total outbreaks was selected to avoid scanning outside the study area (Sauders et al. 2003).

The temporal window was also set to be less than 50% of the study period (Kulldorff 2006). The maximum temporal scanning window was determined by a temporal risk window, which is defined as the period that an infected cluster remains infectious and the virus could be spread to other clusters. In this study, the maximum temporal scanning window (temporal risk window) was assumed to be 30 days, starting one day after a initially defined first lesion date and ending 21 days (the incubation period) after a slaughter and disinfection period (assumed to be 9 days).

Other options in the SaTScan software were selected as follows: (i) Retrospective Analysis was selected to allow both “alive” and “historic” clusters to be detected; (ii) Scan for High Rates was selected for cluster detection; (iii) Time Aggregation was not applied, as the maximum temporal scanning window was set at 30 days and the disease did not vary considerably over time; (iv) the number of Monte Carlo Simulations was set at 999; (v) the Most Likely Clusters and Non-Overlapping Secondary Clusters were selected. For mapping purposes, significant clusters ( $p \leq 0.05$ ) were classified into five risk levels, according to the relative risk obtained from the analysis result.

### **Deduce the role of wild birds in H5N1 spread**

To deduce the role of wild birds in H5N1 spread over long distance, bird migration patterns were compared with the disease trajectory. All regional and local clusters identified along flyways that follow the migration routes were considered potentially related to wild birds. To deduce the role of wild birds in H5N1 spread over short distance, we examined whether the local clusters were found at sites where wild birds are known to overwinter and at times when migratory birds were present.

## **RESULTS**

### **Epidemic curves**

Visual inspection of the epidemic curves (Fig. 3) indicated three epidemic phases at global level, i.e. phase I extending from week 50 (2003) to week 15 (2004), phase II from week 21 (2004) to week 16 (2005), and phase III from week 23 (2005) to week 51 (2006). Asia was involved in all disease outbreak phases, with peaks observed in week 4 (2004) during phase I, in week 41 (2004) during phase II and in week 46 (2005) during phase III. European and African outbreaks were only reported in phase III. The epidemic in Europe lasted from week 37 (2005) to week 26 (2006), with three peaks in week 2, 8 and 20 (2006). The epidemic in Africa overlapped with the European epidemic and lasted from week 2 to week 23 (2006), with a peak in week 12. All three epidemic phases revealed a high occurrence of H5N1 outbreaks in winter and early spring (i.e., from October to March).

### **Dynamics of space-time H5N1 clusters and correlated flyways**

A total of 143 space-time clusters ( $p \leq 0.05$ ) were identified by the space-time permutation scan statistic, with 20, 20 and 74 local clusters, and another 8, 12 and 9 regional clusters forming for the three phases respectively. The temporal dynamics of the identified space-time clusters in the three global epidemic phases were displayed in Figure 4, and overlaid with the five correlated flyways. (i.e., the East Asia Australian, the Central Asian, the Black Sea Mediterranean, the East Atlantic and the East Africa West Asian flyway).

#### *Global H5N1 epidemic phase I*

During the first stage of the disease outbreak, in December 2003 (Fig. 4Ia), significant clusters were identified in Indonesia, Korea and Vietnam, with low risk of infection in southeast Asia and high risk in Korea. In January 2004 (Fig. 4Ib), the virus circulated throughout Vietnam at both local and regional level, forming six low risk clusters. Meanwhile, another low risk regional cluster occurred in southeast China. In February 2004 (Fig. 4Ic), one local cluster was identified in Indonesia, followed by one regional cluster in Thailand. Then, medium and high risk clusters started appearing in Cambodia and Japan. In April 2004 (Fig. 4Id), another high risk local cluster was identified in Thailand.

During this phase, only one major migrating bird flyway (the East Asia Australian flyway) was affected.

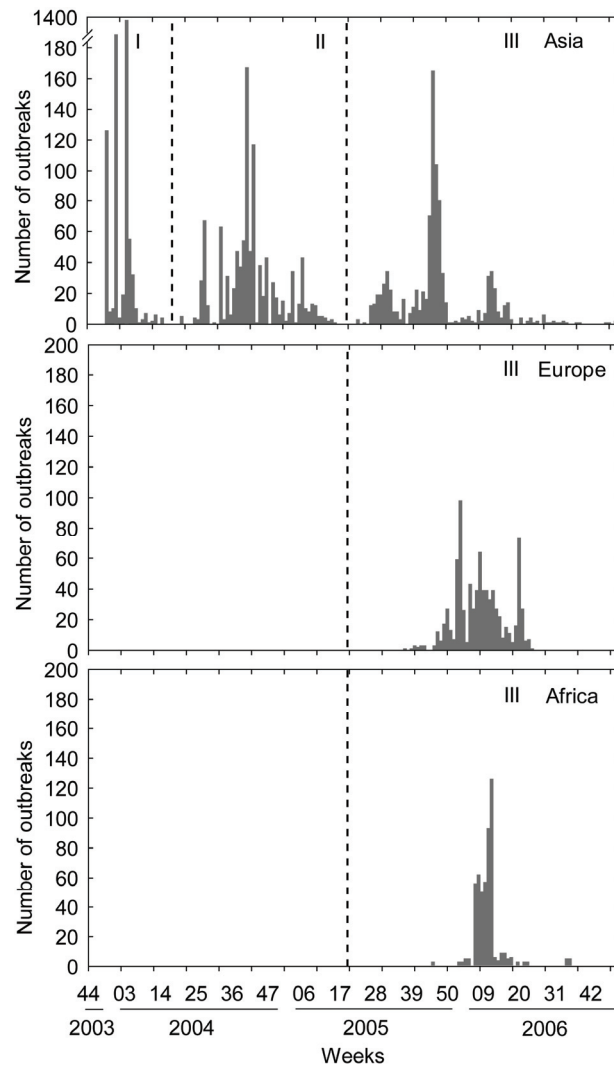


Fig. 3: Epidemic curves of reported H5N1 outbreaks from December 2003 to December 2006, displayed respectively for Asia, Europe and Africa. The three epidemic phases are separated by dashed lines.

#### *Global H5N1 epidemic phase II*

From March to May 2004 the H5N1 virus showed little activity, but then low and medium risk clusters started appearing in Thailand between June and December 2004 (Fig. 4IIa-g). Medium risk clusters were also identified in Malaysia in September (Fig. 4IIId) and in Indonesia in December of that same year (Fig. 4IIg). In January and February 2005 (Fig. 4IIh-i), 10 disease clusters were located in Vietnam, at both local and regional level, with low and medium risk factors. The disease reoccurred in Thailand in February (Fig. 4IIi),

and in Indonesia in February and March 2005 (Fig. 4IIi-j), forming one and six clusters, respectively.

As during the first phase, the H5N1 virus was limited to the East Asia Australian flyway.

*Global H5N1 epidemic phase III*

As in 2004, no H5N1 clusters were identified during April, May or June 2005. In July and August 2005 (Fig. 4IIia-b), eight clusters were located in western Russia and Kazakhstan. In southeast Asia, clusters were identified in Thailand between July and September (Fig. 4IIia-c), reappearing in south Indonesia during October (Fig. 4IIId). Eighteen clusters were identified in Vietnam between October and December 2005, forming either a low or a medium risk, at both local and regional level (Fig. 4IIIf). In October 2005, another medium risk cluster was located in western Russia (Fig. 4IIId). Thereafter, some sporadic outbreaks were reported in the Black Sea region, leading to four clusters (two of them high risk) in Ukraine and two medium risk clusters in Romania in December 2005 (Fig. 4IIIf). Disease clusters were also identified in the eastern Mediterranean in January 2006 (two of them low risk and five medium risk clusters) and in Nigeria, Africa, where three local clusters occurred in January and February 2006 (Fig. 4IIIf-h).

In February 2006 (Fig. 4IIIf), more disease clusters were located in the eastern Mediterranean region, forming one high risk local cluster in Italy and four low and medium risk clusters (at both local and regional level) in Egypt. Within the same month, two local clusters were identified in Slovenia and France, with a high and low risk factor, respectively (Fig. 4IIIf). By March 2006 there were outbreaks in northern Europe (Fig. 4IIIf), with two local clusters (medium to high risk) and one low risk regional cluster in Denmark. Another six clusters (low to medium risk) appeared in Egypt during March (Fig. 4IIIf). In April 2006 (Fig. 4IIIf), two medium risk local clusters were located in Sudan, as well as one medium risk local cluster in Pakistan. Meanwhile in Asia, one local cluster was identified in Hong Kong in February 2006 (Fig. 4IIIf), and three clusters (at both local and regional level) in Myanmar in March and April (Fig. 4IIIf-j). The disease appeared in Romania (five low to medium risk clusters) in May (Fig. 4IIIfk), and in neighbouring Hungary (one medium risk cluster) in June 2006 (Fig. 4IIIf). After a relatively long period with low virus activity (July to October 2006), one final high risk local cluster was identified in Korea in November 2006 (Fig. 4IIIfm).



During this third epidemic phase, the H5N1 virus circulated chronologically, via the East Asia Atlantic flyway to the Central Asian flyway, the Black Sea Mediterranean flyway, the East Atlantic flyway and finally the East Africa West Asian flyway.

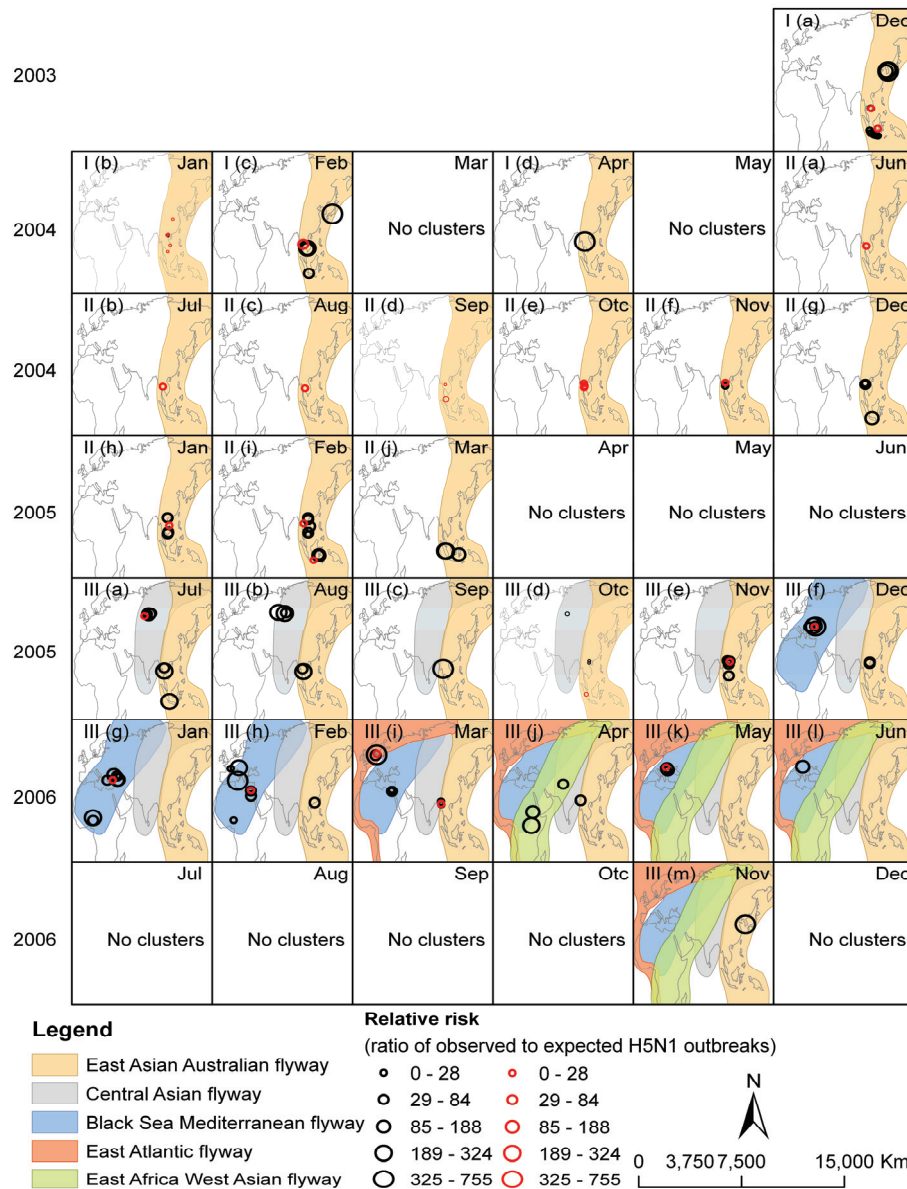


Fig. 4: Monthly dynamics of significant space-time clusters ( $p \leq 0.05$ ) in each epidemic phase. The size of the circle is determined by the value of the relative risk (ratio of observed to expected outbreaks) and overlaid with the correlated flyways. Black circles indicate local clusters (radius  $\leq 10$  km) and red circles indicate regional clusters (radius  $> 10$  km).

### **Space-time H5N1 clusters along and across flyways**

Based on the five identified flyways (Fig. 4), the sum of clusters occurring per month within each flyway was plotted. A chronology emerges, indicating the spread of disease clusters along and across flyways, as shown in Figure 5.

Disease clusters appeared throughout the East Asia Australian flyway during the whole study period, with peaks in December 2003, February 2005 and November 2005. In the Central Asian flyway, disease clusters started emerging in July 2005 and waned in October 2005. In the Black Sea Mediterranean flyway, clusters lasted from December 2005 to March 2006. Finally, clusters appeared in the East Atlantic and East Africa West Asian flyways in March and April 2006, respectively.

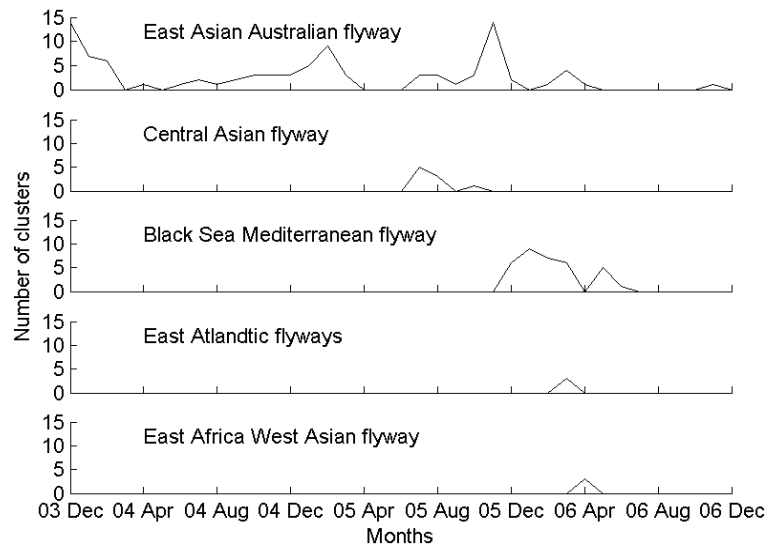


Fig. 5: Space-time H5N1 clusters along and across the flyways

### **Dynamics of space-time H5N1 clusters and seasonal bird migration**

Six H5N1 cluster patterns were found to be associated with the seasonal migration of waterbirds (Fig. 6). In February 2004, a northbound spread of disease clusters was identified from Indonesia, via Thailand and Cambodia, to Japan. This disease pattern coincided with the wild bird spring migration northwards via the East Asia Australian flyway (Fig. 6a). During the autumn migration (August to November in 2004), the birds migrated southwards via the East Asia Australian flyway, which coincided with the disease spreading into southeast Asia (Fig. 6b), as space-time clusters shifted from Thailand (August), via Malaysia (September), to south Indonesia (December).

In the autumn of 2005 (Fig. 6c), a similar pattern was detected, in Thailand (September) and Indonesia (October), the disease clusters again following a north-south direction, overlapping with the birds' migration route via the East Asia Australian flyway.

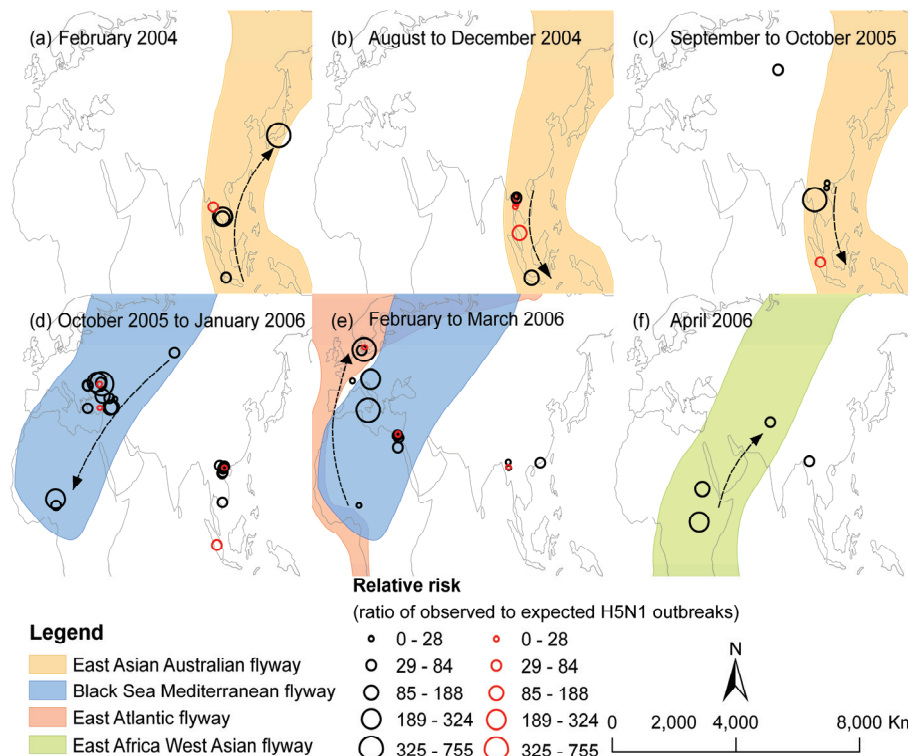


Fig. 6: Dynamics of significant space-time H5N1 clusters overlaid with correlated flyways during migratory seasons. The size of the circle is determined by the value of the relative risk (ratio of observed to expected outbreaks). Black circles indicate local clusters (radius  $\leq 10$  km) and red circles indicate regional clusters (radius  $> 10$  km). Black arrows show the movement direction of disease clusters

The disease clusters detected in western Russia (October 2005), the Black Sea region (December 2005) and Nigeria (January 2006), occurred in a northeast to southwest direction and coincided with peak migratory movements via the Black Sea Mediterranean flyway during autumn (Fig. 6d). During spring 2006 (Fig. 6e), space-time clusters were detected from Italy (February), Slovenia and neighbouring countries (February), to Denmark (March), as well as from Nigeria (February) to Denmark (March), following south-north directions. The spatio-temporal dynamics of these disease clusters correspond with the timing and direction of bird migration along both the Black Sea Mediterranean flyway and the East Atlantic flyway. In

April 2006, disease clusters were first observed in Sudan, and then in Pakistan (Fig. 6f), which coincides with the direction and timing of birds migrating over the East Africa West Asian flyway.

## **DISCUSSION**

The H5N1 virus outbreaks can be divided into three global epidemic phases. By displaying the monthly dynamics of the space-time clusters of the disease and the matching migratory flyways of wild birds, a quantitative description is presented of H5N1 transmission at a global scale, and the possible relationship between migratory birds and the spread of the disease is revealed. The H5N1 outbreaks were mainly concentrated in winter and early spring (from October to March), suggesting a seasonally higher risk of infection.

### **The possibility of long-distance virus spread by migratory birds**

A chronology of H5N1 infections developed from the East Asia Atlantic flyway to the Central Asian flyway, the Black Sea Mediterranean flyway, the East Atlantic flyway and finally the East Africa West Asian flyway, suggesting that the H5N1 virus may be transmitted by wild birds via the different flyways. Possible reasons are that migratory birds may become infected when sharing breeding grounds in Siberia, as well as overlapping flyways creating opportunities for birds to come into contact with each other.

The correlation between disease cluster patterns and seasonal wild bird migration suggests that wild birds may spread the virus over long distances. Some of our results are consistent with a previous report (Gilbert et al. 2006b), indicating that *Anatidae* may spread the virus from Russia and Kazakhstan to the Black Sea region during the autumn migration. We found that the virus also appeared in Nigeria after the Black Sea region had been infected. The pandemic in Nigeria occurred in January 2006, after the migratory season, but the virus may have already been present in the population of birds overwintering in Nigeria, with outbreaks not occurring until some time after the arrival of the migratory birds. A similar delay was observed in Romania, where the virus was first detected in October 2005, but the first high risk cluster was not detected till December 2005. Ducatez et al. (2006) found that the three H5N1 lineages found at two farms in Nigeria were independently introduced through routes coinciding with the migratory bird flyway. Our findings also support a previous study which suggest that the African sublineages emerged outside of Africa but followed the east African west Asian and Black Sea Mediterranean flyways, as at least two of the sublineages

isolated in western Africa also circulated in Germany during 2006 (Ducatez et al. 2007). However, human mobility can not be excluded completely, as Nigeria imports large numbers of poultry, without rigorous bio-security safeguards, from different countries in the world (Ducatez et al. 2006). The long-distance disease spread does coincide with the seasonal pattern of bird migration over the Black Sea Mediterranean flyway.

All this evidence supports the hypothesis, that the spread of the H5N1 virus is associated with migratory movements of birds via well-known flyways. Though our study attempts to seek the role of wild birds in spreading H5N1 virus by correlative studies, this of course does not constitute "proof". In other words, human mobility cannot be ruled out as a vector in the observed patterns of virus transmission. Besides, some spatio-temporal patterns of the disease clusters, such as in Egypt (February 2006) and Sudan (March 2006), do not follow the dominant flyway direction from South to North trend expected for that time of the year, which may be caused by poultry transportation with H5N1 virus.

Latitudinal and unusual bird movements may facilitate the H5N1 virus spread as well. Gilbert et al. (2006b) suggested that the western European pandemic was caused by unusual waterfowl movements, due to unseasonably cold weather in the Black Sea area, where the virus was already established. However, detailed and localized bird movement patterns are required to further test this hypothesis, which is beyond the scope of the current study.

### **The possibility of short-distance virus spread by migratory birds**

Migratory birds could be involved in short-distance virus spreading, especially where disease clusters were detected in overwintering areas. A large number of disease clusters was detected during winter and spring in important staging and overwintering regions for migratory waterbirds (<http://ramsar.wetlands.org/Default.aspx>), such as Xuan Thuy Natural Wetland Reserve in Vietnam, Chany Lakes in Russia, Kizilirmak Delta in Turkey, Aquatic-cliff complex of Karadag in Ukraine, South Funen Archipelago in Denmark, Vendicari in Italy and Maldunba Lake in Nigeria. By sharing staging and overwintering sites, migratory waterbirds come into contact with other flocks of birds, as well as with free-ranging poultry (Gilbert et al. 2006a; Onozuka and Hagihara 2008), facilitating the spread of disease. In addition, the outbreaks in western Europe and central Asia (Fig. 5), showed few or no infections in poultry, suggesting that wild birds have to play a role in the virus transmission.

Infected migratory birds may facilitate the virus spread by contaminating overwintering sites, as the virus may survive without a host for extended periods, especially at low temperatures. Stallknecht et al. (1990) determined that water with an initial concentration of  $1 \times 10^6$  TCID<sub>50</sub> (50% Tissue Culture Infective Dose) could remain infectious for up to 207 days at 17°C or up to 102 days at 28°C (Stallknecht et al. 1990). As the H5N1 virus remains virulent in bird faeces for at least 35 days at 4°C and 6 days at 37°C (OIE 2008), the frequent reoccurrences of disease clusters in Thailand (June to December 2004, July to September 2005), west Russia (July, August and October 2005), Vietnam (October to December 2005) and the Black Sea region (May and June 2006), suggest that local outbreaks may be caused by viruses surviving in contaminated areas.

Both local and regional clusters were assumed to be associated with epidemic risks in this study. However, we cannot exclude the possibility of some clusters, especially local ones, having no relationship with bird migration. Distinguishing epidemic risk clusters from endemic risk clusters requires extra data, such as distribution pattern of genetic lineage. This study explored the maximally possible role of wild bird in spreading H5N1 virus at either regional or local scales. We recommend confirming these findings by using genetic lineage distribution patterns to exclude endemic risks in future studies.

In conclusion, the spread of the H5N1 virus, as quantified by the space-time clusters, was associated with the timing, location and direction of continental bird migration, thereby suggesting that wild birds spread H5N1 over long distances. Disease clusters were also detected at sites that are known overwintering areas, and at times when these areas were frequented by migratory birds, thereby suggesting that wild birds are involved in short distance H5N1 spread, as well.

## **ACKNOWLEDGEMENTS**

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## **Chapter 3**

### **Environmental factors influencing the spread of the highly pathogenic avian influenza H5N1 virus in wild birds in Europe**

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This chapter is based on: Yali Si, Tiejun Wang, Andrew K. Skidmore, Willem F. de Boer, Lin Li, and Herbert H.T. Prins, 2010. Environmental factors influencing the spread of the highly pathogenic avian influenza H5N1 virus in wild birds in Europe, *Ecology and Society*, 15(3): 26.

## **ABSTRACT**

A large number of occurrences of the highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds were reported in Europe. The relationship between the occurrence pattern and environmental characteristics has, however, not yet been explored. This research uses logistic regression to quantify the relationships between anthropogenic and physical environmental factors, and HPAI H5N1 occurrences in wild birds in Europe. Our results indicate that HPAI H5N1 occurrences in wild birds in Europe are highly correlated with increased normalized difference vegetation index (NDVI) in December, intermediate NDVI in March, lower elevations, increased minimum temperatures in January, and reduced precipitation in January. A predictive risk map of HPAI H5N1 occurrences in wild birds in Europe is generated on the basis of five key environmental factors. Independent validation of the risk map showed the predictive model to be of high accuracy (79%). The analysis suggests that HPAI H5N1 occurrences in wild birds are strongly influenced by the availability of food resources and facilitated by increased temperatures and reduced precipitation. We therefore deduced that HPAI H5N1 occurrences in wild birds in Europe are probably caused by contact with other wild birds and not by contact with domestic poultry. These findings are of importance in the global surveillance of HPAI H5N1 occurrences in wild birds.

## **INTRODUCTION**

The global spread of the highly pathogenic avian influenza (HPAI) H5N1 in poultry, wild birds and humans, poses a pronounced panzootic threat and a serious public health risk. During the second half of 2005, the HPAI H5N1 virus was detected outside Asia, appeared in Russia, and then arrived in Romania in October (Gilbert et al. 2006a). In 2006, the disease became panzootic around the Black Sea region, the Mediterranean region, Western Europe and Africa (Si et al. 2009). Kilpatrick et al. (Kilpatrick et al. 2006) suggested that movements of wild birds and trade in domestic poultry and wild birds form potential agents for global dispersion of the HPAI H5N1 virus. An efficient surveillance and disease control system requires a greater understanding of the mechanism responsible for the spread of the HPAI H5N1 virus. Two different hypotheses are currently under investigation, namely that human transport of infected domestic poultry is the underlying mechanism responsible for the spatial pattern in the disease occurrence, or that wild birds (mainly waterfowl) are spreading the disease. These two hypotheses are not mutually exclusive as the two mechanisms interact.

Because the HPAI H5N1 virus is associated with both migratory and domestic bird populations, it is likely that environmental factors play a significant role in the spread of the disease. A number of efforts have been made to investigate the influence of environmental factors on HPAI H5N1 occurrence. The occurrence of the HPAI H5N1 virus in Nigeria and neighbouring countries in West Africa has been linked to differences in plant phenology and land-surface reflectance (Williams et al. 2008). In Southeast Asia HPAI H5N1 outbreaks correlated with free-range duck farming and rice-paddy cultivation (Gilbert et al. 2006b; Gilbert et al. 2008). In China, annual precipitation, the minimum distance to national highways, and the interaction between minimum distance to the nearest lake and wetland were correlated with the risk of HPAI H5N1 occurrence (Fang et al. 2008). Landuse patterns, the occurrence of seasonal wetlands, backyard poultry and animal husbandry, as well as the density of human population were identified to be associated with the presence of the HPAI H5N1 virus in the Indian subcontinent (Adhikari et al. 2009). In Bangladesh, HPAI H5N1 outbreaks in backyard chickens were associated with offering the chickens slaughter remnants of purchased chickens, the vicinity to water bodies, and contact with pigeons (Biswas et al. 2009). In the Middle East and northeast Africa, HPAI H5N1 cases were shown to occur in areas with large seasonal variation in NDVI values (Williams and Peterson 2009). Inconsistencies in predictions based on HPAI H5N1 occurrences were also reported in different sub-

regions, suggesting certain environmental factors may be of greater importance in some areas than others (Williams and Peterson 2009). Risk factors affecting one specific area may not affect the distribution of the virus elsewhere. The correlation between environmental factors and the occurrence of the HPAI H5N1 virus in Europe has yet to be investigated. Studies referred to above were mainly based on outbreaks in poultry, and revealed that both anthropogenic and physical environmental factors have some bearing on the disease outbreaks, suggesting that both domestic poultry and wild birds facilitate the spread of the HPAI H5N1 virus in poultry. The influence of environmental factors affecting the occurrence of the HPAI H5N1 virus in wild birds, however, is not clearly understood.

Wild birds are capable of excreting abundant viruses (e.g. in their faeces) before and after the onset of clinical signs (Keawcharoen et al. 2008), or even asymptotically (Chen et al. 2006). They are suspected of spreading the virus over either long or short distances (Si et al. 2009). Kilpatrick et al. (2006) even suggested that migratory wild birds formed the prime spreading agent of the HPAI H5N1 virus in Europe. Traditional surveillance and control measures may successfully constrain infection by the HPAI H5N1 virus in domestic poultry but not in wild birds. Given the mobility of wild birds and the challenge of tracing different populations, it is of great importance to identify which environmental factors correlate with the occurrence of the HPAI H5N1 virus in wild birds. This knowledge may assist in setting priorities for mitigating action and developing necessary precautionary measures against future disease outbreaks in wild birds. In this way, the risk of domestic poultry infections through contact with wild birds could be reduced as well.

In contrast to Asia and Africa, Europe has reported large numbers of HPAI H5N1 occurrences in wild birds. Distinct disease patterns were observed between wild birds and domestic poultry outbreaks (Fig. 1). In Europe wild bird infections were mainly found in the northwest, while poultry outbreaks were largely observed in the southeast. Most countries reporting wild bird infections, found few or no poultry outbreaks nearby. The highest density of HPAI H5N1 occurrences in wild birds was reported in Germany, with approximately 40% of all occurrences in wild birds in Europe from 2005 to 2008. Observing the temporal distribution of the monthly occurrences of the HPAI H5N1 virus in Germany (Fig. 1), the HPAI H5N1 virus was first reported in wild birds and also mainly circulated among wild birds (with few poultry events reported in between). This implies that wild birds may be mainly infected through direct or indirect contact with other wild birds, rather than through contact with domestic poultry. Wild birds



therefore may play a prominent role in the spread of the HPAI H5N1 virus amongst wild birds in Europe.

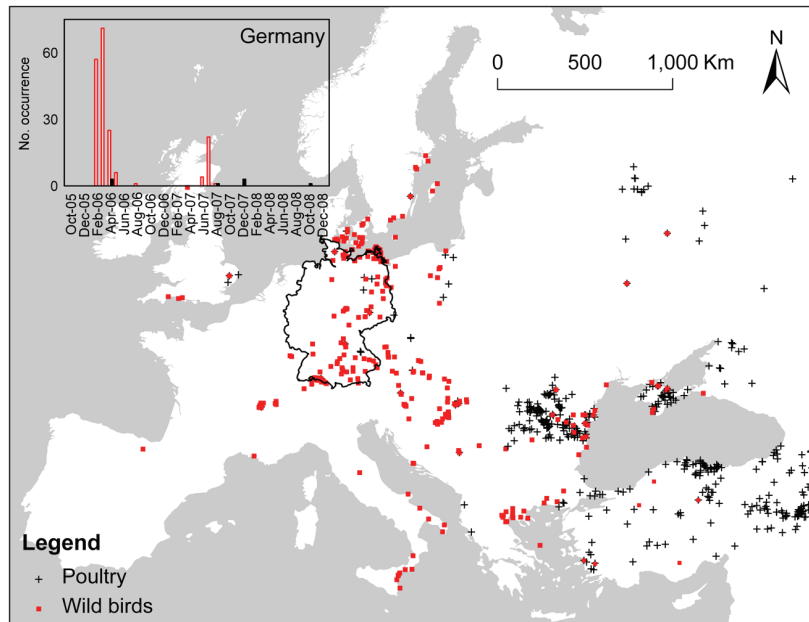


Fig. 1: Spatial distribution of the HPAI H5N1 virus reported in wild birds (red squares) and domestic poultry (black crosses) in Europe from 2005 to 2008. The black boundary indicates Germany and the bar plot illustrates the monthly occurrences of the HPAI H5N1 virus in wild birds (red bar) and domestic poultry (black bar) in Germany

If wild birds act as the main spreading agent in the European panzootic in wild birds, then the disease pattern is expected to be strongly influenced by the distribution and movement of wild birds. The distribution and movement of wild birds depends on the availability of natural resources, which can be strongly correlated with physical environmental factors, such as climate, topography, feeding sites, and wetlands (Owen and Black 1990). These factors could therefore be used as predictor variables in the analysis of the risk of HPAI H5N1 occurrence in wild birds. In contrast, trade in domestic poultry mainly relies on anthropogenic factors. These factors may therefore not affect the HPAI H5N1 occurrence pattern in wild birds in Europe. This study aims to examine the key environmental factors associated with the occurrence of the HPAI H5N1 virus in wild birds in Europe, and to map the risk of disease occurrences in wild birds in Europe on the basis of identified explanatory variables.

## METHODS AND MATERIALS

### Data

Data on the occurrence of the HPAI H5N1 virus in wild birds in Europe consisted of 467 confirmed events reported from 2005 to 2008, provided by EMPRES-I, a global animal health information system of FAO's Emergency Prevention Programme for Transboundary Animal Diseases (<http://empres-i.fao.org/empres-i/home>). Environmental data were categorized into anthropogenic and physical environmental subsets, corresponding to the two disease spreading agents (i.e. poultry and wild birds). Table 1 shows the environmental data sets used for this study.

Table 1: Data sets used to generate environmental variables for the analysis of HPAI H5N1 occurrences in wild birds in Europe, with data format and source

Category	Description of data sets	Format	Data producer
Anthropogenic environmental data	Cities	Polygon	ESRI
	Metropolises	Polygon	ESRI
	Roads	Polyline	ESRI
	Highways	Polyline	ESRI
	Railways	Polyline	ESRI
	Human population density in 2005	Raster	CIESIN, FAO, CIAT
	Poultry density in 2005	Raster	FAO
Physical environmental data	Digital elevation model	Raster	WORLDCLIM
	Global lakes and wetlands database	Raster	WWF, ESRI, CESR
	Ramsar sites	Point	Wetlands International
	Mean annual potential evapotranspiration	Raster	CGIAR-CSI
	Mean annual aridity index	Raster	CGIAR-CSI
	Mean monthly precipitation	Raster	WORLDCLIM
	Mean monthly maximum temperature	Raster	WORLDCLIM
	Mean monthly minimum temperature	Raster	WORLDCLIM
	Monthly NDVI	Raster	NASA

The anthropogenic environmental data sets comprise roads, highways, railways, locations of cities and metropolises, as well as human population and poultry density. The first five factors were selected because distance to transportation routes and cities was found to be significantly associated with HPAI H5N1 outbreaks in China (Fang et al. 2008). Human population density was included because this may indicate higher trade activity. Human population density was significantly associated with the HPAI H5N1 outbreaks in Southeast Asia and India (Adhikari et al. 2009; Gilbert et al. 2006b; Gilbert et al. 2008). Poultry density was included as larger flocks of birds tend to be at higher risk of disease outbreaks (Gilbert et al. 2006b).

The physical environmental data sets comprise a digital elevation model (DEM) (a digital representation of ground surface topography or terrain), a dataset with the location of lakes and wetlands, Ramsar sites, and information about potential evapotranspiration, aridity, monthly precipitation, monthly minimum and maximum temperatures and monthly normalized difference vegetation index (NDVI), an index that is closely correlated with photosynthetic mass calculated from the reflectance in the visible and near-infrared domains (Tucker 1979). In this study, time series NDVI was derived from Moderate Resolution Imaging Spectroradiometer (MODIS) satellite imagery. The topographic data was included because it was found to be a significant risk predictor in Southeast Asia (Gilbert et al. 2008). Because suitable habitats are concentrated in the lowlands, elevation influences the availability of food resources and shelter for waterfowl. The distribution of lakes, wetlands, and Ramsar sites was included because such areas are important for migratory and local waterfowl and provide potential, suitable habitats. Water bodies and wetlands were also found to be significantly associated with HPAI H5N1 outbreaks in China, India and Bangladesh (Adhikari et al. 2009; Biswas et al. 2009; Fang et al. 2008). Potential evapotranspiration, aridity and precipitation were included because lower levels of moisture and precipitation may affect the availability of food resources, and thereby influence the distribution of wild birds. Precipitation was found to be an important risk factor affecting the distribution of the HPAI H5N1 virus in China (Fang et al. 2008). Monthly minimum and maximum temperatures were selected because temperature pattern changes may contribute to an increase in disease occurrences and the spread of the HPAI H5N1 virus among live birds (Liu et al. 2007). Killer et al. (2009) also found that cold weather may trigger winter movements of migratory birds and thereby contribute to the spread of bird-transmitted diseases outside the actual migration period. The occurrence of the HPAI H5N1 virus in Africa and the Middle East has been linked to differences in plant phenology using time series NDVI data (Williams et al. 2008; Williams and Peterson 2009), so we also included time series NDVI data, as they correlate strongly with the availability of food resources, and thereby with waterfowl distribution and movement. Two additional composite variables (i.e. poultry density associated with the distance to the nearest lake or wetland and poultry density associated with the distance to the nearest Ramsar site) were created in order to investigate potential linkages between domestic poultry and waterfowl. Poultry distribution associated with the location of wetlands and Ramsar sites could be an entry gate for virus exchange between these two disease spreading agents.

Table 2: Summary of environmental variables used in the analysis of HPAI H5N1 occurrences in wild birds in Europe

Category	Description of variables	Abbreviation	Unit
Anthropogenic environmental variables	Distance to the nearest city	City	km
	Distance to the nearest metropolis	Metro	km
	Distance to the nearest road	Road	km
	Distance to the nearest highway	Highway	km
	Distance to the nearest railway	Railway	km
	Human population density in 2005	Hpopden	p/km <sup>2</sup>
Physical environmental variables	Poultry density in 2005	Poultryden	p/km <sup>2</sup>
	Distance to the nearest lake and wetland	GLWD	km
	Distance to the nearest Ramsar site	Ramsar	km
	Digital elevation model	DEM	m
	Slope aspect	Aspect	°
	Slope gradient	Slope	°
	Mean annual potential evapotranspiration	Mapet	mm/km <sup>2</sup> /year
	Mean annual aridity index	Maaridity	No unit
	Mean monthly precipitation	PrecJan to Dec	mm
	Mean monthly minimum temperature	TminJan to Dec	°C*10
	Mean monthly maximum temperature	TmaxJan to Dec	°C*10
Interaction variables	Monthly NDVI	NDVIJan to Dec	No unit
	Poultry density * distance to the lake and wetland	PoultrydenW	No unit
	Poultry density * distance to the Ramsar site	PoultrydenR	No unit

### Data pre-processing

Layers detailing the distance to the nearest city, metropolis, road, highway, railway, lake or wetland, and Ramsar site were generated in a geographic information system (GIS) with a spatial resolution of 1 km. Layers depicting slope aspect (defined as the compass direction of the maximum rate of change) and slope gradient (defined as the maximum rate of change in altitude) were also generated from the DEM. To diminish noise caused mainly by remnants of clouds, a clean and smooth 12-month NDVI time series was reconstructed on the basis of four 12-month NDVI time series data sets from 2005 to 2008 by employing an adaptive Savitzky-Golay smoothing filter, using the TIMESAT package (Jonsson and Eklundh 2004). Two composite variables were constructed by multiplying poultry density with the distance to the nearest lake or wetland and to the nearest Ramsar site. Table 2 summarizes the anthropogenic, physical, and interaction variables used in this study.

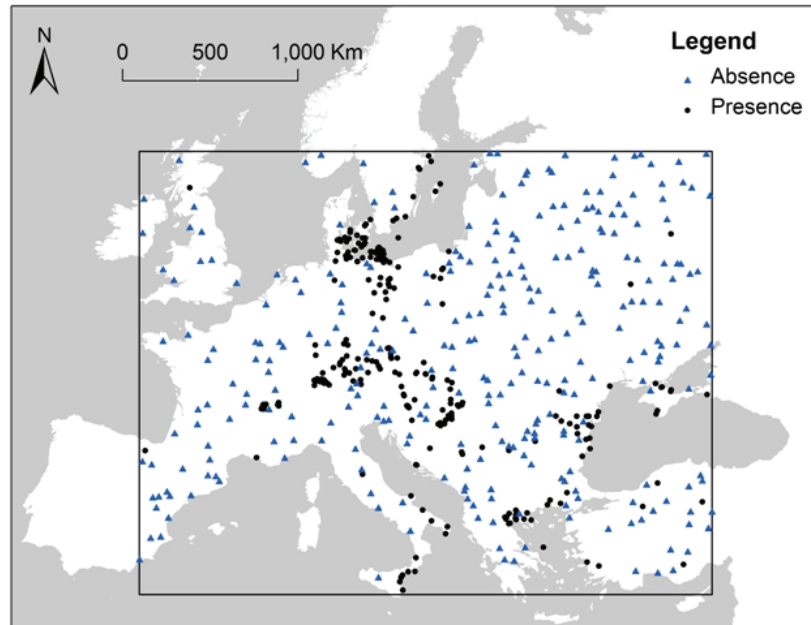


Fig. 2: Distribution of presence (black squares) and absence (blue triangles) of HPAI H5N1 occurrences in wild birds from 2005 to 2006 in Europe (one training subset). The box indicates the maximum geographic range of HPAI H5N1 occurrences in wild birds from 2005 to 2008.

Logistic regressions were carried out to examine the relationship between explanatory factors and the occurrence of the HPAI H5N1 virus in wild birds. Duplicate occurrences of HPAI H5N1 cases in wild birds from the same locality were discarded, resulting in 320 unique geographic coordinates. A total of 296 locations in 2005 and 2006 were assigned as HPAI H5N1 presence data for model training, and an additional 24 locations in 2007 and 2008 were utilized for validation. As Europe adopted a 10 km surveillance zone policy (Pittman and Laddomada 2008), 10 km radius buffers were generated around the HPAI H5N1 virus presence locations. Absences drawn from too small an area can produce spurious models while absences drawn from too large an area can lead to artificially inflated test statistics as well as potentially less informative response variables (VanDerWal et al. 2009). The areas within the maximum geographic range of wild bird infections in Europe from 2005 to 2008, except for the HPAI H5N1 presence buffers, were thus defined as non-panzootic areas. In order to select absence data in a way that is fully representative of disease absence, 9000 absence locations were generated randomly in these non-panzootic areas. The minimum distance between each pair of absence locations was set at 20 km, to avoid overlapping of the surveillance buffers.

The minimum distances were then extracted from the distance layers (i.e. distance to the nearest city, metropolis, road, highway, railway, lake or wetland, and Ramsar site,) for all presence and absence locations. Furthermore, using zonal statistics, we calculated mean values of a 10 km buffer zone of all presence and absence locations for the following variables: human population density, poultry density, elevation, slope aspect, slope gradient, potential evapotranspiration, aridity index, precipitation, minimum temperature, maximum temperature, and NDVI.

Mcpherson et al. (2004) demonstrated that optimal models developed from logistic regressions had intermediate prevalences (50%) and large sample sizes (300-500). We therefore applied a bootstrapping procedure in which, together with the 296 presence locations, 296 absence locations were randomly selected with replacement from the 9000 absence locations. This process was repeated 1000 times, creating 1000 subsets for model training. Fig. 2 shows the presence and absence of HPAI H5N1 occurrences in wild birds from 2005 to 2006 in Europe (an example of one training subset).

### **Statistical analysis**

We examined the linear and quadratic effects of each variable on the occurrence of disease separately using logistic regression models. This process was repeated 1000 times using different training subsets, reporting mean values of odds ratios (OR), 95% confidence intervals (CIs) of OR, p value, pseudo  $R^2$ , Akaike's information criterion (AIC), the area under the Receiver Operating Characteristic curve (AUC), and Kappa for each variable. Odds ratios were used to evaluate the impact of predictor variables. The further below 1.0 the odds ratio is, the greater the effect of that variable in reducing the odds of disease presence, while the higher the odds ratio is above 1.0, the greater the effect of that variable in increasing the odds of disease presence. An odds ratio of 1 corresponds to an explanatory variable which does not affect the dependent variable. Variables yielding non-significant changes in log-likelihood were excluded from further analysis.

The Akaike's Information Criterion (AIC) (Akaike 1974) is a measure of the goodness of fit of an estimated statistical model. The chosen model should be the one that minimizes the Kullback-Leibler distance between the model and the truth. AUC measures the ability of a model to discriminate between sites where a species is present, versus those where it is absent (Hanley and McNeil 1982). It provides a single measure of overall accuracy of model fit that is not dependent upon a particular threshold. It ranges from 0 to 1, with

values larger than 0.5 indicating a performance better than random. Kappa (Cohen 1960), a chance-corrected measure of agreement, provides an index that considers both omission and commission errors. We calculated a maximum Kappa for each model by calculating Kappa at all possible thresholds and identifying both the maximum Kappa and the threshold at which this occurred.

Autocorrelation and multi-collinearity were assessed by examining Moran's I (Moran 1950) and the variance inflation factor (VIF) (Stine 1995), respectively. A negative value of Moran's I indicates negative autocorrelation and a positive value reveals positive autocorrelation; no autocorrelation is present when the value of Moran's I equals zero. The VIFs were calculated for each predictor as the inverse of the coefficient of non-determination ( $1/(1-R^2)$ ) for a regression of that predictor on all others. VIF is a positive value representing the overall correlation of each predictor with all others in a model. Collinearity is present when the VIF for at least one independent variable is large. All reported Moran's I and VIF values are mean values of 1000 repeated calculations using the 1000 training subsets.

For all significant variables, a pre-selection was carried out to remove the ones with relatively high collinearity and/or relatively high autocorrelation. Highly correlated variables were removed by sequentially dropping the variable with the lowest impact, recalculating the VIFs and repeating this process until all VIFs were smaller than 10. Generally,  $VIF > 10$  indicates "severe" collinearity (Kutner et al. 2004). When two variables had comparable impacts, the one with the lower p value was preferentially selected. Variables with a Moran's I larger than 0.5 or smaller than -0.5 were not considered for selection. A multiple stepwise logistic regression was carried out by using the pre-selected variables. For each of the pre-selected variables, a quadratic term was included if the quadratic effect was stronger than the linear effect. This stepwise process was repeated 1000 times using the different training subsets. The frequency of each variable being selected was calculated on the basis of applying 1000 best stepwise logistic regression models, ranked by AIC. The mean p value and its 95% CIs were calculated for each selected variable. Variables yielding non-significant effects in the stepwise logistic regression models were discarded. The remaining variables were identified as key risk factors. A multiple logistic regression was carried out using these key risk factors. This process was repeated 1000 times using the different training subsets. The mean values of coefficients, OR, 95% CIs of OR, p value, pseudo  $R^2$ , AIC, AUC and Kappa were used as indicators of model performance. A ROC plot was generated by plotting all sensitivity values (true

positive fraction) on the y axis against their equivalent (1-specificity) values (false positive fraction) for all available thresholds on the x axis. One thousand ROC curves were calculated using the 1000 training subsets.

On the basis of the model derived from the multiple logistic regression models, a risk map of HPAI H5N1 occurrence in wild birds was generated with a spatial resolution of 1 km. The risk map was then classified into four levels (i.e. very high, high, medium and low risk) for validation by using independent occurrences (i.e. HPAI H5N1 occurrences in wild birds from 2007 to 2008). A 10 km buffer zone was generated around each validating occurrence and the mean risk value in each buffer zone was calculated. The percentage of buffer zones occurring at high and very high risk areas was then calculated to evaluate the accuracy of the predictive map.

All statistical analyses were conducted using R statistical software ([www.r-project.org](http://www.r-project.org)) with additional packages for some of the specific analyses.

## **RESULTS**

The linear and quadratic logistic regression analyses demonstrated that physical environmental variables were substantially correlated with the occurrence of HPAI H5N1 in wild birds (Table 3), but no anthropogenic variables selected in this study were significantly associated with HPAI H5N1 occurrences. The composite variables, indicating interactions between poultry and waterfowl, yielded non-significant effects on HPAI H5N1 occurrence in wild birds. Positive linear associations were found between wild bird HPAI H5N1 occurrences and minimum temperature, cold season maximum temperature, and cold season NDVI. Negative linear associations were detected between wild bird HPAI H5N1 occurrence and elevation, slope aspect, slope gradient, and cold season precipitation. Among the linear associated physical environmental variables for the HPAI H5N1 occurrences in wild birds, the NDVI in December revealed the strongest effect, with an odds ratio of 30.4 (95% CI 6.6 -141.74). Significant quadratic effects were observed between disease occurrences and minimum temperature, cold season maximum temperature, cold season NDVI, and warm season NDVI. The strongest quadratic effect was observed in the NDVI in March.



Table 3: Variables significantly associated with HPAI H5N1 occurrences in wild birds reported by the linear and quadratic logistic regression analyses using 1000 bootstrapping training datasets. All values in the table are mean values obtained from 1000 runs of the model. Italics indicate quadratic effects. Variables marked in bold were included in the final model.

Variable	OR <sup>†</sup>	Linear and quadratic logistic regressions					Moran's I		VIF (all)	Stepwise selections		
		95% CI OR <sup>†</sup>	P value	Pseudo R <sup>2</sup>	AIC	AUC $\pm$ SD	Kappa $\pm$ SD	I		VIF (selected)	Times	P value
<b>DEM</b>	<b>0.989</b>	<b>0.984</b>	<b>0.995</b>	<b>&lt;0.001</b>	<b>0.09</b>	<b>0.65</b>	<b>0.02</b>	<b>0.3</b>	<b>0.04</b>	<b>0.08</b>	<b>11.65</b>	<b>0.08</b>
Slope	0.8	0.728	0.88	<0.001	0.06	0.61	0.02	0.17	0.04	0.13	5.29	0.13
Aspect	0.998	0.997	0.999	0.007	0.03	0.58	0.02	0.14	0.04	0.12	1.23	0.12
<b>MpreJan</b>	<b>0.984</b>	<b>0.976</b>	<b>0.991</b>	<b>&lt;0.001</b>	<b>0.05</b>	<b>0.55</b>	<b>0.02</b>	<b>0.18</b>	<b>0.03</b>	<b>0.25</b>	<b>46.02</b>	<b>2.95</b>
MpreFeb	0.983	0.974	0.992	0.005	0.04	0.54	0.02	0.15	0.03	0.26	42.08	-
MpreMar	0.985	0.976	0.995	0.029	0.03	0.53	0.02	0.14	0.03	0.31	32.31	-
MpreApr	0.985	0.974	0.996	0.046	0.02	0.59	0.02	0.21	0.04	0.21	11.37	-
MpreOct	0.989	0.982	0.997	0.036	0.02	0.54	0.02	0.13	0.04	0.27	13.73	2.58
MpreNov	0.99	0.983	0.997	0.035	0.02	0.52	0.02	0.14	0.03	0.24	23.79	-
MpreDec	0.985	0.979	0.992	0.001	0.04	0.55	0.02	0.15	0.03	0.21	47.98	-
TminJan	1.013	1.008	1.018	<0.001	0.08	0.63	0.02	0.36	0.03			
<b>TminJan</b>	<b>1.027</b>	<b>1.019</b>	<b>1.035</b>	<b>&lt;0.001</b>	<b>0.23</b>	<b>0.71</b>	<b>0.02</b>	<b>0.38</b>	<b>0.04</b>	<b>0.36</b>	<b>467</b>	<b>0.36</b>
TminJan2	0.407	0.318	0.522	<0.001							<b>818</b>	<b>0.017</b>
TminFeb	1.013	1.008	1.017	<0.001	0.07	0.61	0.02	0.35	0.03		601	0.026
TminFeb	1.026	1.018	1.034	<0.001	0.22	0.69	0.02	0.37	0.04	0.49	524.7	-
TminFeb2	0.406	0.314	0.525	<0.001								
TminMar	1.016	1.01	1.022	<0.001	0.07	0.62	0.02	0.35	0.03			
TminMar	1.033	1.023	1.043	<0.001	0.21	0.66	0.02	0.36	0.04	0.46	363.9	-
TminMar2	0.441	0.347	0.561	<0.001								
TminApr	1.013	1.006	1.021	0.012	0.03	0.57	0.02	0.21	0.03			
TminApr	1.022	1.012	1.032	<0.001								
TminApr2	0.758	0.652	0.881	0.005	0.06	0.56	0.02	0.22	0.03	0.31	228	-
TminMay	1.011	1.004	1.019	0.034	0.02	0.55	0.02	0.15	0.03	0.32	295.2	-
TminJun	1.014	1.007	1.022	0.006	0.04	0.58	0.02	0.17	0.03			
TminJun	1.022	1.012	1.033	<0.001	0.05	0.57	0.02	0.18	0.03	0.31	355.3	-
TminJun2	0.818	0.703	0.952	0.041								
TminJul	1.013	1.006	1.02	0.01	0.03	0.59	0.02	0.19	0.04			
TminJul	1.023	1.013	1.033	<0.001	0.06	0.58	0.02	0.2	0.04	0.27	359.6	-
TminJul2	0.799	0.69	0.926	0.019								
TminAug	1.015	1.008	1.022	0.002	0.04	0.62	0.02	0.27	0.04			
TminAug	1.031	1.019	1.042	<0.001	0.08	0.62	0.02	0.27	0.04	0.26	543.9	-
TminAug2	0.739	0.636	0.859	0.002								
TminSep	1.018	1.011	1.025	<0.001	0.07	0.66	0.02	0.33	0.04			
TminSep	1.04	1.029	1.052	<0.001	0.14	0.66	0.02	0.33	0.04	0.25	272.8	-
TminSep2	0.657	0.567	0.76	<0.001								
TminOct	1.019	1.013	1.026	<0.001	0.08	0.67	0.02	0.32	0.04			
TminOct	1.04	1.029	1.05	<0.001	0.16	0.67	0.02	0.5	0.04	0.27	156.17	-
TminOct2	0.649	0.563	0.748	<0.001								

# Environment influencing H5N1 in wild birds in Europe

TminNov	1.016	1.01	1.022	<0.001	0.07	795	0.64	0.02	0.34	0.03	0.34	143.6	-						
TminNov	1.034	1.043	<0.001	<0.001	0.16	752	0.65	0.02	0.47	0.33									
TminNov2	0.624	0.533	0.73	<0.001															
TminDec	1.008	1.003	1.012	0.013	0.03	811	0.59	0.02	0.34	0.03									
TminDec	1.022	1.015	1.029	<0.001	0.16	750	0.62	0.02	0.35	0.03	0.42	340.5	-						
TminDec2	0.506	0.415	0.616	<0.001															
TmaxJan	1.008	1.004	1.013	0.004	0.04	807	0.59	0.03	0.36	0.03	0.46	713.2	-						
TmaxJan	1.022	1.015	1.029	<0.001	0.2	729	0.66	0.02	0.39	0.04									
TmaxJan2	0.42	0.333	0.531	<0.001															
TmaxFeb	1.008	1.004	1.012	0.003	0.04	807	0.58	0.02	0.34	0.03									
TmaxFeb	1.019	1.013	1.025	<0.001	0.19	737	0.64	0.02	0.36	0.04	0.53	594.4	-						
TmaxFeb2	0.437	0.348	0.55	<0.001															
TmaxMar	1.009	1.005	1.013	0.004	0.04	808	0.58	0.02	0.28	0.03									
TmaxMar	1.016	1.01	1.022	<0.001	0.16	753	0.64	0.02	0.3	0.03	0.47	182.5	3.47	867	0.015	0.069			
TmaxMar2	0.456	0.36	0.577	<0.001											860	0.016	0.066		
TmaxOct	1.007	1.002	1.012	0.05	0.02	816	0.56	0.02	0.24	0.03	0.46	141.7	-						
TmaxOct	1.005	1	1.009	<0.001	0.08	788	0.58	0.02	0.25	0.03									
TmaxOct2	0.638	0.534	0.763	<0.001															
TmaxNov	1.008	1.003	1.012	0.017	0.03	813	0.58	0.03	0.31	0.02									
TmaxNov	1.02	1.013	1.026	<0.001	0.14	763	0.61	0.02	0.32	0.03	0.44	262.4	-						
TmaxNov2	0.549	0.455	0.663	<0.001															
TmaxDec	1.008	1.003	1.012	0.013	0.03	812	0.58	0.03	0.34	0.03									
TmaxDec	1.022	1.015	1.029	<0.001	0.16	750	0.62	0.02	0.35	0.03	0.43	376.3	-						
TmaxDec2	0.508	0.417	0.619	<0.001															
NDVJan	82.131	14.274	477.48	<0.001	0.15	754	0.69	0.02	0.3	0.04	0.36	161.5							
NDVJan2	0.415	0.324	0.533	<0.001															
NDVIFeb	8.325	2.2	31.558	0.047	0.02	816	0.57	0.02	0.21	0.04									
NDVIFeb	79.637	13.711	468.14	<0.001	0.15	756	0.68	0.02	0.29	0.04	0.3	361	-						
NDVIFeb2	0.428	0.335	0.548	<0.001															
NDVIMar	13.368	2.92	61.357	0.03	0.02	814	0.57	0.02	0.2	0.04									
NDVIMar	111.33	15.489	811.8	0.001	0.14	760	0.66	0.02	0.27	0.04	0.28	87.07	2.41	997	0.001	0.014			
NDVIMar	0.445	0.348	0.57	<0.001											1000	<0.001	0.003		
NDVJun	0.03	0.004	0.263	0.008	0.06	798	0.62	0.02	0.21	0.04	0.24	79.06	-						
NDVJun2	0.668	0.563	0.793	<0.001															
NDVJul	0.006	0.001	0.046	<0.001															
NDVJul2	0.533	0.436	0.652	<0.001	0.11	775	0.64	0.02	0.26	0.04	0.26	183.2	3.56	672	0.038	0.125			
NDVJul2														805	0.029	0.1			
NDVTagu	0.015	0.002	0.113	<0.001	0.09	787	0.62	0.02	0.24	0.03	0.22	101.13	-						
NDVTagu2	0.572	0.467	0.701	<0.001	0.03	812	0.58	0.02	0.19	0.04									
NDVINov	23.449	4.637	119.04	0.014	0.03	803	0.62	0.02	0.22	0.04	0.43	142.5	-						
NDVINov	15.49	2.708	89.102	0.048	0.05	803	0.62	0.02	0.22	0.04									
NDVINov2	0.759	0.628	0.919	0.036															
NDVIDec	30.448	6.566	141.74	0.004	0.04	807	0.59	0.02	0.22	0.04	0.45	99.86	3.26	980	0.005	0.037			
NDVIDec	28.187	5.417	147.905	0.008	0.07	797	0.64	0.02	0.25	0.04									
NDVIDec2	0.72	0.591	0.877	0.016															

<sup>†</sup> Calculated using z-score standardized variables to avoid too small or too large OR values  
<sup>‡</sup> All p values of 95% lower CI are less than 0.001

Eight out of fourteen pre-selected variables were discarded as they showed non-significant effects (upper 95% CI of p value > 0.05) in the process of stepwise selection (Table 3). Five key risk factors (i.e. elevation, precipitation in January, minimum temperature in January, NDVI in March (including the square of NDVI in March), and NDVI in December) were selected as inputs for the multiple logistic regressions. Using bootstrapping training subsets 1000 models were fitted, after which the mean value of each output parameter was calculated (Table 4). The results showed all key risk factors (i.e. lower elevation, reduced precipitation, a higher minimum temperature, an intermediate NDVI in spring, and a higher NDVI in winter) consistently associated with the occurrence of the HPAI H5N1 virus in wild birds. The predictive power of the fitted models is good as measured by AUC with a mean value of 0.81 and a Kappa value with a mean of 0.52 (Fig. 3).

Table 4: Summary of the multiple logistic regression models for the occurrence of the HPAI H5N1 virus in wild birds in Europe from 2005 to 2006. All values in the table are mean values obtained from 1000 runs of the model

	B	OR†	95% CI OR†	P value	AIC ±	Pseudo R <sup>2</sup> ± SD	AUC ± SD	Kappa ± SD
Model				<0.001				
Intercept	-10.86			<0.001				
Dem	-0.001	0.999	0.998	0.999	0.016		0.81	0.52
PrecJan	-0.037	0.963	0.95	0.976	<0.001	638	0.38 ±	±
TminJan	0.025	1.026	1.018	1.034	<0.001	± 25	0.04	±
NDVIMar	50.6	1.17	0.059	23.63	<0.001		0.02	0.03
SNDVIMar	-56.36	0.408	0.307	0.543	<0.001			
NDVIDec	6.102	1318.596	100.248	17685.59	<0.001			

† Calculated using z-score standardized variables to avoid too small or too large OR values

A predictive risk map of HPAI H5N1 occurrences in wild birds in Europe was generated on the basis of the model derived from the multiple logistic regression analysis (Fig. 4). The validation samples of HPAI H5N1 occurrences in wild birds were found for 79% (19/24) in the predicted high or very high risk areas (i.e. predictive risk > 0.4).

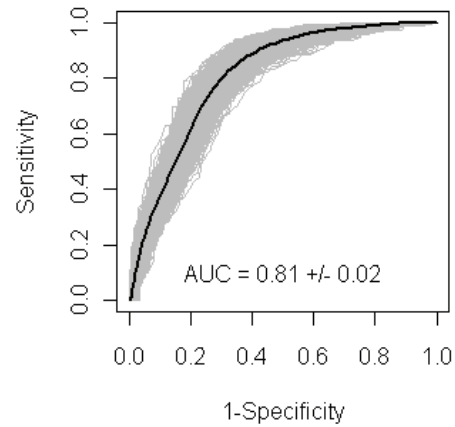


Fig. 3: Received Operating Characteristic (ROC) curves (abbreviated AUC) of the predictive power of the multiple logistic regression models on the presence/absence of the HPAI H5N1 virus in wild birds in Europe

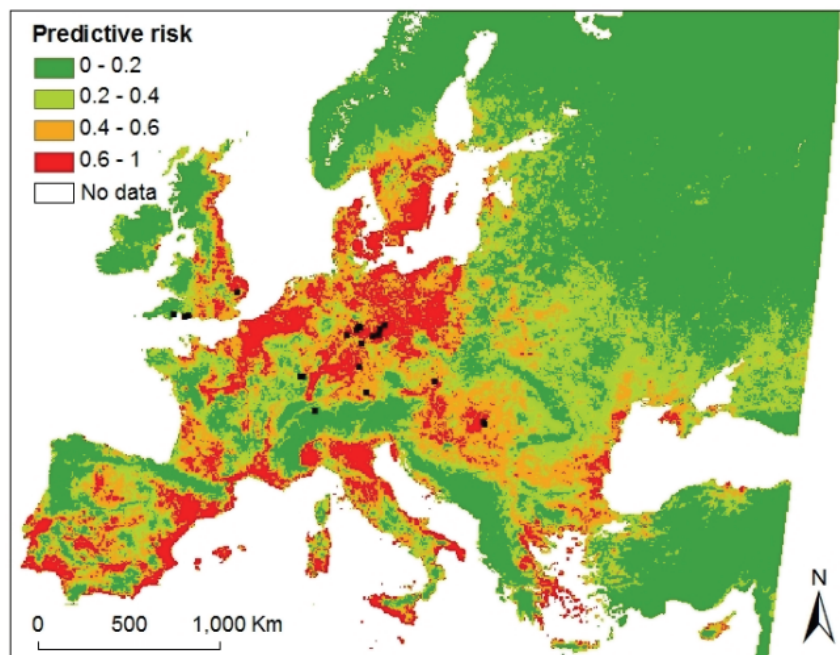


Fig. 4: Predictive risk map of HPAI H5N1 occurrences in wild birds in Europe. Black dots indicate HPAI H5N1 occurrences in wild birds in 2007 and 2008.

## **DISCUSSION**

The results presented in this paper have shown that HPAI H5N1 infections in wild birds in Europe occur under consistent and predictable environmental circumstances. The key environmental factors affecting the occurrence of HPAI H5N1 infections in wild birds in Europe are an increased NDVI in December, an intermediate NDVI in March, low elevation, an increased minimum temperature in January, and reduced precipitation in January. We therefore suggest that occurrences of HPAI H5N1 in wild birds in Europe are strongly influenced by the availability of food resources, and facilitated by increased temperatures and the reduced precipitation.

Elevation consistently showed a negative association with HPAI H5N1 occurrences in wild birds in Europe. Positive associations with higher NDVI and higher temperature during the winter were found, when a large number of wild birds overwinter and stage in Europe. The increased number of bird populations during winter may also increase the disease risk in Europe. As wetlands, rivers, canals, ponds and irrigated networks are concentrated in lowlands, flat plains, deltas, and coastal areas (Gilbert et al. 2006b; Gilbert et al. 2008), flat areas in combination with increased winter NDVI and increased winter temperature indicate increased resources for waterfowl in cold seasons.

Waterfowl utilize small plants (e.g. grasses and herbaceous plants) rather than larger plants (e.g. bushes and forest). During spring, the growing season for vegetation, areas with intermediate NDVI values may indicate the availability of herbaceous plants, while areas with high NDVI values may be dominated by larger plants. During the maturation stage of vegetation in summer, herbaceous plants may yield relatively low NDVI values compared to other larger plants. This may explain the significant quadratic associations observed between NDVI in spring and summer, and the occurrence of HPAI H5N1 in wild birds. The risk of HPAI H5N1 infections, however, is not increased in areas closer to wetlands (identified as a non-significant variable), probably because wetlands are so extensive in Europe, both in panzootic and non-panzootic areas.

This study highlights that climatic factors substantially contribute to HPAI H5N1 occurrences in wild birds. In agreement with recent findings from China (Fang et al. 2008), we also found that precipitation is negatively associated with the risk of HPAI H5N1 occurrences in wild birds in Europe, maybe because lower precipitation leads to a higher concentration of birds in the limited

suitable habitats, and therefore increased opportunities for contact. Areas with higher minimum temperatures, especially during the cold season, consistently show higher risk of disease occurrence, as higher temperature can stimulate viral activity. A confounding factor is that wild birds seek refuge from cold weather and congregate in warmer areas as temperatures drop, resulting in a higher probability of disease occurrence. Previous studies have suggested that the western European panzootic was caused by unusual waterfowl movements, due to extreme cold weather in the Black Sea area, where the virus was already established (Gilbert et al. 2006a; Keller et al. 2009). Hence, dramatic drops in temperature may trigger the spread of HPAI H5N1 viruses (Liu et al. 2007).

The analyses in this study have demonstrated that the risk of HPAI H5N1 infections in wild birds is affected only by selected physical environmental factors, indicating that the HPAI H5N1 occurrence in wild birds in Europe may be caused mainly by contact with infected wild birds. The surveillance and control measures in Europe (Pittman and Laddomada 2008; World Organization for Animal Health (OIE) 2009) may influence the occurrence pattern of HPAI H5N1 in wild birds. Our results show that HPAI H5N1 occurrences in wild birds in Europe are not related to any of the anthropogenic environmental factors selected in this study. Composite variables linking poultry density and location of wetlands yielded non-significant effects, suggesting generally few interactions between domestic poultry and waterfowl. One reason may be that biosecurity measures (e.g. the quarantine of free ranging poultry) successfully limited contact between domestic poultry and wild birds in poultry areas (Sinclair et al. 2006). This suggests that the role of domestic poultry as one of the spreading agents may be effectively minimized in wild bird infections in most European regions. Wild birds themselves may therefore play a prominent role in the occurrence of HPAI H5N1 in wild birds in Europe.

The heterogeneity in surveillance and control measures across Europe may lead to a bias in reporting and differences in sensitivity to risk factors. For example, the interaction effect between domestic poultry and waterfowl appears to be more pronounced in some counties (e.g. Romania and Ukraine) than other counties (e.g. France and Switzerland), depending whether the occurrence of HPAI H5N1 in domestic poultry and wild birds overlap (Fig. 1). A previous study has suggested that the Danube River Delta (an area associated with both waterfowl and domestic poultry) played a critical role in the introduction and initial spread of HPAI H5N1 in Romania (Ward et al. 2008). The same interaction may potentially occur in other countries,

but stricter quarantine measures may reduce the chance of contact between domestic and wild birds there.

In line with previous studies (Fang et al. 2008, Gilbert et al. 2008), we demonstrated that the logistic regression model was quite robust in identifying the environmental factors influencing the spread of HPAI H5N1 and predicting the risk of HPAI H5N1 virus occurrence in Europe. Compared with one previous study (Fang et al. 2008), which reported the quadratic effect did not perform significantly better than a linear association for any of the selected environmental factors in the logistic regression model, we found that only the NDVI in March yielded a consistent quadratic effects. A possible reason is that the quadratic effect of NDVI might be stronger in areas with HPAI H5N1 occurrence in wild birds than those with domestic poultry. To gain more insight in non-linear responses of the HPAI H5N1 virus to environmental factors, other analytic tools (e.g. neural network, GARP or Maxent) should be considered.

By simply focusing on the spatial position and environmental characteristics of sites where disease occurs, any occurrence can create a non-random distribution that appears predictive, and independent testing and repeated challenging are needed to evaluate models (Peterson and Williams 2008). We therefore investigated the relationship between environmental factors and HPAI H5N1 occurrences using statistically independent occurrences (i.e. space-time clusters of disease occurrence identified by space-time scan statistics). The results indicate only cold season temperature (minimum and maximum) and cold season NDVI yielded significant effects. The occurrence risk was largely underestimated by the model fitted using space-time clusters and the map of predictive risks shows a low accuracy (25%). By discarding sporadic H5N1 occurrences and aggregating individual occurrence into space-time clusters, a large part of the information was lost, which could be potentially important. Small sample sizes may also influence the model's predictive power and the sensitivity of risk factors. Models fitted using original disease occurrences, accessing maximum information, showed high accuracy (79%) of the predictive model after independent testing, suggesting that the identified key environmental factors are consistently affecting the occurrence of HPAI H5N1 in wild birds.

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## Chapter 4

### **Influence of refuges, distance to roost, and food resources on the distribution of barnacle geese (*Branta leucopsis*) in the northern Netherlands**

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This chapter is based on: Yali Si, Andrew K. Skidmore, Tiejun Wang, Willem F. de Boer, Albertus G. Toxopeus, Martin Schlerf, Mandy Oudshoorn, Susan Zwerver, Henk van der Jeugd, Klaus-Michael Exo and Herbert H.T. Prins, Influence of refuges, distance to roost, and food resources on the distribution of barnacle geese in the northern Netherlands, ARDEA (in revision).

## **ABSTRACT**

In Europe, the highest density of herbivorous waterfowl is found in The Netherlands, where the heavily fertilized agricultural land provides attractive foraging areas. To reduce damage to agriculture, 80 000 hectares of fields have been specifically designated as goose accommodation areas. These areas are largely in addition to semi-natural areas, where geese are already protected. Little is known, however, about whether geese intensively utilize these refuges and how forage conditions influence the distribution of geese in these areas. In this study we used satellite tracking data and field measurements of forage quality and quantity to investigate the effects of refuges, distance to roost, and food resources on the distribution of barnacle geese *Branta leucopsis* in the northern part of the Netherlands. A strong preference for refuges was consistently observed from February until May. In the Lauwersmeer area in particular, a significantly high grazing intensity was observed in areas located within 2 km from roosts. No relationship was found between forage quality and grazing intensity. Significant quadratic relationships were found between forage quantity and goose distribution, revealing a pronounced preference for sites with an intermediate forage quantity. A general linear model, using forage quantity and distance to roost as predictive variables, explained 60% of the variance in goose grazing intensity. The effectiveness of the refuge system for goose management in the northern part of the Netherlands is emphasized by this study. Distance to roost should be viewed as an important factor when designating refuges in the future. Improving forage quality may not efficiently increase the capacity of refuges, whereas maintaining grasslands of intermediate height and biomass is critical for increasing the capacity of refuges. Sward height manipulation can thus be an important tool in luring geese away from non-refuge areas.

## INTRODUCTION

The populations of herbivorous waterfowl along the western Palaearctic continental flyway have increased strongly over the last decades due to changes in land use and hunting regimes (Madsen *et al.* 1999; van Eerden *et al.* 2005). Agricultural practices, together with protection measures, have enhanced the capacity of winter and spring habitats for herbivorous waterfowl (van Eerden *et al.* 2005). This success of wild bird protection, however, also led to increasingly frequent claims of agricultural damage (Leistra *et al.* 2008). The conflict with agriculture has intensified because herbivorous waterfowl (mainly geese and wigeons) feed to a large extent on agricultural land. The establishment of a refuge system to accommodate these birds is regarded to be an effective, long term solution to the problem (McKay *et al.* 2001; Owen 1977; Owen 1980). In several European countries (e.g. The Netherlands and United Kingdom), large areas of agricultural land, together with some natural or semi-natural areas have been designated as refuge areas (McKay *et al.* 2001). Feeding areas are provided for herbivorous waterfowl within these refuges and farmers are compensated for allowing waterfowl on their fields. The farmers have to abide by certain management rules in an effort to lure birds away from surrounding farmlands in order to decrease agricultural damage there. In addition, some species of waterfowl are disturbed or can be shot outside of refuges. The creation of refuges is, hence, expected to significantly affect the distribution of herbivorous waterfowl.

Distribution of avian herbivores is influenced by food resources (forage quantity and quality, plant species and plant distribution), environmental conditions (distance to water and roosts, predator risk and human disturbance), and animal characteristics (cognitive abilities, social organization, intra -and interspecific competition) (Fryxell 1991; Kurvers *et al.* 2009; Raquet *et al.* 1998). As food is the main driver of animal activities, it is reasonable to expect that animal distribution is largely influenced by food availability (Fryxell 1991; Prins and Ydenberg 1985). However, in order to save travelling time and energy, waterfowl prefer to forage close to their roosts (Owen 1980; Owen *et al.* 1987; Vickery and Gill 1999). Furthermore, waterfowl are very sensitive to human disturbance, especially those species that remain heavily hunted at their wintering grounds (Hockin *et al.* 1992; Owen 1980; Vickery and Gill 1999). The country authorities assumed that a higher level of disturbance in non-refuges would stimulate geese and wigeons to forage mainly in the designated feeding areas within refuges. In these refuges, the influence of food resources and distance to roost are therefore

expected to be more pronounced than in the surrounding non-refuge areas.

Optimal foraging theory predicts that animals spend more time in patches that enable them to have a higher energy intake rate than the average rate of all patches combined (de Boer and Prins 1989; Emlen 1966; MacArthur and Pianka 1966; Prins 1996; Raouquet et al. 1998). The theory was firstly developed for animals that forage on prey of a high and relatively uniform nutritional content. The application of optimal foraging theory to herbivores raises the forage quality issue as the diet is relatively low in protein (Raouquet *et al.* 1998). For many herbivores, nutrient limitation is an important constraint influencing foraging behaviour and thereby distribution (Buchsbaum et al. 1981; Durant et al. 2004; Stephen and Krebs 1986). Particularly nitrogen content of forage is a limiting factor (Bos et al. 2005; Durant et al. 2004; Ydenberg and Prins 1981). Small herbivores such as waterfowl require a higher concentration of nutrients in their diets than large herbivores, as they are less capable of utilizing poor quality plants (Durant et al. 2004; Prins and van Langevelde 2008; Prins and Ydenberg 1985). For instance, for the barnacle goose (*Branta leucopsis*) protein supply is not sufficient in foods containing less than 15% crude protein (Amano et al. 2004; Prop and Deerenberg 1991). Furthermore, waterfowl have short food retention times of only few hours (McKay et al. 1994; Prop and Vulink 1992), and therefore absorb only easily digestible components, resulting in a high throughput and defecation rate (one dropping every 3-5 minutes) (McKay et al. 1994; Owen 1980). Such a digestive system requires high ingestion rates (Karasov 1990).

Digestibility appears to depend mainly on food chemistry (McKay *et al.* 1994). It is well-known that young plant material has a high protein content, a low tensile strength and a low fibre content and is easier to peck and digest (Riddington *et al.* 1997). This condition, however, rapidly changes with age, as structural components such as cellulose, hemicelluloses and lignin are incorporated into cells (Rockwood 1974; Ydenberg and Prins 1981). Mature patches with high plant biomass therefore tend to be of poorer quality. In the trade-off between forage quality and quantity, avian herbivores select feeding sites of intermediate biomass in order to maximize their digestible nutrient intake (Durant et al. 2004; Heuermann 2007), as predicted in the forage maturation hypothesis (Fryxell 1991). In high biomass sites, the preference decreases as a result of the nutritious constraint (poorer quality) and an intake constraint (difficulties in handling long leaves, and longer time needed for cropping, swallowing, and searching) (Hassall et al. 2001; Heuermann 2007;

van der Graaf et al. 2006; van der Wal et al. 1998). At intensively managed agricultural land, where high biomass levels could be combined with high quality food, the trade-off is not so much about decreasing quality (because farmers prevent that through fertilization) but about increased handling time in the high biomass sites, which reduces the net intake rate (the so-called spaghetti-effect).

Many studies have been carried out to investigate the influence of food resources on foraging behaviour of herbivorous waterfowl. A dome-shaped response was observed between vegetation standing crop and geese grazing intensity (or food intake rate) (Heuermann 2007; van de Koppel et al. 1996; van der Wal et al. 1998). Riddington et al. (1997) found that on unfertilized plots, shorter swards were preferred but on fertilized plots, medium and longer swards were preferred. Hassall *et al.* (2001) showed that brent geese (*Branta bernicla*) select intermediate height swards, as they trade off the maximization of energy intake against nitrogen absorption rates. Some studies found that smaller bodied geese select shorter swards than larger bodied ones in order to maximise digestible nitrogen intake rate (Durant et al. 2004; Heuermann 2007). Bos *et al.* (2005) reported that food quality was an important parameter in patch choice of brent geese, acting at all forage biomass levels. Van der Graaf *et al.* (2007) demonstrated that herbivorous waterfowl trade off forage quality and quantity by selecting plots with the highest standing crop of nitrogen in their spring, staging and breeding sites. These studies were all executed under experimental conditions and lay the foundation for investigating the distribution of herbivorous waterfowl at a habitat scale. However, scaling-up of these experiments to a habitat level may reveal important differences from the factors that attract geese to a field (Vickery and Gill 1999).

In Europe, the highest density of herbivorous waterfowl is found in The Netherlands, where the heavily fertilized agricultural land provides attractive foraging areas (Madsen and Fox 1995; Prins and Ydenberg 1985; van Eerden et al. 2005; Ydenberg and Prins 1981). The increasing number of wintering geese and wigeons has led to an increase in the damage of local agricultural crops (Groot Bruinderink 1989) and hence an increase in the amount of compensation paid to farmers. To solve this problem, from 2005 onwards 80 000 hectares of fields (mainly agricultural land) have been designated as accommodation areas (Leistra *et al.* 2008), largely in addition to already existing protected semi-natural areas (e.g. salt marshes, fresh-water marshes, and some extensively managed grasslands). We investigate whether birds intensively utilize these refuges and

how forage conditions and distance to roosts influence their distribution. The answers to these questions have conservation implications, which can be used to further improve wildfowl refuge management.

## **METHODS**

### **Study area**

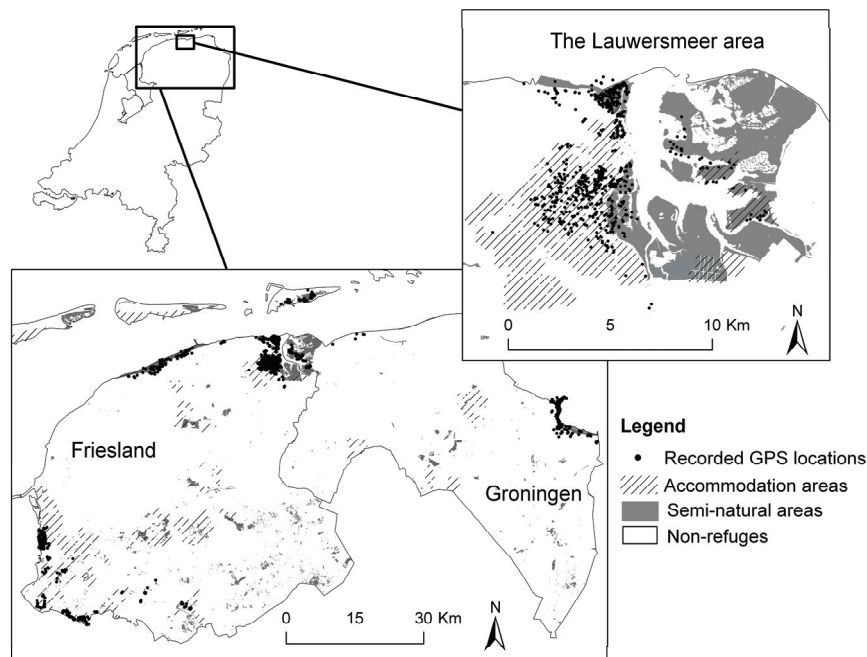


Fig. 1: Spatial distribution of recorded GPS locations of barnacle geese in Friesland and Groningen in the northern part of the Netherlands. The records stem from the period from the 1st of February to the 18th of May 2008, after which the geese migrated towards Russia. The Lauwersmeer area indicates the area for field sampling. The locations of semi-natural areas were extracted from the Dutch National Landuse Database.

The study area is situated in the northern part of the Netherlands, in the provinces of Groningen and Friesland (Fig. 1). Field sampling of forage quality and quantity was only carried out in and around the Lauwersmeer area. Agricultural land in accommodation areas are managed by local farmers. In accommodation areas, wildfowl are not allowed to be disturbed from the 1st of November to the 1st of April. Semi-natural areas are managed as nature reserves by different organizations, some of which allow grazing by naturalized cattle year-round. In these nature reserves waterfowl are fully protected. Just as in accommodation areas, the agricultural land located in non-



accommodation areas are managed by regular fertilization, mowing, and cattle grazing. A variety of scaring methods have been developed for the non-refuge areas (e.g. gas canons, scarecrows, dog chasing). Yet in these areas, killing by shooting is permitted if chasing the geese away by other means does not result in reduced damage to agriculture (except for some particular species that are fully protected in the Netherlands). The plant community in agricultural fields is dominated by *Lolium perenne* and *Poa pratensis* (Prins and Ydenberg 1985). Low and middle height plant species (useful for herbivorous waterfowl) in semi-natural areas mainly consist of *Festuca rubra*, *Puccinellia maritima*, *Elymus repens*, *Halimione portulacoides*, *Trifolium repens*, *Plantago maritima*, and *Triglochin maritima* (Esselink 2000).

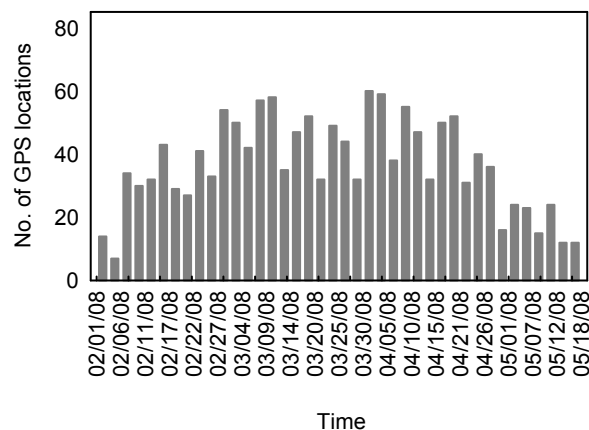


Fig. 2: Temporal distribution of the recorded GPS locations of barnacle geese in the provinces of Friesland and Groningen in the northern part of the Netherlands from 1st of February to 18th of May 2008. The bars indicate the number of locations received every three days.

### Satellite tracking data of barnacle geese

In order to catch and fix transmitters on barnacle geese, a license under the law "Flora en Fauna Wet", number FF75A/2007/056 and approval from the Dutch Ethical Committee under protocol number CL 0703 was obtained. A license to conduct this study in the Wadden Sea, a Natura2000 area, was obtained from the Province Friesland, number 00692701. In January 2008, eight adult barnacle geese were caught in the Lauwersmeer area and fitted with 30g solar-powered GPS PTT (Microwave Telemetry, Inc., Columbia, MD, USA). The transmitters were fastened in a way to maximize freedom of movement. The transmitters recorded GPS locations four times per day (at 7:00, 10:00, 13:00, and 16:00 MET), and the collected data, including goose ID, date, time, longitude, latitude, speed, course and

altitude, was transmitted every three days. A total of 1468 GPS locations were recorded in the provinces of Friesland and Groningen from the 1st of February to 18th of May in 2008, after which all of the tracked geese had left the Netherlands (Fig. 1). The temporal distribution of the recorded GPS locations is displayed in blocks of three days in Fig. 2, indicating the continuity of records.

### **Field data collection**

Fieldwork was conducted in March and April of 2008. Spring was selected because the feeding behaviour of geese in the spring staging areas is important for the accumulation of body reserves and affects subsequent breeding success (Ebbinge and Spaans 1995). Moreover, only heavily fertilized agricultural land is grazed by barnacle geese in winter (Prins and Ydenberg 1985; van der Graaf 2006), but both agricultural and semi-natural areas are utilized during spring (Bos and Stahl 2003; Spaans and Postma 2001). The foraging areas of barnacle geese are up to 7.5 km from their roost sites (Owen et al. 1987; Vickery and Gill 1999). Therefore, for geese roosting on the Lauwersmeer lake, a 7.5 km buffer was generated from the lake sides to define the maximum extent of the potential feeding area. Non-refuge areas were defined as those areas within the mentioned perimeter of the 7.5 km buffer, but outside of accommodation areas, semi-natural areas and the Lauwersmeer lake (Fig. 3).

A stratified sampling design was adopted, based on two strata: refuges and non-refuges. In total, 20 random locations were generated, with 15 located in refuges and 5 in non-refuges (Fig. 3). For each location, 5 random sample plots were generated, resulting in 75 samples in refuges, and 25 samples in non-refuges.

Three vegetation variables were measured: green biomass, sward height and nitrogen concentration. Green biomass and sward height were used as forage quantity indicators, whereas nitrogen concentration was used as a measure of forage quality as crude protein is determined by multiplying total nitrogen in the sample by 6.25. Samples were taken from a plot of 1 × 1 m. Because of the homogeneity of the swards, we assumed that small sampling areas (i.e. 0.1 × 0.1 m) adequately represent the biomass level of the bigger plots (i.e. 1 × 1 m), a similar strategy as adopted in previous studies (Harwood 1977; Owen 1971). A 0.1 × 0.1 m area within each sample plot was clipped to ground level using hand shears, and samples were stored in sealed plastic bags. Soon after collecting, the green portions were separated by hand and non-green parts were discarded. The remaining green portions were dried at 70°C for 48 h and weighted afterwards. Sward height was measured directly

(Stewart *et al.* 2001): a ruler was vertically pushed through the sward, until it came to rest on the soil surface, and by placing a piece of carton on top of the vegetation, the sward height was read off the ruler. Sward height was recorded as a mean of ten random positions within each sample plot. Twenty leaf samples per plot were collected for nitrogen analysis by taking leaves between the side of the forefinger and the thumb, in an attempt to simulate goose grazing. Nitrogen concentration (%) was analyzed in the Resource Ecology Group laboratory of Wageningen University using a SkalarSan-Plus auto analyzer, after destruction with a mixture of  $H_2SO_4$ , selenium and salicylic acid (Novozamsky *et al.* 1983).

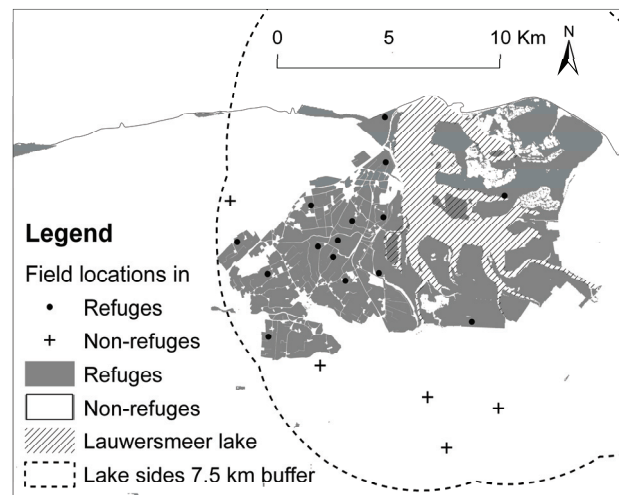


Fig. 3: Distribution of twenty sampling locations in refuges and non-refuges in the Lauwersmeer area. All field locations were situated within a 7.5 km buffer from the lake sides.

### Pre-processing of satellite tracking data

Previous studies indicate that geese forage during 70% of the daylight period in the spring staging period (Black *et al.* 1991; Prins *et al.* 1980; van der Graaf 2006). We therefore assumed that from March to April, the geese locations recorded at 7:00, 10:00, 13:00, and 16:00 MET per day were all grazing locations. In order to exclude locations recorded during flight, GPS locations associated with a speed of more than 1 km per hour were excluded. GPS locations corresponding to the time of field sampling March and April 2008, were imported into ArcGIS software as point data ( $n = 1025$ ). This point data layer depicted bird grazing at specific locations. To quantify the spatial distribution of birds, a grazing intensity map (the number of recorded GPS locations of barnacle geese per  $km^2$ ) was generated, using a fixed kernel density estimator with 95% space-use

contours. The fixed kernel density estimator is a commonly applied space-use estimator in wildlife studies and has been used to estimate resource selection (Marzluff et al. 2004; Millspaugh et al. 2006). By overlaying the vegetation sample data on the generated density map, the grazing intensity for each vegetation sample was extracted.

### **Statistical analysis**

To compare the level of geese preference in refuges and non-refuges, all recorded GPS locations from the 1st of February to the 18th of May were utilized. The numbers of locations recorded in refuges and non-refuges for each month were calculated. Thereafter we analysed data at the Lauwersmeer area and compared the forage conditions in refuges and non-refuges. All field data were tested for normality using a Kolmogorov-Smirnov test. Percentage data (nitrogen concentration) were arcsine-square root transformed (Zar 1999) as necessary. To test whether the forage quality and quantity were different within and outside the refuges, a one-way ANOVA was used, with nitrogen concentration, green biomass and sward height as dependent variables, respectively, and being located inside or outside refuges as a fixed factor.

To test how the distance to the nearest roost influences the grazing intensity of barnacle geese in the Lauwersmeer area, the number of recorded GPS locations was calculated within 7 distance buffers ranging from 1 to 7.5 km, with an increase of 1 km. As areas close to the roost tend to attract more geese, a distance threshold was identified to distinguish areas with high cumulative grazing intensity from low intensity, based on the number of recorded GPS locations in each distance buffer. The field samples were hence categorized into two groups: within and beyond the distance threshold. The difference of grazing intensity in these two groups was tested using a one-way ANOVA, with grazing intensity as a dependent variable and within than or beyond the distance threshold as a fixed factor. Categorizing the field samples into two groups of different distance to the roost may relax the influence of the distance to roost on the grazing intensity of geese. The relationship between forage conditions and grazing intensity would hence be more pronounced in each subset than in the pooled data.

Geese prefer sites of higher forage quality when nitrogen is in limited supply but do not distinguish when overall forage quality is sufficiently high. Previous studies found a positive relationship between forage quality and goose grazing intensity for nitrogen concentration in green leaves below 2.4% (Prop and Deerenberg 1991), but above 3.2% (National Research Council 1994) no

relationship was expected. We described this response by a linear-plateau model. For the first linear stage, a linear function was fitted when nitrogen concentration was lower than 3.2%, and for the second plateau stage, a horizontal line was fitted ( $y$  equals the mean value of grazing intensity) when the nitrogen concentration was above 3.2%. Meanwhile, geese prefer sites of intermediate forage quantity and their foraging efficiency drops at high sward height because of increased handling time (Heuermann 2007; van de Koppel et al. 1996; van der Wal et al. 1998). A quadratic regression was therefore utilized to approximate a Holling's IV functional response to describe this dome-shaped function (Durant et al. 2003).

A general linear model was fitted using grazing intensity as the dependant variable and several independent variables: nitrogen content, green biomass, square of green biomass, sward height, square of sward height, and categorized distance to roost. The non-significant variables were then excluded and a final general linear model was built to predict the grazing intensity of barnacle geese in the Lauwersmeer area.

## RESULTS

### Goose distribution and forage conditions in refuges and non-refuges

Table 1: The difference in forage quality and quantity in or outside the refuges at the Lauwersmeer area (The Netherlands). Significance level of ANOVA test: \*  $P < 0.05$ , \*\*  $P < 0.01$ , ns = not significant

	Field type	Mean	95% CIs of mean	n	$F_{1,98}$	P
Nitrogen (%)	Refuges	3.9	3.7 – 4.1	98	0.55a	ns
	Non-refuges	4.1	3.8 – 4.4			
Green biomass (g DW m <sup>-2</sup> )	Refuges	90.2	72.9 – 107.5	100	9.88	**
	Non-refuges	141.5	118.7 – 164.3			
Sward height (cm)	Refuges	4	3.0 – 4.9	100	5.94	*
	Non-refuges	6.1	5.2 – 6.9			

<sup>a</sup>  $F_{1,96}$ , calculated using transformed data

In the provinces of Friesland and Groningen, the number of recorded GPS locations of barnacle geese observed in refuges was 259 (94%), 357 (83%), 339(72%) and 76 (70%) from February to May 2008, respectively (Fig. 4a). Within the 7.5 km potential grazing buffer around the Lauwersmeer lake, the number of recorded GPS locations of barnacle geese observed in refuges in the same periods was 224 (96%), 247 (94%), 154 (87%), and 0 respectively (Fig. 4b). There was no significant difference in forage quality between refuges and

non-refuges, but the green biomass and sward height in refuges were significantly lower than those in non-refuges (Table 1).

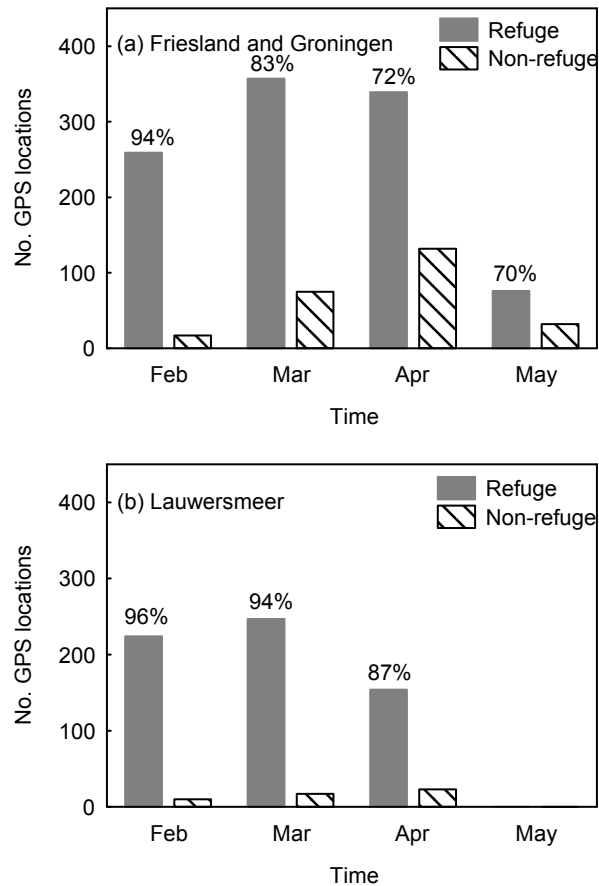


Fig. 4: Monthly numbers of recorded GPS locations of barnacle geese in the provinces of Friesland and Groningen and the Lauwersmeer area from the 1st of February to the 18th of May 2008. The percentage of locations recorded in refuges for each month is shown above each bar.

### **Relationship between distance to roost, food, and grazing intensity**

The number of recorded GPS locations of barnacle geese in different distance buffers revealed a high cumulative grazing intensity in fields within 2 km from the roost, accounting for 83% of the total recorded locations in the Lauwersmeer area (Fig. 5a). The field samples were therefore categorized into two groups: within 2 km and beyond 2 km from the nearest roost. A significantly higher grazing intensity was observed for samples collected within 2 km from the nearest roost than for those beyond 2 km (Fig. 5b:  $F_{1,53} = 22.03$ ,  $P < 0.001$ ).

The relationships between forage quality, quantity, and grazing intensity were analysed on the basis of two subsets: samples collected within and beyond 2 km from the roost. No significant relationship was found between forage quality and grazing intensity in areas within ( $P = 0.48$ ) and beyond 2 km to the roost ( $P = 0.86$ ), thus a horizontal line was fitted (Fig. 6a). Significant quadratic relationships (Table 2) were found between forage quantity (i.e. green biomass and sward height) and grazing intensity, in areas located within and beyond 2 km from the roost (Fig. 6b-c). Areas located within 2 km from the roost have a consistently higher cumulative grazing intensity than areas located beyond 2 km (Fig. 6). In areas located within 2 km from the roost the highest grazing intensity was observed at a green biomass value of approximately 80 g DW m<sup>-2</sup> and a sward height about 3 cm and in areas located beyond 2 km from the roost at a value of approximately 90 g DW m<sup>-2</sup> and a height of about 4 cm.

Table 2: Parameter estimates and statistics for the regression models for forage quantity and geese grazing intensity. Significance level of ANOVA test: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Roost	Variable	Model	R <sup>2</sup>	N	F	P
Distance < 2km	Green biomass	$y = 1.29 + 0.20x - 0.01x^2$	0.28	30	5.27	*
	Sward height	$y = -7.44 + 11.78x - 1.92x^2$	0.57	30	17.98	***
Distance > 2km	Green biomass	$y = 0.66 + 0.07x - 0.0004x^2$	0.26	25	3.94	*
	Sward height	$y = -1.60 + 2.77x - 0.34x^2$	0.48	25	10.3	**

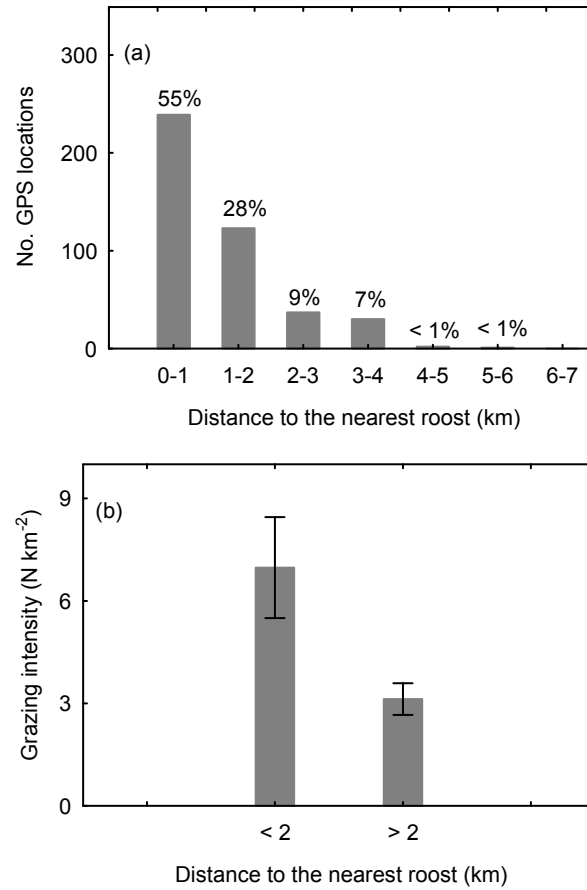


Fig. 5: Numbers of recorded GPS locations of barnacle geese in different distance buffers (a) and the difference of grazing intensity ( $5.26 \pm 3.57$ ) in field samples located within and beyond 2 km from the roost (b) in the Lauwersmeer area.



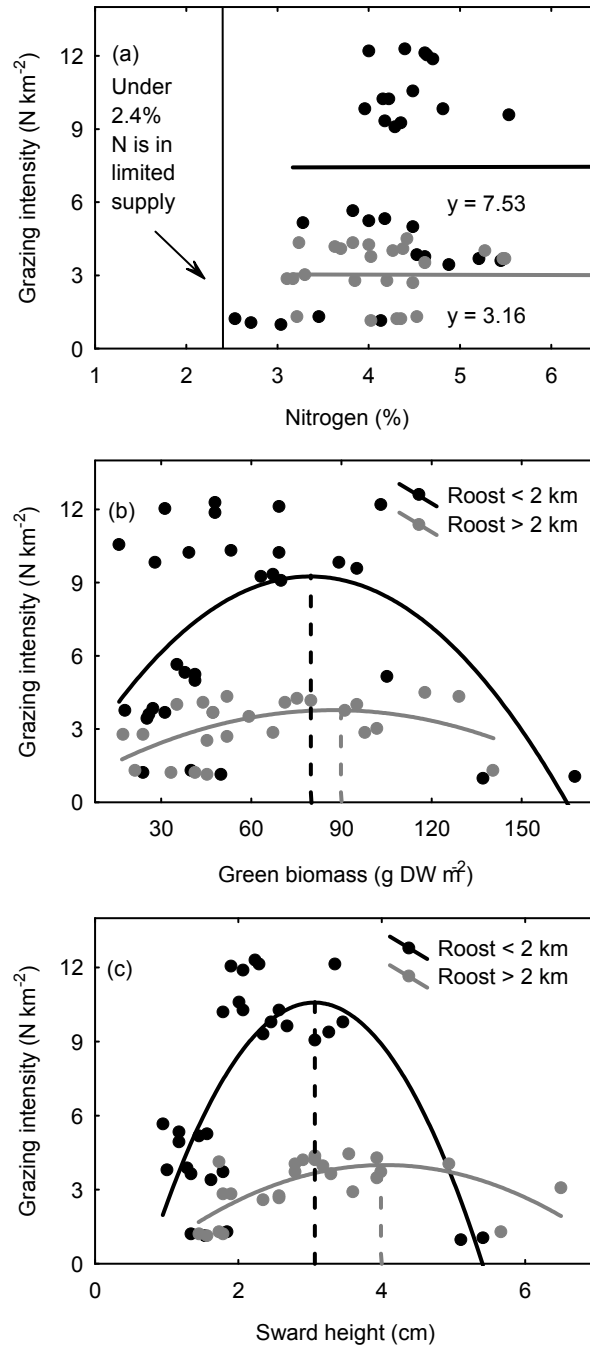


Fig. 6: Grazing intensity as a function of nitrogen content (a), green biomass (b), and sward height (c) in the Lauwersmeer area in spring 2008. Dashed lines indicate the forage quantity corresponding to the highest grazing intensity.

Consistent with the previous univariate analysis, forage nitrogen concentration does not affect the grazing intensity of geese using a general linear model ( $P = 0.54$ ). Forage nitrogen concentration was hence excluded from the final model. By including green biomass, square of green biomass, sward height, square of sward height and distance to the nearest roost as input variables, the final model explained 60% of variance in geese grazing intensity in the Lauwersmeer area. Geese grazing intensity increased with an increasing forage quantity and decreased after it reached the optimal forage quantity that can give maximum intake rate (Table 3).

Table 3: Parameter estimates and statistics for the general linear models for predicting geese grazing intensity using forage quantity and roost distances. Significance level: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Parameter	B	SE	F <sub>1,53</sub>	P	N	R <sup>2</sup>
Model	-	-	14.51	***	55	0.6
Intercept	-7.82	1.98	8.08	**		
Sward height	4.96	1.25	15.54	***		
Sward height <sup>2</sup>	-0.61	0.18	11.99	**		
Green biomass	0.1	0.03	6.77	*		
Green biomass <sup>2</sup>	-0.001	0.0002	10.44	**		
Distance to roost	5.25	0.72	51.88	***		

## DISCUSSION

Barnacle geese intensively utilize refuge areas in the northern part of the Netherlands. A significantly higher cumulative grazing intensity was observed in areas located within 2 km from the roost than in those areas located beyond 2 km. Regarding the influence of food resources on the distribution of geese within refuges, forage quality did not affect grazing intensity because of a generally sufficient nitrogen supply. On the other hand, an intermediate level of forage quantity (with a green biomass of 80-90 g DW m<sup>-2</sup> and a sward height of 3-4 cm) was highly preferred over either lower or higher levels.

Barnacle geese prefer refuges over non-refuges. Apparently, geese are accustomed to utilizing the designated accommodation areas, implying that the establishment of the refuge system in the northern part of the Netherlands is effective. Methods to scare geese developed for non-refuge areas may play a prominent role in chasing birds away. Unsuitable forage conditions in non-forage areas, however, also influence their distribution. These areas were unsuitable because of the high forage quantity (mean sward height equals 6 cm) (Heuermann 2007). This high sward height may be the

result of the low level of goose grazing, as once geese have selected specific areas as foraging area, they tend to maintain the swards at an optimal height and consistently grouped in these areas (Bos *et al.* 2004). Other ungrazed areas preserving a high sward height remain unsuitable, irrespective of the comparable forage quality (Spaans and Postma 2001). From February to May, utilization of refuges decreased, possibly because geese expanded their distribution from mainly agricultural land to both agricultural and semi-natural lands, and some non-refuges nearby would be temporarily occupied.

Distance to the nearest roost significantly influenced the cumulative grazing intensity of barnacle geese in the Lauwersmeer area. Areas located within 2 km from the roost were highly preferred by geese than areas located beyond 2 km from the roost, and geese seldom use areas located more than 4 km away from their roosting areas. This finding is comparable with the observations in the United Kingdom, where barnacle geese were found to feed within an average distance of 3.6 km (SD 1.9 km) from their roosts (Owen *et al.* 1987). Geese were also observed to select slightly shorter grasses and less dense biomass in areas located close to roosts than those located further away (Fig. 6), suggesting a trade-off between flying costs and foraging intake. Besides forage conditions, distance to roost should be viewed as an important factor when designating refuges for herbivorous waterfowl in future.

In refuges located in the Lauwersmeer area, the sites that geese select are all of sufficiently high forage quality (nitrogen content from 2.6% to 5.5%). Note that the limiting level for barnacle geese is 2.4% of nitrogen content (Amano *et al.* 2004; Prop and Deerenberg 1991) as calculated from 15% of crude protein. Indeed, no relationship was observed between forage quality and grazing intensity, suggesting food quality does not influence geese distribution. This is different from previous observations of field experimental studies carried out in the Netherlands (Bos *et al.* 2005; van der Graaf *et al.* 2007), which demonstrated a significantly higher preference for fertilized plots than for control plots. A possible reason is that for the experimental studies, switching between plots does not require additional travel energy. However, at the habitat level, geese would select closer patches rather than the further ones if forage quality is sufficiently high in the entire area.

The significant relationship between forage quantity and grazing intensity observed in the Lauwersmeer area reveals that intermediate biomass and intermediate sward height are highly preferred by barnacle geese. The highest grazing intensity was observed at sites with sward heights of about 3 to 4 cm, which is consistent with the

optimum sward height for barnacle geese reported in previous research (Durant *et al.* 2004). Fitting Holling's IV curves (Heuermann 2007) resulted in similar optima for Canada geese (*Branta canadensis*) the size of barnacle geese (at sward heights of about 3 cm and biomass of about 90 g DW m<sup>-2</sup>) as deduced from the quadratic functions in this study (i.e. 3 cm and 80 g DW m<sup>-2</sup> within and 4 cm and 90 g DW m<sup>-2</sup> beyond 2 km from the roost). Our findings emphasize the importance of intermediate sward height and grass biomass in determining the distribution of geese at the habitat level. Sward height manipulation can thus be an important tool in luring geese to refuge areas, thereby reducing grazing in agricultural land. Indeed, the barnacle geese's preference of swards of about 3 to 4 cm tall appears to be shorter than the optimal sward heights for agricultural production.

## **CONCLUSION**

This study demonstrates how refuges, distance to roost and food resources affect the distribution of spring staging barnacle geese in the northern part of the Netherlands. Geese intensively utilize refuges, underlining the importance and effectiveness of the refuge system. Areas located within 2 km from the roost showed significantly higher accumulative grazing intensity than areas located further away, emphasizing the importance of foraging areas located close to the roost. Forage quality did not play a role in the distribution of birds, implying that increase in the forage quality will not efficiently increase the capacity of the refuges in northern Netherlands. A pronounced preference of sites with an intermediate forage quantity was found, thereby suggesting that maintaining grasses in refuges at an intermediate height and biomass is critical for increasing the capacity of the refuges. As optimal sward heights for agricultural production is higher than for geese foraging, sward height manipulation can be an important tool in luring geese to refuge areas and reducing grazing in agricultural land in non-refuges.

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## **Chapter 5**

### **Mapping spatio-temporal variation of grassland quantity and quality using MERIS data and the PROSPECT+SAIL model**

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This chapter is based on Yali Si, Martin Schlerf, Raul Zurita-Milla, Andrew Skidmore, and Tiejun Wang, Mapping spatio-temporal variation of grassland quantity and quality using MERIS data and the PROSPECT+SAIL model (in revision).

## **ABSTRACT**

Accurate estimates of the quantity and quality of grassland, as it varies in space and time and from regional to global scales, furthers our understanding of the movement of migratory birds and the role of migratory birds in infectious avian diseases. The Medium Resolution Imaging Spectrometer (MERIS) is a promising sensor for measuring and monitoring grassland quality and quantity due to its high spectral resolution, medium spatial resolution and a two- to three-day repeat cycle. However, thus far the multi-biome MERIS land products have limited consistency with *in-situ* measurements of leaf area index (LAI), while the multi-biome canopy chlorophyll content (CCC) has not been validated yet with *in-situ* data. This study proposes a single-biome approach to estimate grassland LAI (a surrogate of grass quantity) and leaf chlorophyll content (LCC) and CCC (surrogates of grass quality) using the inversion of the PROSPECT+SAIL model and MERIS reflectance. Both multi-biome and single-biome approaches were validated using multi-season *in-situ* data sets and temporal consistency was analyzed using time-series of MERIS data. The single-biome approach showed a consistently better performance for estimating LAI ( $R^2=0.70$ , RMSE=1.06, NRMSE= 17%) and CCC ( $R^2=0.60$ , RMSE=0.36, NRMSE=22%) compared with the multi-biome approach (LAI:  $R^2=0.36$ , RMSE=1.77, NRMSE=28%; CCC:  $R^2=0.47$ , RMSE=1.33, NRMSE=84%). However, both single-biome and multi-biome approaches failed to retrieve LCC. The multi-biome LAI was overestimated at lower LAI values ( $< 2$ ) and saturated at higher LAI values ( $\geq 4$ ), and the multi-biome CCC was consistently overestimated through the whole data range. Similar temporal trajectories of grassland LAI and CCC estimated were observed using these two approaches, but the multi-biome trajectory consistently produced larger values than the single-biome trajectory. The spatio-temporal variation of grassland LAI and CCC estimated by the single-biome approach was shown to be closely associated with agricultural practices. Our results underline the potential of accurate mapping of grassland LAI and CCC using the PROSPECT+SAIL model and MERIS satellite data. A time-series of grassland quantity and quality maps may form the basis towards an improved understanding of the movement of migratory waterfowl, as well as the spread of infectious avian disease associated with these herbivores.

## INTRODUCTION

The estimation of the biophysical and biochemical properties of vegetation has proven useful for a large variety of agricultural, ecological, and meteorological applications (Asner 1998; Houborg et al. 2007). Among the many vegetation variables, leaf area index (LAI), leaf chlorophyll content (LCC) and canopy chlorophyll content (CCC) are of prime interest (Bacour et al. 2006; Houborg et al. 2007). LAI is defined as the single sided leaf area per unit of horizontal ground, which characterizes the surface of exchanges for mass and energy between the Earth's surface and the atmosphere (Chen and Black 1992). LAI can be used as a surrogate of grass quantity, as it is often highly correlated with biomass. LCC is defined as the sum of the content of chlorophyll a and chlorophyll b per unit of leaf area and is often strongly related to leaf nitrogen content, thus referring to grass quality (Daughtry et al. 2000; Yoder and Pettigrew-Crosby 1995a). The CCC is defined here by multiplying LAI by LCC. Both LCC and CCC can be used as indicators of vegetation stress and productivity (Carter 1994; Norman et al. 1995; Zarco-Tejada et al. 2004b).

With regard to ecological applications, the spatio-temporal variation of vegetation quantity (biomass or LAI) and quality (nitrogen or chlorophyll content) are key factors affecting the migration activity of herbivores (Fryxell and Sinclair 1988; Wang et al. 2010). For herbivorous waterfowl (mainly geese, duck, and swans), grasslands along their migratory flyways offer wintering, staging, and breeding habitats (Eichhorn et al. 2006; van der Graaf et al. 2007; van Eerden et al. 2005). The movement of migratory waterfowl is driven by the availability and condition of grassland (Owen 1980). Within the context of the "forage maturation hypothesis" (Wilmschurst et al. 1995) and the "green wave hypothesis" (Owen 1980), avian herbivores select forage of intermediate biomass and high nitrogen content. During their spring migration, waterfowl follow a northerly directed "green wave" of highly nutritious plant tissue, as seasonality influences the phenology of the vegetation. The availability of forage with intermediate quantity and high quality therefore is the most important factor determining the migratory schedule of avian herbivores (van der Graaf et al. 2007).

Climate change modifies plant phenology (van der Graaf 2006) and land use changes may relocate the distribution of suitable foraging sites (van Eerden et al. 2005). These changes are expected to dramatically influence bird migration activities (van der Graaf 2006). Accurate estimates of spatio-temporal variation of grassland quality

and quantity from regional to global scales are required to predict the movement of migratory birds. An important motivation for understanding the dynamics of waterfowl migration is that these birds are the spreading agent for infectious avian diseases such as avian influenza (Si et al. 2009; Si et al. 2010).

Remote sensing can be used to estimate the spatio-temporal variation of grassland quantity and quality through repeatable measurement, at a relatively costs compared to physical field measurements (Mutanga et al. 2004). The Medium Resolution Imaging Spectrometer (MERIS), operating in the visible and near-infrared reflective spectral range, has high spectral resolution, moderate spatial resolution and a two to three days repeat cycle. Fifteen spectral bands, centered at 412, 442, 490, 510, 560, 620, 665, 681, 709, 754, 760, 779, 865, 900 nm, are typical about 10 nm wide (Rast et al. 1999). The MERIS pixel size is approximately 300 m at full spatial resolution (FR) and 1200 m for reduced resolution (RR). We use FR data because lower spatial resolution tends to increase the land cover mixture effect. The three-day repeat cycle allows tracing vegetation phenology.

Traditional remote sensing methods used for extracting biophysical and biochemical ecosystem characteristics rely on the observed spectral features via an empirical relationship linking the variables of interest to a combination of radiometric measurements (i.e., vegetation indices) (Darvishzadeh et al. 2008b; Dorigo et al. 2007; Van Der Meer et al. 2001). However, empirical relationships are site, time, and vegetation specific (Baret and Guyot 1991; Gobron et al. 1997). Radiative transfer models aim to generalize empirical results and improve the reliability of vegetation parameter retrieval, in order to cope with a wide range of situations. Physically-based models describe the transfer and interactions of radiation inside the canopy based on physical laws and offer an explicit connection between the biophysical and biochemical variables of vegetation and canopy reflectance (Baret et al. 2000; Darvishzadeh et al. 2008a; Houborg et al. 2007). Therefore, the inversion of the physically based combined leaf-canopy model was selected for this study, as the large-scale movement of migratory birds requires an algorithm that can be applied from regional to global scales, as well as mapped at different times of the year.

A number of studies have estimated vegetation properties via radiative transfer models. Most studies were focused on crops (Casa 2004; González-Sanpedro et al. 2008; Houborg et al. 2009; Houborg and Boegh 2008; Koetz et al. 2005), forests (Moorthy et al. 2008;

Schlerf and Atzberger 2006; Zarco-Tejada et al. 2004a) or a combination of different vegetation types (Bacour et al. 2006; Houborg et al. 2007). Few studies have explored the potential of radiative transfer models to estimate properties of grassland (Jacquemoud et al. 2009). Two studies specifically dealing with grassland successfully retrieved LAI and CCC at the field level (Darvishzadeh et al. 2008a; Vohland and Jarmer 2007). These studies were validated either by a limited number of samples (Vohland and Jarmer 2007) or by single-season *in-situ* measurements (Darvishzadeh et al. 2008a). As different plant species show specific morphological and anatomical features, which again are modified by nutrient supply and change according to phenological development (Vohland and Jarmer 2007), it is desirable to extend the validation procedure by increasing the number of sites and *in-situ* measurements.

A multi-biome MERIS product has been generated by Bacour et al. (2006) to estimate global vegetation characteristics, by using the inversion of the PROSPECT+SAIL model with a neural network approach. Due to the limited number of validation points used by Bacour et al (2006), it is difficult to draw definitive conclusions about the accuracy of the multi-biome approach. Previous validation of the multi-biome LAI against *in-situ* measurements showed an overestimation at lower values of LAI and saturation at higher values of LAI (Bacour et al. 2006; Canisius et al. 2010). It was concluded that none of the MERIS LAI algorithms currently used meet the performance requirements set by the Global Climate Observing System (Canisius et al. 2010). Moreover, the estimation of the multi-biome CCC has not yet been validated against *in-situ* measurements. As the multi-biome approach operates across different biomes, a single-biome approach may increase the accuracy in estimating grassland properties.

The objectives of this study were i) to develop a single-biome approach for the retrieval of grassland properties (i.e., LAI, LCC, and CCC), using the inversion of the PROSPECT+SAIL model from MERIS FR imagery; ii) to compare the performance of multi-biome and single-biome approaches against *in-situ* measurements over multiple seasons; iii) to map the spatio-temporal variation of grassland properties using time-series MERIS imagery; and analyze the temporal consistency of multi-biome and single-biome approaches.

## **MATERIALS AND METHODS**

### **Study area**

The study area is situated in the northern Netherlands, in the provinces of Groningen and Friesland (Fig. 1). The area is a core wintering and spring staging site for migratory waterfowl (Bos et al. 2008; van Eerden et al. 2005). Two types of grassland are present in the study area. The agricultural grassland, accounting for 70% of the grassland in the field sampling area, is managed by local farmers by regular fertilization, mowing, and cattle grazing. The remaining semi-natural grassland is managed as natural reserves and in some of these naturalized cattle is allowed to graze year-round. The plant community in agricultural fields is dominated by *Lolium perenne* and *Poa pratensis* (Prins and Ydenberg 1985), while in semi-natural areas it is dominated by *Festuca rubra*, *Puccinellia maritima*, *Elymus repens*, *Trifolium repens*, *Plantago maritima*, and *Triglochin maritima* (Esselink 2000).

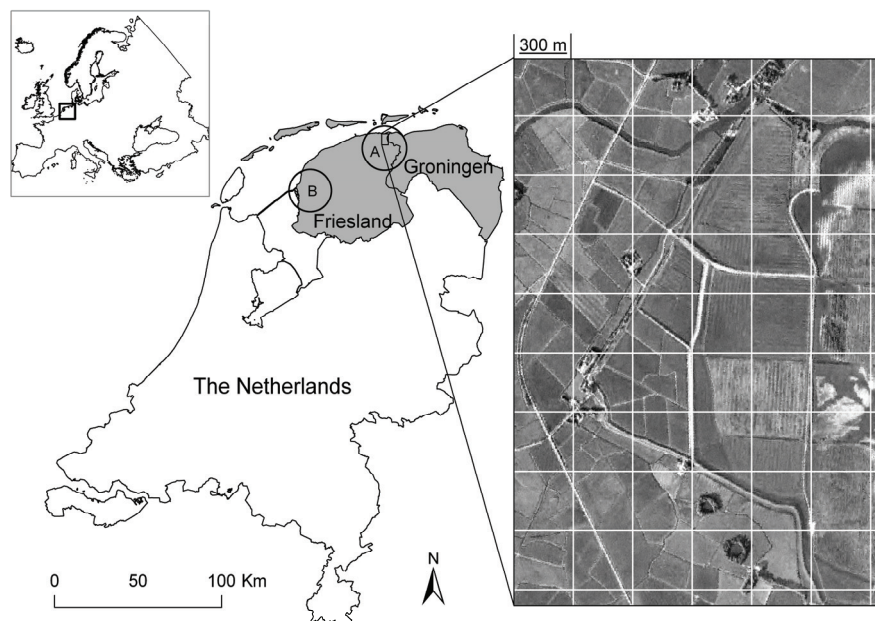


Fig. 1: Location of the study area and the two field sampling areas: Anjumerkolken (A) and Donlaburen-Ferwoude (B). The aero-photo, covered by a grid (representing 300 x 300 m MERIS pixels), illustrates the heterogeneity level of the landscape at the MERIS level.

### **Sampling design**

The fieldwork was conducted in April to June 2008. A total of 30 grassland fields were selected, of which 23 are distributed in agricultural grassland and 7 in semi-natural grassland. Plots of 300

by 300 m were designed and within each plot, five quadratic subplots of 1 by 1 m were established, of which one was situated in the center and four in each corner. A GPS was used to locate the position of each subplot in the field. The vegetation measurements at the plot level were obtained by averaging the measurements of the five subplots.

### **LAI and chlorophyll measurement**

LAI was measured using a Plant Canopy Analyzer LAI-2000 (LICOR Inc., Lincoln, NE, USA), which determines effective LAI using measurements of diffuse solar radiation above and below the grass canopy. The LAI was measured under overcast sky conditions between 10:00 and 16:00, using a view restrictor of 90°. The average LAI was calculated in each subplot, based on one above canopy measurement and five below-canopy measurements. Special attention was taken to ensure stable sky conditions between the above and below canopy measurements. When calculating the LAI, none of the outer rings were eliminated in the gap-fraction inversion. Despite the non-random distribution of leaves, no corrections for clumping effects were applied. These were assumed to be compensated by the overestimation of LAI through grass stems.

A portable SPAD-502 chlorophyll meter (Minolta, Japan) was used for the measurement of LCC. The SPAD measures a unitless value which is highly correlated with leaf chlorophyll content. For each subplot, the SPAD reading was calculated based on the average of 30 randomly selected leaf readings within the subplot. In order to convert the unitless SPAD readings into LCC ( $\mu\text{g cm}^{-2}$ ), a total of 36 leaf samples were collected during the field campaign. The samples were measured with SPAD and subsequently placed in plastic bags inside a portable ice box and transported to the laboratory for LCC measurements. The total chlorophyll a and b contents were then extracted using the dimethyl-sulphoxide solvent method, calculated on the basis of coefficients published by Wellburn (1994). In accordance with Markwell's formulation (Markwell et al. 1995), an exponential equation ( $C_{ab} = 84.8 * \exp(0.00702 * \text{SPAD}) - 82.01$ ) was found to best describe the relationship between the calculated LCC ( $\mu\text{g cm}^{-2}$ ) and the SPAD readings (Fig. 2). The CCC for each subplot was obtained by multiplying LCC by corresponding LAI. The statistical description of field measurements of LAI, LCC, SPAD, and CCC can be found in Table 1.

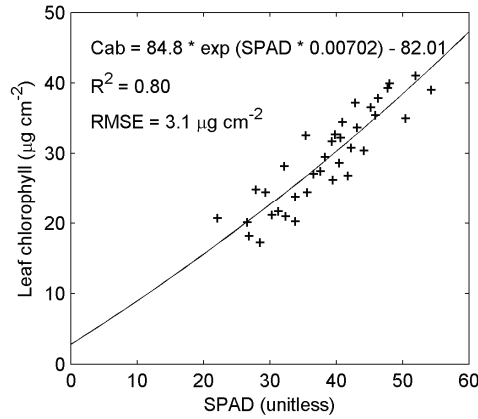


Fig. 2: The empirical relationship between SPAD readings and leaf chlorophyll content based on leaf samples collected during the field campaign in 2008

Table 1: Summary statistics (minimum, mean, maximum, standard deviation, and range) of the field measurement of grass leaf area index (LAI), leaf chlorophyll content (LCC), SPAD readings, and canopy chlorophyll content (CCC).

Measured variables	Min	Mean	Max	StDev	Range
April (n = 30)					
LAI (m <sup>-2</sup> m <sup>-2</sup> )	0.35	0.9	1.66	0.39	1.32
SPAD (unitless)	18.56	37.13	48.5	6.02	29.94
LCC (µg cm <sup>-2</sup> )	21.9	30.16	37.02	3.63	15.12
CCC (g m <sup>-2</sup> )	0.1	0.28	0.54	0.14	0.44
June (n = 30)					
LAI (m <sup>-2</sup> m <sup>-2</sup> )	0.43	3.06	6.77	1.78	6.34
SPAD (unitless)	18.56	37.45	48.67	6.16	30.11
LCC (µg cm <sup>-2</sup> )	14.59	28.48	37.34	4.58	22.75
CCC (g m <sup>-2</sup> )	0.12	0.86	1.69	0.5	1.57
Pooled (n = 60)					
LAI (m <sup>-2</sup> m <sup>-2</sup> )	0.35	1.97	6.77	1.67	6.43
SPAD (unitless)	18.56	38.35	48.67	5.44	30.11
LCC (µg cm <sup>-2</sup> )	14.59	29.32	37.34	4.18	22.75
CCC (g m <sup>-2</sup> )	0.1	0.57	1.68	0.47	1.59



**Soil reflectance measurement**

The spectral reflectance of bare soil was acquired from the vegetation free subplots using a GER 3700 spectroradiometer (Geophysical and Environmental Research Corporation, Buffalo, New York). The spectra were then smoothed using a moving Savitzky-Golay filter (frame size 15 data points, 2nd degree polynomial) (Savitzky and Golay 1964) and resampled to MERIS wavebands. The mean reflectance spectrum was calculated from individual subplot spectra to represent the soil optical properties in the study area.

**MERIS imagery pre-processing**

Five MERIS FR L1b top of atmosphere radiance images, captured on 11th of February, 9th of April, 8th of June, 9th of September, and 26th of December 2008, were acquired respectively. The images of April and June were taken within 10 days of the field sampling time. The SCAPE-M (self-contained atmospheric parameters estimation from MERIS data) algorithm proposed by Guanter et al. (2008) was utilized to convert level 1b top of atmosphere to top of canopy reflectance, correcting distortions caused by the interaction between solar radiation and atmospheric components. The pixels affected by cloud contamination were also masked during the process of atmospheric correction using SCAPE-M. The MERIS reflectance of each subplot was extracted from the April and June corrected images. The reflectance at the plot level was obtained by averaging the reflectance of five subplots. Two approaches have been proposed for generating MERIS multi-biome products using either MERIS L1b images or MERIS L2 images as input (Bacour et al. 2006). L1b images were used to generate the MERIS multi-biome products in this study, as previous study demonstrated that LAI estimates were nearly identical ( $R^2 > 0.98$ ) using either L1b or L2 MERIS images as input in the multi-biome approach (Canisius et al. 2010).

The MERIS bands selected for this study are the same 11 spectral bands utilized for generating multi-biome products (Bacour et al. 2006) and are centered at 490 nm, 510 nm, 560 nm, 620 nm, 665 nm, 681 nm, 709 nm, 754 nm, 779 nm, 865 nm, and 885 nm. The remaining 4 bands were removed because one is an oxygen absorption band (760 nm), one is a water absorption band (900 nm), and two are strongly affected by atmosphere effects (412 nm, 442 nm) and they only provide marginal information (Clevers et al. 2007).

**The PROSPECT+SAIL radiative transfer model**

A combination of the PROSPECT leaf optical properties model (Jacquemoud and Baret 1990) and SAIL canopy reflectance model (Kuusk 1991; Verhoef 1984, 1985), has been validated for different

kinds of vegetation and is therefore considered to be suitable for applications in grassland properties retrieval in terms of both accuracy and running time (Jacquemoud et al. 2000). Leaf optical properties are specified by four parameters: the leaf structural parameter,  $N$  (unitless); the leaf chlorophyll  $a + b$  concentration,  $LCC$  ( $\mu\text{g cm}^{-2}$ ); the dry matter content,  $C_m$  ( $\text{g cm}^{-2}$ ); and the equivalent water thickness,  $C_w$  ( $\text{g cm}^{-2}$ ) (Jacquemoud et al. 2000). The top of canopy reflectance simulation involves three parameters:  $LAI$  ( $\text{m}^2 \text{m}^{-2}$ ); mean leaf inclination angle,  $ALA$  (deg), assuming an ellipsoidal distribution of foliage elements (Campbell 1986); and the hot spot size parameter,  $hot$  ( $\text{m m}^{-1}$ ), implemented by Kuusk (1991). Geometrical parameters include sun zenith angle,  $t_s$  (deg); sensor viewing angle,  $t_o$  (deg); relative azimuth angle,  $\phi$  (deg); and fraction of diffuse incoming solar radiation,  $skyl$ . A soil brightness parameter,  $scale$ , was utilized to account for the changes induced by moisture and roughness in soil brightness (Atzberger et al. 2003; Darvishzadeh et al. 2008a).

### **The single-biome approach**

The single-biome approach is based on a previous study (Darvishzadeh et al. 2008a) which retrieved biophysical and biochemical parameters in heterogeneous grassland at the field level using the inversion of the PROSPECT+SAIL model. The look-up table (LUT) inversion method was selected because it yielded good retrieval performances in estimating grassland properties at the field level (Darvishzadeh et al. 2008a; Vohland and Jarmer 2007). The LUT is a conceptually simple technique, which potentially overcomes limitations of iterative optimization algorithms and reduces the risk of converging to a local minimum which is not necessarily close to the actual solution (Kimes et al. 2000).

Physically based modelling can suffer from the ill-posed problem during the inversion (Atzberger 2004), where the inversion solution is not always unique, as various combinations of canopy parameters may yield almost similar spectra (Weiss and Baret 1999). Previous studies suggested that utilizing prior information is an efficient way of solving the ill-posed problem and of improving the accuracy of the estimated canopy variables (Combal et al. 2003). The LUT generated for the retrieval of grassland properties from MERIS reflectance was optimized according to prior knowledge and the existing literature (Bacour et al. 2006; Darvishzadeh et al. 2008a; Vohland and Jarmer 2007). The geometrical observation parameters and soil reflectance were updated based on the image capture information and the field situation. The geometry of observation, including sun zenith angle, sensor viewing angle and relative azimuth angle, is driven by the

ENVISAT orbit and MERIS swath. Specific ranges of geometry variables were generated based on the range of study area and the date of image capture (Table 2). The input parameters, leaf structural parameter, leaf chlorophyll *a* + *b* content, dry matter content, equivalent water thickness, soil brightness parameter, hot spot size parameter, and fraction of diffuse incoming solar radiation were defined as for the previous field level study (Darvishzadeh et al. 2008a) (Table 2). Considering the changes caused by different grass species and phenological development, broader ranges of the leaf angle (20-70°) and LAI (0.1-8.0 m<sup>2</sup> m<sup>-2</sup>) were utilized compared to Darvishzadeh et al. 2008a. The leaf water content was coupled to the dry matter content in a ratio of 4:1 according to a similar study (Vohland and Jarmer 2007), which demonstrated a clear improvement for LAI estimation in grassland sites.

For each parameter, 100,000 values were drawn randomly within the specific ranges and a total of 100,000 canopy reflectances were generated based on these parameters. To select the optimal spectra corresponding to a given measurement, the RMSE (root mean square error) between measured and modeled spectra was calculated. Previous research found the median of the first 100 matching spectra showed best measures for estimating the grass properties (Darvishzadeh et al. 2008a). The estimated parameter was obtained by calculating the median from the first 100 matching parameter. The estimated LAI, LCC, and CCC in April and June were validated against the *in-situ* measurements.  $R^2$ , RMSE and NRMSE (NRMSE = RMSE / range of the parameter as measured in the field) were adopted to evaluate the performance of the approach.

### **The MERIS multi-biome products**

The MERIS multi-biome products were generated based on the PROSPECT+SAIL model, simulating spectro-directional variation of the reflectance whilst training a neural network to estimate vegetation properties from MERIS reflectance (Bacour et al. 2006). Four variables (i.e., LAI, CCC, fraction of absorbed photosynthetically active radiation, and vegetation cover) were retrieved by the multi-biome approach (Bacour et al. 2006) and the first two variables were analyzed in this study. The multi-biome algorithm is implemented in the BEAM toolbox (ESA) "TOA\_VEG Processor" (<http://www.brockmann-consult.de/cms/web/beam>) and was used to estimate LAI and CCC from five MERIS L1b images. To facilitate comparison, LCC maps were calculated by dividing the CCC maps by the LAI maps. For each sample plot location, the values of LAI, LCC, and CCC, as estimated by the multi-biome approach, were extracted

from the April and June maps and validated against the field measurements.

Table 2: Ranges for input parameters used for generating look up table (LUT) using the PROSPECT+SAIL model simulating MERIS reflectance in the single-biome approach

Parameter	Abb.	Unit	Range or fixed value
Leaf			
Leaf structural parameter	<i>N</i>	No dimension	1.5-1.9
Leaf chlorophyll content	<i>LCC</i>	ug cm <sup>-2</sup>	15-55
Equivalent water thickness	<i>C<sub>w</sub></i>	g cm <sup>-2</sup>	0.01-0.02
Dry matter content <sup>a</sup>	<i>C<sub>m</sub></i>	g cm <sup>-2</sup>	0.0025-0.005
Canopy			
Leaf area index <sup>b</sup>	<i>LAI</i>	m <sup>2</sup> m <sup>-2</sup>	0.1-8.0
Mean leaf inclination angle <sup>c</sup>	<i>ALA</i>	Deg	20-70
Hot spot size parameter	<i>hot</i>	m m <sup>-1</sup>	0.05-0.1
Soil			
Soil brightness parameter	<i>scale</i>	No dimension	0.5-1.5
External			
Sun zenith angle <sup>d</sup>	<i>t<sub>s</sub></i>	Deg	46.98-47.94; 31.37-32.33
sensor viewing angle <sup>d</sup>	<i>t<sub>o</sub></i>	Deg	1.08-13.64; 18.96-29.80
relative azimuth angle <sup>d</sup>	<i>phi</i>	Deg	-131.94--117.4; -135.20--133.14
Fraction of diffuse incoming solar radiation	<i>skyl</i>	No dimension	0.1

<sup>a</sup> Coupled with equivalent water thickness in a ratio of 4:1; range in DR-LUT: 0.01-0.02

<sup>b</sup> Range in the field-level study: 0.3-7.5

<sup>c</sup> Range in the field-level study: 40-70

<sup>d</sup> The first range applies to the April image while the second range applies to the June image

### **Temporal consistency of the multi-biome and single-biome approaches**

The agricultural areas and the semi-natural grasslands (including salt marshes, herbaceous vegetation, and swamp vegetation) were extracted from the Dutch National Landuse Database (LGN 4, 25m spatial resolution). The extracted grassland mask was resampled to a 300 m spatial resolution and then applied to the 5 atmospherically corrected MERIS images. Time-series maps describing the spatio-

temporal variation of grassland quantity and quality were produced using 5 masked grassland images as input. The mean and the standard deviation of time-series maps generated using single-biome and multi-biome approaches were calculated and the temporal consistency was then compared.

## RESULTS

### Estimation of grass variables using the single-biome and multi-biome approaches

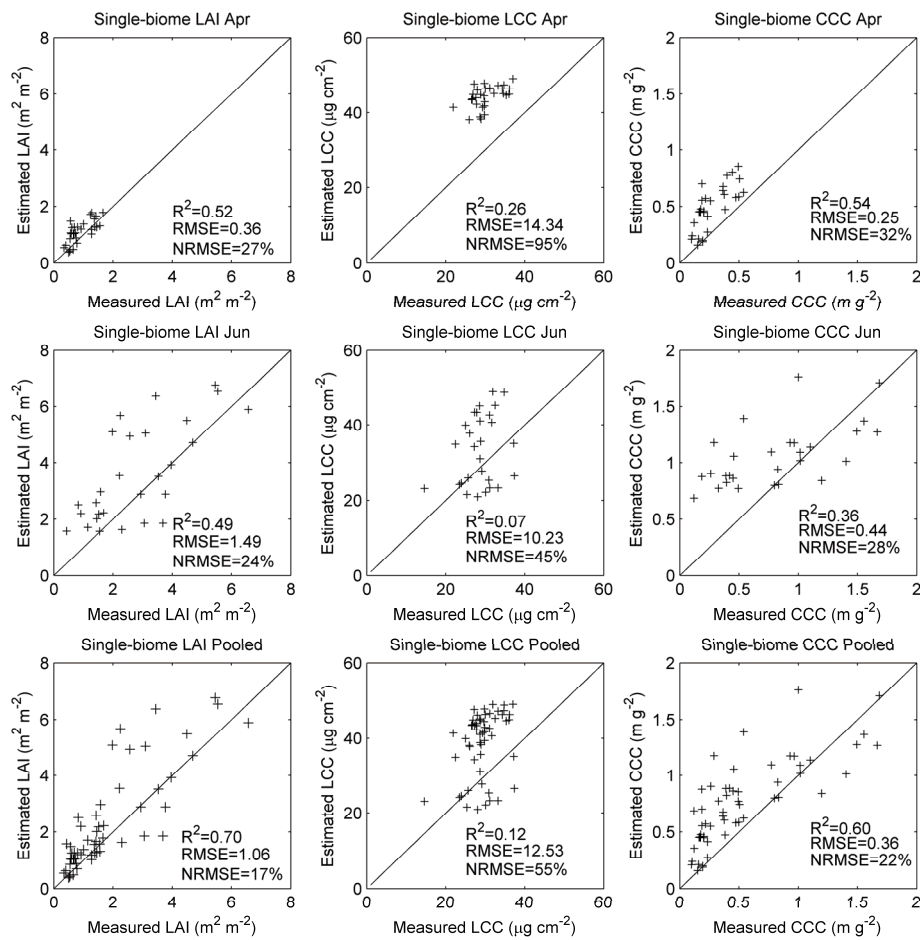


Fig.3: *In-situ* measured vs. estimated grass leaf area index (LAI), leaf chlorophyll content (LCC), and canopy chlorophyll content (CCC), using the single-biome approach in April, June, and April and June pooled

The relationships between *in-situ* measured grass variables and estimates using the single-biome approach are shown in Figure 3. A

good relationship was observed between estimated and measured LAI using the April data, while a relatively larger RMSE was observed using the June data. A poor relationship was observed between the estimated and measured LCC in both single season and pooled data sets. The estimates of canopy chlorophyll content showed an intermediate accuracy with a slightly overestimation using the April data and a relatively larger RMSE using the June data. An intermediate accuracy was found using the pooled data, with a slightly overestimation at the lower CCC ( $< 1$ ).

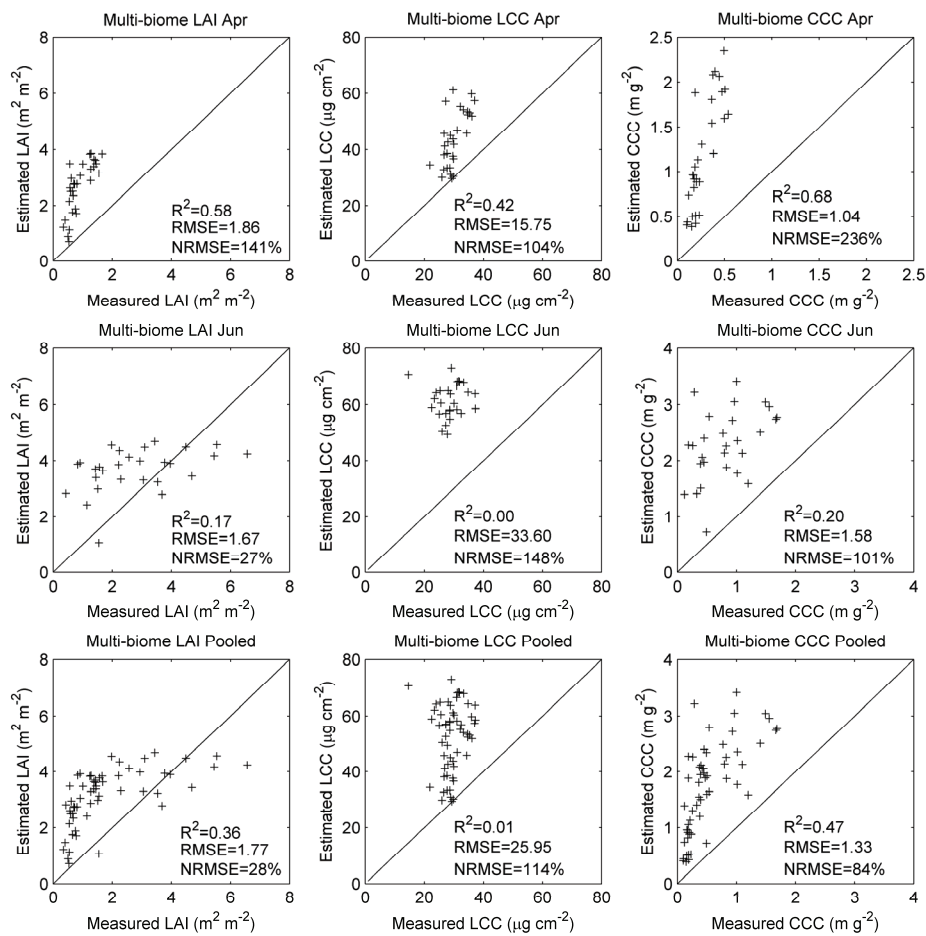


Fig.4: *In-situ* measured vs. estimated grass leaf area index (LAI), leaf chlorophyll content (LCC), and canopy chlorophyll content (CCC), using the multi-biome approach in April, June, and April and June pooled.

The relationships between the *in-situ* measured grass variables and estimates using the multi-biome approach are shown in Fig. 4. LAI was heavily overestimated at a level lower than 2 and saturated at a

level around or higher than 4. Similar to the single-biome approach, the retrieval of the LCC also failed at the MERIS level, using the multi-biome approach. Both LCC and CCC were greatly overestimated using either single season or pooled data sets.

### **Predicted spatio-temporal variation of grass properties using the single-biome approach**

The time-series single-biome LAI maps (Fig. 5.) show a low LAI in February, an increased LAI in April, a peak LAI in June and September, and the lowest LAI in December. The time-series single-biome CCC (Fig. 6.) maps show a similar temporal trend with the seasonal variation of LAI.

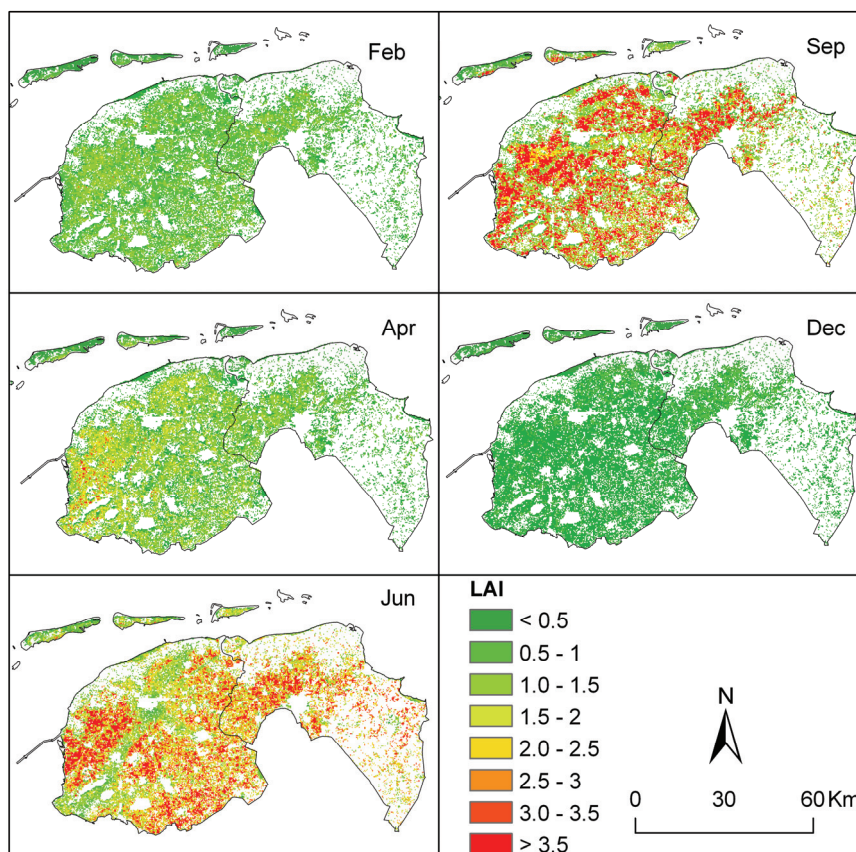


Fig. 5: Time-series maps of single-biome leaf area index (LAI) ( $\text{m}^2 \text{m}^{-2}$ ) for the grassland in the northern Netherlands in 2008.

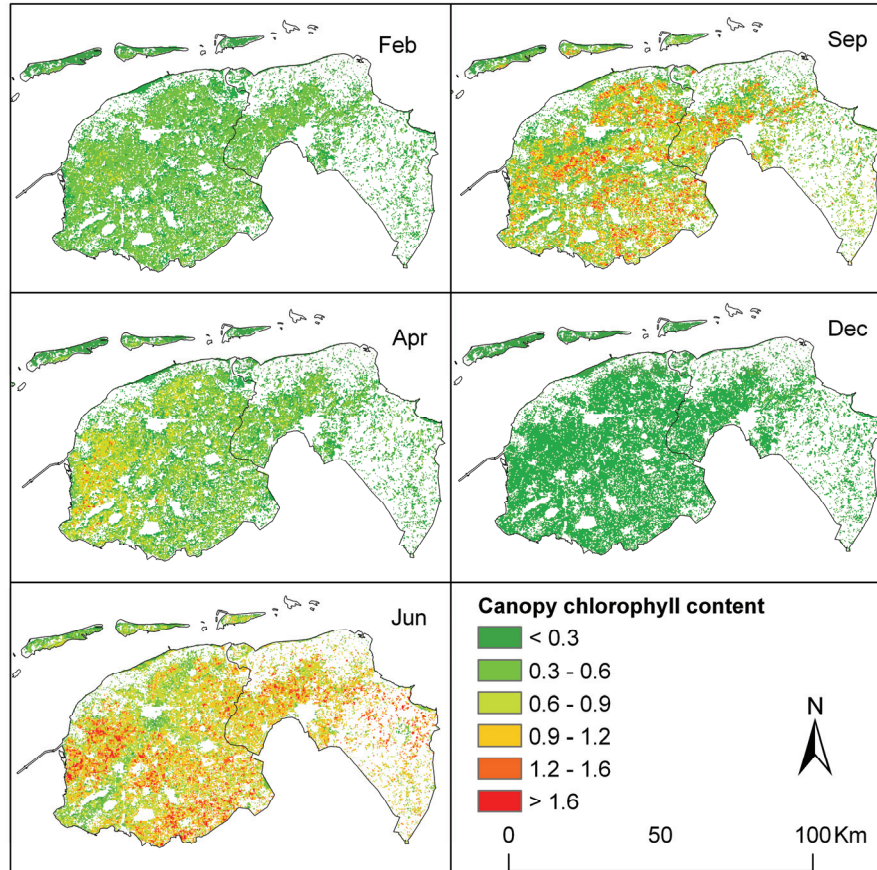


Fig. 6: Time-series maps of single-biome canopy chlorophyll content (CCC) ( $\text{g m}^{-2}$ ) for the grassland in the northern Netherlands in 2008.

### **Comparison of temporal consistency of the single-biome and multi-biome approaches**

The spatio-temporal variation of grassland LAI and CCC estimated by the single-biome and multi-biome approaches are shown in Fig. 7. Similar trajectories were observed for both approaches, but the multi-biome trajectory values were consistently higher. A larger spatial variation was found in single-biome LAI in September and June compared to the other seasons.



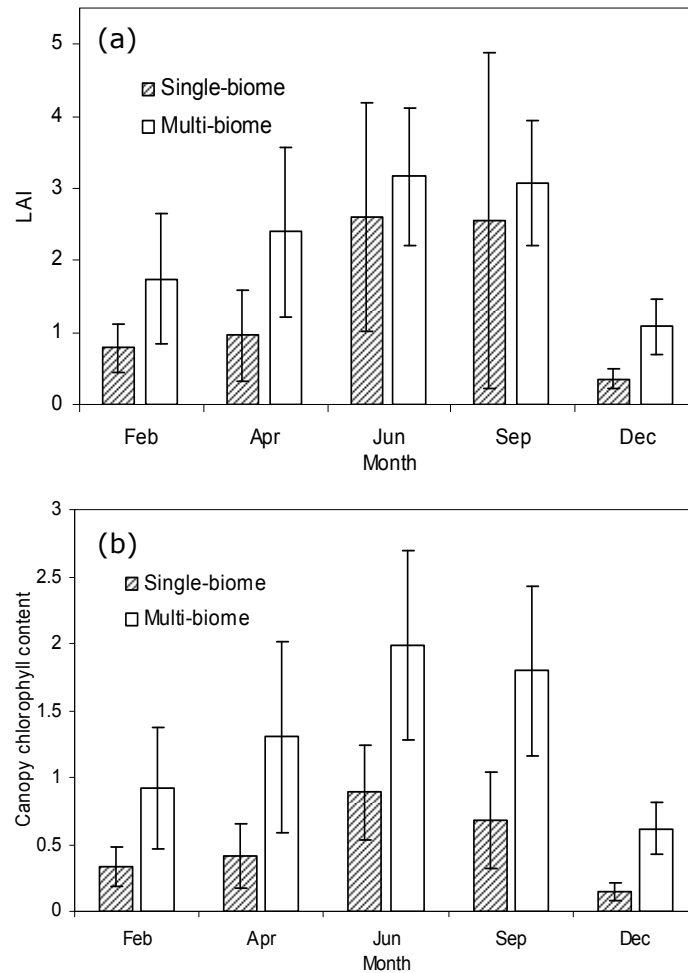


Fig. 7: Temporal variation of grassland leaf area index (LAI) (a) and canopy chlorophyll content (CCC) (b) in the northern Netherlands in 2008 estimated by the single-biome (striped bars) and the multi-biome approach (solid bars), showing mean and standard deviation.

## DISCUSSION

This study demonstrates the ability to accurately estimate grassland LAI and CCC at a regional scale, using the PROSPECT+SAIL model and MERIS FR imagery. The single-biome approach showed good accuracy in LAI estimates ( $R^2=0.70$ , RMSE=1.06, RRMSE= 17%) and intermediate accuracy in CCC estimates ( $R^2=0.60$ , RMSE=0.36, RRMSE=22%). The multi-biome approach performed less accurate. Our results also show that the temporal trajectory of grassland

quantity and quality estimated by the single-biome approach is consistent with the multi-biome trajectory. The results are placed in the context of migratory waterfowl grazing, and the potential to monitor how the “green wave” of vegetation drives the migration of herbivorous waterfowl.

The single-biome approach shows a better performance in estimating the spring grassland in April than the summer grassland in June. The complex grass canopy condition in summer may have caused this poorer retrieval, as grassland was frequently mowed in summer by the local farmers, sometimes with dry leaf materials left covering the vegetation canopy. Grasses tend to grow faster after mowing, taking advantage of the warmer temperature and sufficient precipitation in the study area in summer. After mowing, double grass layers (i.e., dried stems and flushed young grasses) were observed during the June field campaign. The mismatch of the sampling date and image capture date may also influence the retrieval accuracy, especially for the warm seasons, when grass grows faster than cold seasons. The change of observation geometry of images captured on different dates may have caused the change of the heterogeneity level of each MERIS pixel (Gomez-Chova et al. 2010), which may also lead to different accuracies in different seasons. Additionally, cloud contaminated pixels may heavily bias the retrieval of grass properties from MERIS measurements. Although we have used an updated cloud filtering techniques coupled in an atmospheric correction process (Guanter et al. 2008), the influence of thin-cloud was still observed in the generated time-series grassland maps. Further efforts should be taken to solve this cloud mask issue before accurate maps can be produced and used (Bacour et al. 2006).

The single-biome estimates show consistently higher accuracy than the MERIS multi-biome global products. The multi-biome product overestimated LAI values when they were smaller than 2, and it saturated at LAI values equals to and larger than 4. This overestimation and saturation of LAI was also observed in the original study (Bacour et al. 2006) in which the multi-biome global product was developed. These same findings were also reported by a previous study validating MERIS multi-biome LAI products across different vegetation species, including crops, pasture and forest (Canisius et al. 2010). Furthermore, the multi-biome CCC was heavily overestimated across the whole data range observed in this study. The single-biome approach proposed by this study showed significant improvement of LAI and CCC estimation at the MERIS level. Applying this approach for global usage requires further validation by including more sites as well as long-term *in-situ* measurements.

The time-series grassland LAI and CCC maps estimated by the single-biome approach are highly associated with the grassland phenology and also influenced by the agricultural practices. The relatively large spatial variation in June and September maps is caused by mowing activity. As frequent fertilization leads to a consistently high quality grass all year round, the temporal variation of CCC is heavily influenced by the variation of LAI. The single-biome temporal trajectory is consistent with the multi-biome trajectory, showing the phenological development of grasses. Because of the overestimation of the multi-biome LAI, the multi-biome trajectory values were consistently larger than the single-biome trajectory values. Besides, the larger standard deviation of June and September LAI estimated by the single-biome approach can be explained by the coexistence of short and tall grasses in the field.

The estimate of LCC failed at the MERIS FR level. One possible reason could be the heterogeneous nature of the surfaces at this medium spatial resolution. Fig. 1 shows the heterogeneity level of landscape under a 300 m spatial resolution, in which many mixed pixels were observed. One possible alternative to overcome the heterogeneity problem is to make use of data fusion techniques (Zurita-Milla et al. 2009). Nevertheless, the failure of estimating LCC at the space level is expected, as difficulties in estimating LCC for grasslands were also reported at the field level (Darvishzadeh et al. 2008a). The poor signal propagation from leaf to canopy scale reported by previous studies (Asner 1998; Yoder and Pettigrew-Crosby 1995b) may also contribute to the poor retrieval at the MERIS level. Besides, the frequent fertilization in the study area may also lead to a relatively small range of field LCC measurements and thereby cause more difficulty in LCC retrieval.

In conclusion, this study underlines the ability of using the PROSPECT+SAIL model together with MERIS satellite data for accurately estimating the spatio-temporal variation of grassland quantity and quality at the regional scale. The proposed single-biome approach showed consistently higher accuracy of grassland LAI and CCC estimates than the MERIS multi-biome global products. The spatio-temporal patterns of grassland LAI and CCC estimated by the single-biome approach were highly linked to the grassland phenology and showed the influence from the agricultural practices. The time-series of grassland quantity and quality maps generated in this study may facilitate a better understanding of the movement of herbivorous waterfowl and therewith the spread of infectious avian disease.

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## **Chapter 6**

**Synthesis: avian influenza and migratory  
birds from a spatial-ecological perspective**

## INTRODUCTION

Zoonotic diseases (e.g. avian influenza) and vector-borne diseases (e.g. malaria) are inextricably linked to the environment. The ecological processes involved in the life cycle of these diseases include interactions within and between hosts, reservoirs, vectors, agents, pathogens, and the environments associated with disease transmission. Although understanding these ecological processes requires an ecological thinking and spatio-temporal consideration, studies on diseases are still largely the domain of veterinarians and virologists (Olsen et al. 2006). A spatial-ecological perspective of zoonotic and vector-borne diseases may provide insights into unexpected vector/agent control responses, suggest potential implications for proper interventions and contribute to effective management solutions in an ever-changing environment (Ellis and Wilcox 2009). The main objective of this thesis was to investigate, from a spatial-ecological perspective, the interactions among the occurrence of highly pathogenic avian influenza (HPAI) H5N1, the distribution of the spreading agent migratory waterfowl, and environmental factors.

The spatio-temporal dynamics of HPAI H5N1 outbreaks were quantified at a global scale and the links with migratory waterfowl demonstrated in Chapter 2. A risk map for possible occurrence of HPAI H5N1 in wild birds across Europe is presented in Chapter 3, using a number of identified relevant environmental factors. As the activity of spreading agents directly influences the disease prevalence, in Chapter 4 the distribution of migratory waterfowl is modelled at the habitat level, based on both physical and anthropogenic environmental factors (in this case referring to barnacle geese *Branta leucopsis* in the Lauwersmeer area in the northern part of the Netherlands). Food resources are found to be the primary driver of waterfowl distribution as well as the main factor influencing the occurrence of the HPAI H5N1 virus. Spatio-temporal variations of forage quantity and quality were therefore estimated at a regional scale in Chapter 5, which could be used to improve the risk prediction of HPAI H5N1 outbreak. In this final Chapter, the main results from the previous chapters are brought together in order to gain a better understanding of the linkage among HPAI H5N1, migratory waterfowl, and key environmental factors. For each section, the previous scientific gaps now filled by my findings are addressed. The relatedness of the different chapters is shown in figure 1. The practical relevance, including implications for disease surveillance and control, as well as the limitations of our approach

was discussed. Lastly, the main conclusion and a focus for future studies suggested.

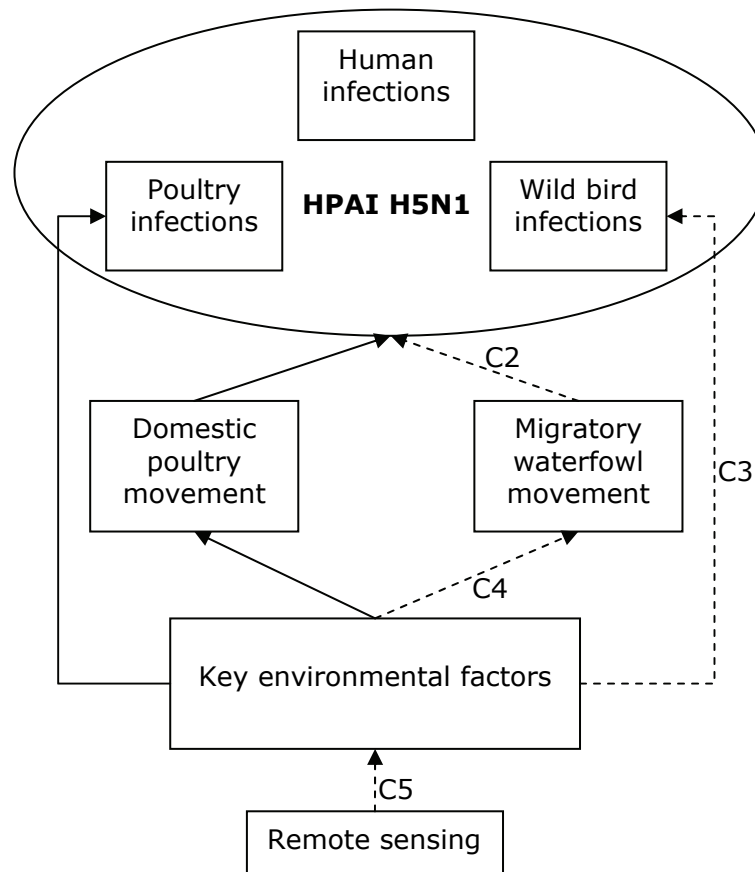


Fig.1: Diagram showing the context of the different thesis chapters, filling previous scientific gaps. C2 - C5 refer to Chapter 2 - Chapter 5, solid arrows indicate issues that have been hypothesized and tested by previous studies and broken arrows indicate issues that require more investigation

### **Spatio-temporal patterns of global HPAI H5N1 outbreaks - Do migratory waterfowl play a role?**

Waterfowl, principally duck, geese, swan, gulls, terns and shorebirds are considered to be the main reservoir of avian influenza (Alexander 2000; Olsen et al. 2006; Suarez 2000; Webster et al. 1992). Though the potential synergism between poultry transportation and waterfowl movement in the spread of HPAI H5N1 has been suggested (Kilpatrick et al. 2006), it still remains a debatable issue whether infected waterfowl are capable of spreading HPAI H5N1 over long-distance (Feare and Yasué 2006; Normile 2006). No direct tests have been conducted to demonstrate the ability of waterfowl to migrate

while being infected with HPAI H5N1. The few studies that attempted to test this hypothesis found the spread of the HPAI H5N1 virus from Russia and Kazakhstan to the Black Sea basin is consistent in space and time with the Anatidae autumn migration routes (Gilbert et al. 2006b). It is more likely that the whooper swan infections in Eastern Asia occurred through contact with asymptomatic migratory hosts, such as wild ducks at or near their breeding grounds (Newman et al. 2009). Significant gaps in our knowledge of the ecology of avian influenza in wild birds are apparent, in particular regarding the risk of the virus being spread by migratory waterfowl (Munster et al. 2007).

Quantifying spatio-temporal patterns is important for our understanding of how spatio-temporal phenomena such as disease occurrence behave. If migratory waterfowl are capable of transmitting HPAI H5N1 viruses, either over long or over short distances, the disease patterns would be influenced by bird distribution and movement. Disease patterns are usually described by statistically significant clustering, a definite, discernible aggregation of points above that would be expected, given an underlying population at risk (Ward 2008). Furthermore, geographical and temporal scales are intimately linked to an accurate understanding of the distribution of vectors/agents, incidence of diseases, and scope of management activities (Ellis and Wilcox 2009). Due to the intercontinental waterfowl migration and the international trade of fowl, the linkage between the waterfowl migration and HPAI H5N1 outbreak patterns has been viewed at a global scale (Chapter 2).

The relationship between waterfowl migration and HPAI H5N1 transmission was revealed by overlapping the dynamics of disease patterns, quantified by the space-time clusters, and the migratory flyways of waterfowl (Chapter 2). Six disease patterns were associated with the timing, location and direction of continental waterfowl migration, suggesting that migratory waterfowl spread HPAI H5N1 over long distances. Disease clusters were also detected at sites that are known overwintering areas at times when these areas were frequented by migratory waterfowl, suggesting that waterfowl are also involved in short-distance disease spread (Chapter 2). Although human mobility cannot be ruled out as the cause of the observed disease patterns, the strong association between waterfowl flyways and disease patterns are indicative of a significant role. For the first time, the linkage between waterfowl migration and HPAI H5N1 outbreak patterns has been investigated at a global scale and covering an extended period of time.

**Risk mapping of HPAI H5N1 occurrence in wild birds - What factors affect the disease occurrence in wild birds?**

A number of environmental factors are closely associated with zoonotic disease, in particular climate, land cover, and landscape patterns (Brownstein et al. 2003; Frank et al. 1998; Lindgren et al. 2000; Randolph 1993). Only recently, and for only few disease systems, have the types, sizes and positions of landscape elements (e.g. habitat patches, physical or biotic gradients, and type of matrix surrounding patches) and their connectivity been considered potentially important drivers of risk or incidence (Ostfeld et al. 2005). In Southeast Asia, HPAI H5N1 outbreaks were highly correlated with free-range duck farming and rice cultivation (Gilbert et al. 2007). Environmental factors were also found to be associated with HPAI H5N1 prevalence in other regions, such as in China, India, the Middle East and Africa (Adhikari et al. 2009; Biswas et al. 2009; Fang et al. 2008; Williams and Peterson 2009). Disease incidence at different locations and times may have very different causes. Certain environmental factors or landscape features may be of greater importance in some areas than others (Williams and Peterson 2009). For example, poultry infections in Thailand were largely associated with rice fields and free grazing ducks in the area (Gilbert et al. 2007). This factor may only affect the areas where duck production operates, such as in Southeast Asia (Gilbert et al. 2008b). Another reason for fewer waterfowl HPAI H5N1 reports in Southeast Asia may occur because of underreporting, due to a lack of interest by authorities in wild waterfowl. In contrast to Asia, Europe has reported large numbers of HPAI H5N1 occurrences in wild birds, whereas most countries that reported wild bird infections found few or no poultry outbreaks nearby (Chapter 3). It was therefore suggested that wild birds are the primary spreading agent of HPAI H5N1 in Europe (Kilpatrick et al. 2006). Previous studies (Gilbert et al. 2006a; Gilbert et al. 2007; Ward et al. 2008a; Williams and Peterson 2009) mainly focused on poultry outbreaks and revealed that both anthropogenic and physical environmental factors have some bearing on the disease incidence. The risk of the HPAI H5N1 virus occurring in wild birds, however, is not clearly understood.

The influence of both physical and anthropogenic environmental factors on the HPAI H5N1 occurrence in wild birds in Europe was investigated and a risk map based on identified factors associated with disease incidence was generated. None of the anthropogenic factors and composite factors (indicating interaction between wild birds and domestic poultry) showed significant effects on the disease occurrence, suggesting poultry transportation did not play a primary role in Europe (Chapter 3). The infections in wild birds were found to

occur consistently under predictable, physical environmental circumstances: an increased NDVI in December, an intermediate NDVI in March, low elevation, an increased minimum temperature in January and reduced precipitation in January (Chapter 3). We therefore suggest that occurrences of HPAI H5N1 in wild birds in Europe are influenced by the availability of food resources and facilitated by increased temperatures and reduced precipitation (Chapter 3). The risk of HPAI H5N1 occurring in wild birds in Europe was mapped based on these identified geographical characteristics and features (Chapter 3).

**Spatial modelling of the disease spreading agent – what factors affect waterfowl distribution?**

As early as in 1930, Klinger pointed out the need to have a thorough knowledge of breeding places and habits of disease vectors in order to apply the most suitable methods to the situation (Klinger 1930). Since then, a large body of ecologically relevant knowledge has accumulated for vector-borne diseases. Basic ecological science has grown in parallel, but neither area has consistently benefited from the knowledge generated by the other (Ellis and Wilcox 2009). Ecological theory can help us understand the distribution and movement of vectors/agents and lead to better control measures. At a population level of ecological research, efforts are typically focused on understanding the dynamics of a particular species and its interactions with the environment (Ellis and Wilcox 2009). This is an important area for vector-borne and zoonotic disease research, because there is a need to understand the factors regulating vector/agent or host populations and abundances, so they can be incorporated in risk predicting (Ellis and Wilcox 2009).

As food is the main driver of animal activities (Fryxell 1991; Prins and Ydenberg 1985), it is a reasonable expectation that animal distribution is largely influenced by food availability. Animal feeding behaviours have been described in ecological theories and hypotheses. Optimal foraging theory predicts that animals spend more time in patches that enable them to have a higher intake rate than the average intake rate of all patches (de Boer and Prins 1989; Emlen 1966; MacArthur and Pianka 1966; Prins 1996; Raoulet et al. 1998). The forage maturation hypothesis (Wilmschurst et al. 1995) predicts that avian herbivores select forage with intermediate biomass and high nitrogen content, whereas the green wave hypothesis (Owen 1980) predicts that migratory birds follow a green wave of highly nutritious plant tissue on their way from temperate staging sites, via stopover sites, to their breeding areas (Fig. 2). Besides food resources, the distribution of waterfowl is also



influenced by environmental conditions, predation risk, and animal characteristics (Fryxell 1991; Kurvers et al. 2009; Raouet et al. 1998).

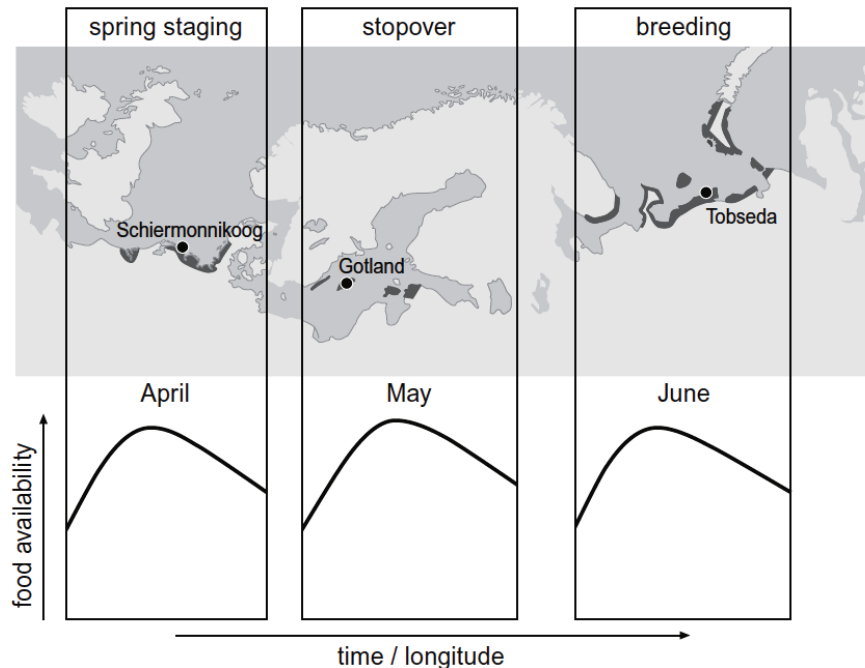


Fig. 2: A schematic overview of the green-wave hypothesis, modified for the flyway of the Russian population of Barnacle Geese *Branta leucopsis*. Three sites are indicated on the map; Schiermonnikoog, the Netherlands, a Wadden Sea staging site; Gotland, Sweden, a Baltic stopover site; and Tobsseda, a Russian breeding site (after van der Graaf 2006)

The migration patterns of birds are also influenced by ecological changes. In Europe, the distribution of waterfowl is influenced by the restructuring of agricultural practices (van Eerden et al. 2005). Profound changes have occurred in the European landscape during the 20th century. Intensification of land use has led to a simplification of the trophic structure of many ecosystems, giving man control over the natural variation in the landscape (van Eerden et al. 2005). Migratory birds are forced to cope with these ever-changing patchy environments, resulting from frequent and often radical shifts in land use (van Eerden et al. 2005). For example, almost 80% of the total flyway population of barnacle geese in north-western Europe relies during the winter on agricultural land in the Netherlands (Madsen et al. 1999; van Eerden et al. 2005). Large numbers of agricultural fields were therefore designated as refuges for wild birds in the Netherlands to reduce damage to the remaining agriculture.

The effects of both physical environmental factors (i.e., food resources, distance to roost) and anthropogenic factors (i.e., refuge locations) on the distribution of migratory waterfowl (in this case barnacle geese) at the habitat level was therefore investigated in Chapter 4. Investigating at the habitat level may reveal important additional insights concerning the factors that attract birds to a particular field (Vickery and Gill 1999), given the previous studies were mainly conducted under field experimental conditions (Bos et al. 2005; Durant et al. 2004; Riddington et al. 1997; van der Graaf et al. 2007). Geese were observed to intensively utilize refuges and showed significantly higher accumulative grazing intensity in areas located within 2 km from the roost (Chapter 4). A pronounced preference for sites with an intermediate forage quantity was found. Forage quality did not play a role in the distribution of birds because the nitrogen level observed in the study area is high and above the Barnacle geese' requirement level (Chapter 4).

**Retrieving key environmental data using remote sensing techniques - How to accurately quantify resource availability?**

In applied and environmental epidemiological studies, up-to-date, accurate measures of environmental conditions that extend over large geographical regions are required. In regard to the HPAI H5N1 virus, food availability primarily determines the distribution of migratory waterfowl as well as the risk of disease occurrence (Chapter 3, 4). Currently, vegetation indices derived from remote sensing imagery are commonly used as a surrogate of food resources in epidemiological and ecological studies (Wang et al. 2010; Williams et al. 2008), and the migration patterns of birds used are general and not yet quantified (Chapter 2). An accurate quantification of forage characteristics is needed to predict migratory waterfowl movement and HPAI H5N1 occurrence. Aerial photography and satellite imagery offer a way of retrieving vegetation characteristics from regional to global scales. Due to its high spectral resolution, moderate spatial resolution and a two to three day repeat cycle, the Medium Resolution Imaging Spectrometer (MERIS) is considered to be a promising sensor to measure and monitor forage quality and quantity. However, the existing MERIS land products which estimate global multi-biome characteristics show limited consistency with *in-situ* measurements (Canisius et al. 2010). A higher accurate estimate of forage properties is required to meet the demands of epidemiological and ecological applications.

A single-biome approach was proposed in Chapter 5 to improve the accuracy of estimating the forage quantity and quality at a regional scale, using the PROSPECT+SAIL model and MERIS imagery. The

physically-based model was utilized because it can cope with a wide range of situations and could be applied from regional to global scales. An intermediate accuracy of forage quantity and quality retrieval was achieved by using the single-biome approach. The *in-situ* validation revealed a consistently better performance of the single-biome approach compared to the multi-biome approach (Chapter 5). The spatio-temporal variation of forage quantity and quality was mapped at a regional scale, revealing the phenology of grasses as well as the effects of agricultural practices (Chapter 5). The way of employing remotely sensed data as described in chapter 5 provides data more relevant to herbivorous waterfowl movement. It will therefore facilitate the prediction of the spread of infectious avian disease.

## **PRACTICAL RELEVANCE**

The role of the short-distance and long-distance spread of the HPAI H5N1 virus by migratory waterfowl as demonstrated in Chapter 2, implies that understanding migratory waterfowl distribution and movement is critical for monitoring HPAI H5N1 prevalence as well as for generating measures for future control. Spatial models built on ecological principles are invaluable tools in quantifying waterfowl distribution. By modelling the spatial distribution of waterfowl, areas where this spreading agent is more likely to aggregate could be identified. A higher density of waterfowl indicates a higher chance of intra- and interspecific contact and thereby a higher risk of infection. Preventive measures, surveillance and early-warning systems should target these areas.

Based on the environmental risk factors of HPAI H5N1 occurrence in wild birds identified in Chapter 3, a more sensitive and cost-effective surveillance systems could be designed. Areas where HPAI H5N1 incursions are more likely may be preferentially sampled during surveillance. By understanding where the disease is more likely to occur, preventive measures can be implemented to reduce the risk of an outbreak. These measures should complement the increased biosecurity provided by keeping poultry indoors, limiting live bird markets and the transportation of fowl, and using prophylactic vaccination.

The effect of scale is a critical issue when integrating ecological approaches with disease analysis. The size of the study area influences which risk factors are relevant. For example, the effect of interaction between domestic poultry and waterfowl in HPAI H5N1 spread was not detected at a continental (European) scale, but a

previous study found that HPAI H5N1 infections poultry occur via exposure to migratory waterfowl at a local scale in Romania (Ward et al. 2008b). If investigations focus only on national or continental data, such associations may be missed (Ward et al. 2008b). The relevance of risk factors at different scales needs to be further tested while control measures developed at a continental level should be adjusted before being applied at local areas.

More effort is needed to accurately quantify the spatio-temporal movement of migratory waterfowl. After modelling the distribution of waterfowl at a habitat level in Chapter 4, the green wave hypothesis needs to be further tested at the landscape and multi-temporal level, to permit accurate modelling of waterfowl distribution. This requires accurately quantified environmental data, such as resource quality and quantity at a landscape scale. Generating these data requires efforts in remote sensing. The role that remote sensing can play in studying the ecology of the avian influenza virus is illustrated in Figure 3. In Southeast Asia, the risk of avian influenza has been predicted based on the distribution of rice fields derived from remote sensing imagery, as these fields provide food resources for free-grazing ducks, an important spreading agent. The spatio-temporal variation of forage quantity and quality estimated in Chapter 5 is placed in the context of monitoring the availability of food resources at a continental scale and then quantifying the migration patterns of waterfowl, which could be used to assist the risk prediction of HPAI H5N1.

A better understanding of the transmission and pathogenesis of HPAI H5N1 in waterfowl, as well as the identification of other virus-permissive bird species are also important (Olsen et al. 2006). For an accurate disease risk mapping based on migration patterns, knowing the target bird species that are capable of transmitting the virus asymptomatically during their migration is critical. Although birds are generally driven by the availability of food resources, different waterfowl species may show significant variation in their ecology and behaviour (Olsen et al. 2006).

Data quality requires more attention with respect to availability and reliability. The quality of information sources is a delicate problem, as the diagnosis methods may vary among countries. The heterogeneity in surveillance and control measures may also lead to a bias of reported cases. A consistent standard of case reporting should be applied across geopolitical boundaries. The basic data requirements for HPAI H5N1 reports should include full and accurate identification of bird species involved, spatially and temporally accurate location

and habitat, date of collection, capture method, sampling method, and population characteristics (Olsen et al. 2006). Besides data quality, the integration and accessibility of data are a common problem. Remotely sensed environmental variables such as air temperature, humidity, and rainfall should be processed and made available in real-time and in a format that can readily use as model input.

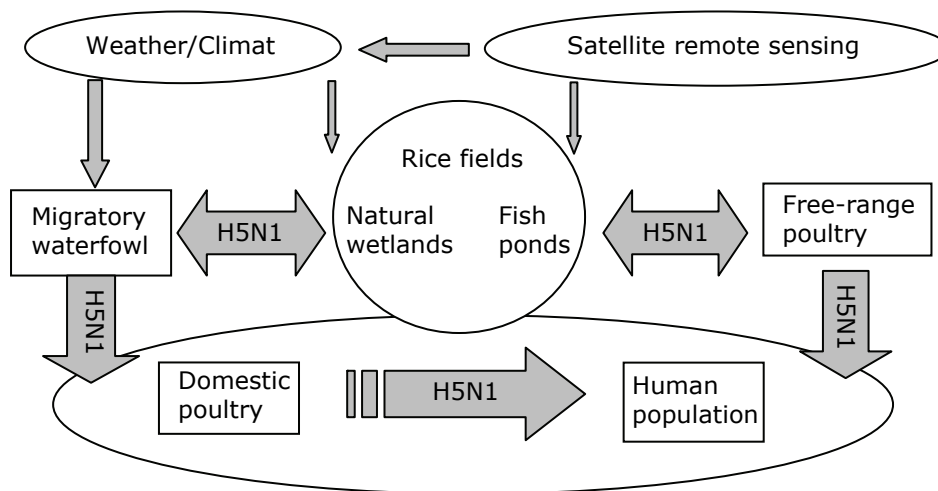


Fig. 3: A schematic diagram that illustrates the role of satellite remote sensing in studying bird migration, agricultural areas, natural wetlands, and epidemiology of the avian influenza virus (adapted from Xiao et al. 2007).

## GENERAL CONCLUSION

This thesis has demonstrated the value of a spatial-ecological perspective in understanding the relation between HPAI H5N1 spread and waterfowl migration. Migratory waterfowl are suggested to play an important role in the global spread of HPAI H5N1, over both short and long distances. The occurrence of HPAI H5N1 in wild birds is influenced by the availability of food resources and is facilitated by increased temperatures and reduced precipitation. Consistent with ecological principles, the spatial distribution of waterfowl is determined by food availability (i.e., grasses with high quality and intermediate quantity), distance to roost, and the availability of refuges. Remote sensing techniques can be used to quantify spatio-temporal variation of forage quantity and quality at a regional scale. This information can further assist in predicting the spreading agent distribution and thus disease occurrence. The use of GIS, remote sensing, relational databases, predictive modelling, spatial analyses,

and geostatistics has proved extremely valuable in exploring the dynamic interactions among HPAI H5N1 occurrences, the spreading agent migratory waterfowl, and the changing physical and anthropogenic environment.

## **OUTLOOK**

Global environmental change, particularly climate change and land use/cover change, has the potential to greatly alter global patterns of avian influenza epidemiology. These changes will shift the distribution, composition, diversity, and migration behaviour of the wild birds that harbour the genetic pool of the avian influenza virus (Gilbert et al. 2008a, Gibbs 2010). Understanding the influence of environmental change on disease incidence patterns, bird migration patterns, and on the distribution of domestic-wild waterfowl contact points is of utmost importance. To accomplish this, real time monitoring of environmental change is necessary. Further advances in GIS, remote sensing, and spatial techniques will allow for the accurate quantification of environmental change.

The requirement for an integrated, transdisciplinary, system-based approach to understand and control infectious disease transmission, has become increasingly obvious due to the wide range of social and biophysical factors involved (Ellis and Wilcox 2009). This thesis has demonstrated that the understanding of the avian influenza puzzle can be improved by integrating concepts and methodology taken from ecology, biology, geography, epidemiology, and spatial science. Further intercommunication among these disciplines, as well as with medicine, medical statistics, anthropology, medical sociology and economics, should be promoted. Furthermore, international and intergovernmental collaboration should be promoted as transmission of infectious diseases is unbound by political boundaries.

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## Author's Biography



Yali Si was born on the 15th of September 1982 in Hubei Province and grew up in Henan Province, China. In 1999, she went to Henan Agricultural University and studied Land Resources Management, where she received her bachelor degree with distinction in 2003. She then moved to Wuhan University in 2004 and enrolled in a joint master's program between Wuhan University and the International Institute for Geo-information Science and Earth Observation (ITC) in the Netherlands. She completed her thesis with distinction in March 2006 and received her M.Sc. degree in Geo-information for Natural Resources and Environment Management. In 2006, she was awarded a four-year doctoral scholarship from the China Scholarship Council, with partial financial support from ITC. She then moved to the Netherlands again in 2007 and started her Ph.D. research at ITC and Wageningen University, which resulted in this thesis. Her research interests include spatial epidemiology, spatial ecology, ecosystem modelling, remote sensing of vegetation, and global change ecology.



## Summary

The global spread of highly pathogenic avian influenza (HPAI) H5N1 poses a significant threat to public health. An efficient surveillance and disease control system relies on our understanding of the ecology of the HPAI H5N1 virus. Domestic poultry and wild birds (mainly waterfowl) are considered as the spreading agents. The role of poultry in the spread of the virus is relatively well understood. However, the role of migratory waterfowl, especially in long-distance transmission, requires further investigation. Environmental factors influencing the occurrence of HPAI H5N1 in wild birds are currently unknown. Furthermore, the spatio-temporal occurrence of waterfowl has not yet been quantified at regional and higher scales. To generate up-to-date, accurate measures of the relevant environmental conditions over large geographic regions, more effort in remote sensing is required.

The aim of this study is to investigate the interactions among the occurrence of HPAI H5N1, the distribution of migratory waterfowl, and environmental factors, from a spatial-ecological perspective.

We find that the outbreak pattern of HPAI H5N1 is highly correlated with waterfowl migration patterns, suggesting that migratory waterfowl play an important role in the global spread of HPAI H5N1 over both short and long distances. Furthermore, wild birds instead of poultry were the main spreading agent causing the 2005-2008 HPAI H5N1 outbreaks in European wild birds. HPAI H5N1 occurrence in wild birds in Europe is influenced by the availability of food resources and facilitated by increased temperatures and reduced precipitation. The spatial distribution of waterfowl at a habitat level is determined by food availability (i.e., grasses with high quality and intermediate quantity), distance to roosts, and the presence of refuges. We use a single-biome approach, which specifically focuses on grassland, rather than the conventional multi-biome approach, which looks at vegetation in general. The single-biome approach performs consistently better and yields an intermediate accuracy of forage quantity and quality retrieval. The estimated spatio-temporal variation of forage quantity and quality is placed in the context of migratory waterfowl grazing, which allows monitoring the availability of food resources continentally. Subsequently, the migration patterns can be quantified and these can further assist the risk prediction of HPAI H5N1 outbreaks.

This study has shown the value of taking a spatial-ecological perspective to the understanding of the interactions among HPAI

### *Summary*

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H5N1 occurrence, waterfowl distribution, and environmental factors. Our findings can be used to improve HPAI H5N1 surveillance and assist in controlling the disease in an ever-changing environment.

## Samenvatting

De wereldwijde uitbreiding van hoog pathogene vogelpest (highly pathogenic avian influenza; HPAI) variant H5N1 vormt een significante bedreiging voor de volksgezondheid. Een efficiënte controle en bestrijding is afhankelijk van ons begrip van de ecologie van het HPAI H5N1 virus. Pluimvee en in het wild levende vogels (met name watervogels) worden gezien als de verspreiders. De rol die pluimvee speelt in het verspreiden van het virus is relatief goed bekend. Echter, de rol van trekvogels, met name in de transmissie over lange afstanden, dient verder te worden onderzocht. Het is momenteel onbekend welke milieu-factoren het voorkomen van HPAI H5N1 beïnvloeden. Bovendien is het spatio-temporele voorkomen van watervogels niet eerder gekwantificeerd op regionale en grotere schaal. Voor het genereren van up-to-date, accurate metingen van de relevante milieu-condities die een uitgebreide geografische regio beslaan, is meer inspanning op het gebied van remote sensing vereist.

Het doel van deze studie is om de interacties te onderzoeken tussen het voorkomen van HPAI H5N1, de verspreiding van trekkende watervogels en milieu-factoren, vanuit spatio-ecologisch perspectief.

We vinden dat het uitbraakpatroon van HPAI H5N1 sterk is gecorreleerd aan de migratiepatronen van watervogels, wat suggereert dat trekkende watervogels een belangrijke rol spelen in de wereldwijde verspreiding van HPAI H5N1, over zowel korte als lange afstanden. Bovendien waren wilde vogels, en niet pluimvee, de voornaamste verspreiders van HPAI H5N1 onder Europese wilde vogels, gedurende de periode 2005-2008. Het voorkomen van HPAI H5N1 bij wilde vogels in Europa wordt beïnvloed door de beschikbaarheid van voedselbronnen en gefaciliteerd door hogere temperaturen en een afname in neerslag. De ruimtelijke verspreiding van watervogels op habitat niveau wordt bepaald door de beschikbaarheid van voedsel (i.e., grassen van hoge kwaliteit en middelmatige kwantiteit), afstand tot roestplaatsen, en aanwezigheid van toevluchtsoorden. We maken gebruik van een single-biome aanpak, die zich specifiek richt op grasland, in plaats van de conventionele multi-biome aanpak, waarbij wordt gekeken naar de vegetatie in het algemeen. De single-biome aanpak presteert consistent beter en levert een middelmatige nauwkeurigheid voor de opbrengst van voedsel kwantiteit en kwaliteit. De geschatte spatio-temporele variatie van voedsel kwantiteit en kwaliteit wordt in de context van het grazen van trekkende watervogels geplaatst, wat het

### *Samenvatting*

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monitoren van de beschikbaarheid van voedsel op continentale schaal mogelijk maakt. Vervolgens kunnen de migratiepatronen worden gekwantificeerd en deze kunnen verder helpen het risico op HPAI H5N1-uitbraken te voorspellen.

Deze studie heeft de waarde aangetoond van het aannemen van een ruimtelijk-ecologisch perspectief in het trachten om de interacties tussen HPAI H5N1 voorkomen, watervogels distributie en het milieu te begrijpen. Onze bevindingen kunnen worden gebruikt om HPAI H5N1 toezicht te verbeteren. en om de bestrijding van de ziekte in een steeds veranderende omgeving bij te staan.



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### **PE&RC Education Certificate**

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks activities)



### **Review of Literature (5 ECTS)**

- Modeling the migration pattern of avian herbivores for avian influenza warning by MERIS (2007)

### **Writing of Project Proposal (7 ECTS)**

- Modeling the migration pattern of avian herbivores for avian influenza warning by MERIS (2007)

### **Post-Graduate Courses (3 ECTS)**

- Basic and advanced statistics course; Wageningen University (2008)

### **Competence Strengthening / Skills Courses (4 ECTS)**

- Scientific writing; ITC (2007)
- Advanced course guide to scientific artwork; Wageningen University (2008)
- Presentation course; ITC (2009)

### **Discussion Group / Local Seminars and Other Scientific Meetings (7.8 ECTS)**

- Fort-nightly PhD discussion group; ITC (2007-2011)
- PhD and research seminars; ITC (2007-2011)
- PhD day in Tropical Ecology; University of Amsterdam (2008)
- MSc day chair; ITC (2007, 2009)

### **PE&RC Annual Meetings, Seminars and the PE&RC Weekend (2.6 ECTS)**

- PhD weekend; Wageningen University (2007)
- Research day of department of Natural Resources (2007-2010)

### **International Symposia, Workshops and Conferences (5 ECTS)**

- XXI ISPRS Conference; Beijing, China (2008)
- Annual Ecology Meeting; The Netherlands (2010)
- XXXVIII ISPRS Conference; Hong Kong, China (2010)